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1 Effects of dietary fatty acids on nutrient digestion, energy partitioning, and milk fat synthesis. A. L. Lock* and J. de Souza, Michigan State University, East Lansing, MI.

Our understanding of fatty acid (FA) digestion and metabolism in dairy cows has advanced significantly in the last few decades. We now recognize that FA, both of dietary and rumen origin, can have different and specific effects on feed intake, rumen metabolism, small intestine digestibility, milk component synthesis in the mammary gland, and energy partitioning between the mammary gland and other tissues. We will present research focusing on specific FA and how dairy cows respond differently to combinations of FA. Recent research has highlighted differences in intestinal digestibility among palmitic acid (C16:0), stearic acid (C18:0), and oleic (cis-9 C18:1) acids, which impacts the amount and profile of absorbed FA available for metabolic purposes. C16:0, C18:0, and cis-9 C18:1 usually comprise the majority of FA present in milk fat and adipose tissue of dairy cows. In addition, these FA comprise the major FA in a wide range of commercially available fat supplements. While these FA have different functions in metabolism, they may also interact with each other by competitive or complementary mechanisms under different physiological conditions. In the mammary gland, milk FA are derived from 2 sources: <16 carbon FA from de novo synthesis in the mammary gland and >16 carbon FA originating from extraction from plasma. 16-carbon FA originate from either de novo or preformed sources. Milk lipid synthesis in the mammary gland is dependent upon the simultaneous supply of short/medium-chain FA and long-chain FA. C16:0 has a higher preference as a substrate to start triglyceride synthesis than C18:0 or cis-9 C18:1. Also, if the amount of preformed FA surpasses the capacity of the mammary gland, these might be redirected to other tissues (e.g., adipose tissue) altering energy partitioning. In the future, the opportunity and challenge will be to continue to improve our understanding of how and which FA affect nutrient digestion, energy partitioning, and milk synthesis in lactating dairy cows and effectively apply this knowledge in the feeding and management of today’s high producing dairy cows.

Key Words: energy partitioning, fatty acids, milk fat synthesis

2 Amino acid uptake by the mammary glands: Where does the control lie? J. P. Cant*,1, J. J. M. Kim1, S. R. L. Cieslar1, and J. Doelman2, 1University of Guelph, Guelph, ON, Canada, 2Nutreco Nederland BV, Boxmeer, the Netherlands.

Milk protein yield responses to changes in the profile of essential amino acids absorbed by the gastrointestinal tract or circulating in blood plasma do not follow the classic limiting amino acid response, in part because of an ability of the mammary glands to modify their blood flow rate and net clearance of amino acids out of plasma. The hypothesis that mammary blood flow is locally regulated to maintain ATP balance accounts for observed changes in flow due to postprandial glucose, insulin and EAA infusions. An additional hypothesis that net mammary uptakes of metabolites from blood are affected by perturbations in their respective arterial concentrations and the rate of mammary blood flow also appears to hold for the energy metabolites glucose, acetate, BHBA and FA. However, net EAA uptakes by the mammary glands are poorly predicted by models considering arterial concentrations and blood flow rates only. Evidence points to intramammary protein synthesis and secretion as the determinant of net EAA uptake. The intracellular signaling network anchored by the mechanistic target of rapamycin complex 1 (mTORC1) stands as an excellent candidate to explain nutritional effects on milk protein synthesis because it integrates information on physiological and nutritional state to affect protein synthesis and cell metabolism, growth, proliferation and differentiation in many cell types. In mammary cells in vitro and in vivo, the mTORC1, integrated stress response, and insulin signaling networks that contribute to regulation of initiation of mRNA translation are responsive to acute changes in nutrient supply and EAA profile. However, after several days of postpruminal infusion of balanced and imbalanced EAA profiles, these signaling networks do not appear to continue to account for changes in milk protein yields. Gene expression evidence suggests that regulation of components of the unfolded protein response that control biogenesis of the endoplasmic reticulum and differentiation of a secretory phenotype may contribute to effects of nutrition on milk protein yield. Connections between early signaling events and their long-term consequences are proposed.

Key Words: mammary blood flow, milk protein synthesis, translational regulation

3 Influences of heat stress on the bovine mammary gland. S. Tao*, R. M. Orellana, X. Weng, T. N. Marins, and J. K. Bernard, University of Georgia, Tifton, GA.

Heat stress (HS) reduces cows’ milk production, resulting in a significant economic loss for the dairy industry. During lactation, HS lowers milk yield by 25–40% with half of the decrease in milk synthesis due to factors unrelated to feed intake. In vitro studies indicate that primary bovine mammary epithelial cells display greater rates of programmed cell death when exposed to high ambient temperature, which may lead to a decrease in total number of milk synthetic cells in the mammary gland (MG) and partially explain the lower milk production of lactating cows under HS. The function of mammary cells is also altered by HS. In response to HS, mammary cells display higher gene expression of heat shock proteins, indicating a need for cytoprotection from protein aggregation and degradation. Further, HS results in increased gene expression but similar protein expression of mammary epithelial junction proteins, and doesn’t substantially influence the integrity of mammary epithelium, indicating an effort to maintain cell-to-cell junction by synthesizing more proteins to compensate for protein loss by HS. Bovine mammary epithelial cells also have reduced gene expression of proteins involved in milk synthesis suggesting that HS directly reduces milk synthetic capacity of MG. During the dry period, HS negatively affects MG development by reducing mammary cell proliferation before parturition, resulting in a dramatic decrease in milk production in the subsequent lactation. In addition to mammary growth, MG of the HS cow has reduced protein expression of autophagy proteins in the early dry period, suggesting HS influences mammary involution. Emerging evidence also indicates that heifers born to late gestation HS cows have lower milk production during their first lactation, implying that the maternal environment may alter MG development of the offspring. It is not clear if this is due to a directly epigenetic modification of prenatal MG development by maternal HS. More research is needed to elucidate the impact of HS on MG development and function.

Key Words: heat stress, mammary gland, lactation
The disparate impacts of inflammatory signaling pathways on lactogenesis, galactopoiesis, and cessation of lactation. B. J. Bradford*1, C. M. Ylioja1, and K. M. Daniels2, 1Kansas State University, Manhattan, KS, 2Virginia Polytechnic Institute and State University, Blacksburg, VA.

Inflammation is a well-characterized process used by the immune system as a component of the response to infection or tissue damage. The repertoire of signals used in immune inflammation, however, is neither limited to immune cells nor confined to adverse health events. Inflammatory signals affect mammogenesis, lactogenesis, lactation, and involution, often in dramatic ways. The role of inflammatory mediators in lactogenesis should not be surprising, given that lactogenic factors such as prolactin and growth hormone utilize cytokine receptors with second messengers that overlap with inflammatory cytokine signaling pathways. Some eye-opening studies have demonstrated that tissue-specific gene knockout mice lacking certain inflammatory mediators completely lack a functional mammary gland. Inflammatory signals are also critical mediators of mastitis-induced decreases in milk synthesis. Evidence for this role ranges from the molecular to the whole-animal level, implicating pattern recognition receptors which trigger inflammatory transcription factors that act as transcriptional repressors for milk synthesis genes. A poorly understood mechanism that contributes to this phenomenon is the transient but dramatic change in methylation of milk component gene promoters, which may or may not revert completely to the pre-mastitis condition after resolution of the inflammation. More recent findings demonstrated that inflammatory mediators such as interleukin-6 are essential for normal mammary involution at the end of lactation. More recent findings demonstrated that the loss of phagocytic cleanup of mammary tissue during involution (triggered by inflammatory signals) dramatically impairs milk production in the subsequent lactation. In closing, emerging data suggest that cellular differentiation processes, including those in the mammary gland, often incorporate inflammatory signaling, and inflammatory links with mammary development likely continue to operate into at least the very early stages of lactation.

Key Words: lactation, development, mastitis

Oxylipids and the regulation of bovine inflammatory responses. L. Sordillo*, Michigan State University, East Lansing, MI.

Inflammation is a critical aspect of the innate immune system that can determine the outcome of several economically important diseases of dairy cattle including mastitis. The purpose of the inflammatory response is to eliminate the source of tissue injury and then return tissues to normal function. Aggressive inflammatory responses, however, can cause damage to host tissues and contribute significantly to the pathophysiology of mastitis. A precarious balance between pro-inflammatory and pro-resolving mechanisms is needed to ensure optimal pathogen clearance and the prompt return to immune homeostasis. Therefore, inflammatory responses must be tightly regulated to avoid bystander damage to the milk synthesizing tissues of the mammary gland. Oxylipids are potent lipid mediators that can regulate all aspects of the inflammatory response. The biosynthetic profiles of oxylipids are dependent on both the availability of diverse polyunsaturated fatty acids substrates and their subsequent metabolism through various oxidizing pathways. Changes in lipid metabolism in dairy cows around parturition due to negative energy balance can profoundly change the composition and concentration of oxylipids in the mammary gland that may be responsible for dysfunctional inflammatory responses during this time. This presentation will provide a brief overview of the role that oxylipids play in contributing to the onset and resolution of inflammation. Factors associated with periparturient cows that can contribute to dysfunctional regulation of inflammation as a function of altered oxylipid biosynthesis and metabolism also will be described. Understanding the role oxylipids may play in mediating the onset and resolution of mastitis is key to developing novel prevention and control programs for the dairy industry.

Key Words: lipid mediator, inflammation, mastitis
6  **Purposes and types of models.**  M. D. Hanigan*,  *Virginia Tech, Blacksburg, VA.*

The principles of mathematical modeling in agricultural sciences are well described by France and Thornley (1984). They categorized models as static or dynamic, empirical or mechanistic, and deterministic or stochastic, although, in practice, they can fall somewhere in the middle of each. In general, our nutrient requirement models are static, empirical, and deterministic; they provide snapshots in time, do not describe the mechanisms underlying responses, and do not consider the inherent variance intrinsic to biological systems. These models are generally easier to derive, and have served the community very well for more than a century. The Molly cow model is dynamic, mechanistic, and deterministic; it predicts responses through time, is based on the underlying driving elements of digestion and metabolism, but does not represent the biological variation underlying predictions. Dynamic models are very useful when one needs to predict changes over time as compared with representing only the new state after the system is given sufficient time to reach steady state. For example, growth and lactation models are typically dynamic, empirical, and deterministic. They capture the effects of slightly greater growth rates on body weight at any point in the growth cycle, or the effect of greater persistency on overall lactational yield. Static nutrient response models only provide the new rate of growth or milk yield after the animal has consumed the diet long enough to reach a new steady state. They cannot predict full lactation yields. Mechanistic models are often used to represent the effects of underlying behavior on higher level performance, e.g., the effects of passage rate on ruminal digestion or the effects of enzymatic activity of a tissue on metabolism. Such representations may provide more precise predictions of higher level performance, although that generally requires that the mechanisms are well defined and provide unbiased estimates. The models are also very useful to assess the relative importance of more basic information. Addition of stochastic elements to mechanistic models can accommodate known variance in the underlying mechanisms and thus provide confidence intervals for predictions.

**Key Words:** mathematical model, type, review

7  **Dynamic deterministic models.**  T. Hackmann*,  *University of Florida, Gainesville, FL.*

This lesson will demonstrate how to construct dynamic deterministic models, which are popular for mechanistic modeling in nutrition research. This type of model represents a biological system as a set of state variables and simulates how these variables change over time. For example, it can represent the rumen system using state variables for fiber, protein, and starch; subsequently, it can simulate the size of these nutrient pools over a feeding cycle. The model is written formally using differential equations, but it can be drawn first as a compartmental model diagram. In this diagram, each state variable is represented by a rectangle (a pool). Arrows leading to and from a pool represents input and output of material. For the rumen, these arrows commonly represent nutrient intake, digestion, and passage. The diagram is then translated into a set of differential equations. These equations define the change of state variables (pools) over time as the difference between inputs and outputs [i.e., \( \frac{d(\text{state variable})}{dt} = \text{inputs} - \text{outputs} \)]. These inputs and outputs, in turn, are functions of parameters (e.g., digestion and passage rates) and other state variables. After defining values of parameters, the model is solved and used to generate predictions. A simple model may have an analytical solution, but a more complex model must be solved numerically (e.g., with Euler’s method and difference equations). During a demonstration exercise, the speaker will show how to construct a simple (one-pool) model of rumen fermentation by coding difference equations into an Excel spreadsheet. During a hands-on exercise, participants will construct their own, multi-pool model.

**Key Words:** least squares, likelihood, Bayesian

8  **Estimation of parameter values in nutrition models.**  L. Moraes*,  *The Ohio State University, Columbus, OH.*

The use of modeling techniques in animal nutrition relies on the construction of mathematical models determined by a set of parameters. In practice, parameter true values are unknown. Estimators must be obtained with data from designed experiments, observational studies, meta-analysis or another appropriate data generating mechanism. For virtually any type of model, parameter estimates have to be optimal in some sense. For example, linear regression least squares estimates are the minimizers of the squared differences between observations and predictions. In this setting, if model errors are assumed to independent, identically and normally distributed, least squares estimators coincide with maximum likelihood estimators. Maximum likelihood is the standard estimation method for more complex models used in animal nutrition. It seeks parameter values that maximize the likelihood function: a function constructed with the probability density of the observations but as a function of parameters while fixing the data. Nonlinear models are regularly used in the development of mechanistic models as these allow the relationship between variables to be specified by a function that is nonlinear with respect to the parameters. The flexibility of specifying nonlinear functional forms comes with a cost: the function to be optimized is often complex and an analytical solution to the problem is many times not available. Further, several of the mechanistic models used in animal nutrition rely on the use of differential equations that require numerical integration. Parameter estimation in these cases is usually approached by algorithmic optimization of either a likelihood function or a nonlinear least squares cost function. Recently, Bayesian methods have been proposed as estimation approaches for nutrition models as they naturally describe multilevel structures and incorporate prior information in the analysis. This lesson will cover parameter estimation in a variety of models frequently used in animal nutrition as well as demonstration exercises. During a hands-on exercise, workshop participants will estimate parameters in different models using the freely available software R.

**Key Words:** mathematical model, state variable, differential equation

9  **Model evaluation.**  E. Kebreab*,  *University of California, Davis, Davis, CA.*

Statistical measures of model performance commonly compare predictions with observations judged to be reliable. Model evaluation indicates the level of accuracy and precision of model predictions by assessing the credibility or reliability of a model in comparison to real-world observations. Quantitative statistical model evaluation methods can be classified into 3 types including (1) standard regression statistics, which determines strength of linear relationship, (2) error index, which quantifies deviation in observed units, and (3) relative model evaluation that are dimensionless. Within the first category, analysis of residuals
involves regressing residuals against predicted or other model variables. In this method, the model is unbiased if residuals are not correlated with predictions and the slope is not significantly different from zero. Predicted values can also be centered making the slope and intercept estimates in the regression orthogonal and thus, independent. This allows for mean biases to be assessed using the intercepts of the regression equations, and the slopes to determine the presence of linear biases. Mean square error of prediction (MSEP) and its square root (RMSEP) are commonly used methods of evaluation. In general RMSEP values less than half of observed SD may be considered having a good performance. The MSEP can be decomposed into error due to 1) overall bias of prediction, 2) deviation of the regression slope from unity, and 3) disturbance. Examples of the third category include concordance correlation coefficient (CCC), and the Nash-Sutcliffe index (NSE). The CCC can be represented as a product of 2 components (range from 0 to 1 and 1 indicates perfect fit): a correlation coefficient estimate that measures precision and a bias correction factor that indicates how far the regression line deviates from the line of unity. The NSE is a normalized statistic that determines relative magnitude of residual variance compared with observed data variance. During model evaluation, a combination of the methods described above should be used to gain insight on model performance. The hands-on exercises include coding a function to calculate RMSEP, NSE and CCC for a set of data, which will be provided to participants.

Key Words: model performance, modeling, prediction accuracy

10 Example models for ruminant digestion and metabolism .
H. A. Rosow*, Veterinary Medicine Teaching and Research Center, University of California Davis, Tulare, CA.

Mathematical models are a tool to examine existing theories, find gaps in knowledge and explain phenomena of nutrient digestion and metabolism. The model can then produce simulation data to examine model behavior and determine if predictions from such models make biological ‘sense’. The objective of this session is to explore how concepts or theories of nutrient digestion, metabolism and lactation physiology are translated into mechanistic mathematical equations and combined into a whole animal model using the Molly model. Molly is a mechanistic model of a dairy cow composed of a digestive element and an animal element. The digestive element converts chemical composition of the diet to volatile fatty acids, microbial growth and absorbed nutrients using physical attributes of the diet such as proportions of large and small particles and water passage. The animal element converts products from the digestive element into tissues (protein), waste products, heat production or secreted products (milk, milk fat, etc.). In this session, representations of digesta passage, protein synthesis and milk production in Molly will be examined beginning with a conceptual diagram. Then differential equations representing these processes will be described. Finally, because Molly predicts changes in production processes over time, full lactation simulations will be demonstrated to show examples of how passage, protein accretion and milk synthesis change over time. Based on these examples, participants will conduct a simulation exercise which uses concepts of milk synthesis in Molly that were explored previously to predict lactation performance. A compiled version of the Molly program which operates only in the Windows environment is available for download at http://www.vmtrc.ucdavis.edu/laboratories/metabolic/molly.cfm and will be used in the simulation exercise. In the exercise, participants will observe effects of altering milk production processes on production of the dairy cow to understand how metabolic processes can be represented by mathematical equations to provide a conceptual framework that improves our understanding of animal biology.

Key Words: computer simulation model, dairy cow, metabolism

11 Meta-regression analysis of animal nutrition literature .
R. R. White*, Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA.

Quantitative literature summary (meta-analysis) is often used to generate a more comprehensive understanding of system behavior than can be obtained from individual experiments. Although every data set is unique and often requires some individualized analysis, most meta-analytical data can be evaluated using weighted, mixed-effect, regression in a 9-part procedure, described as follows. 1) Search criteria should be clearly defined. 2) The literature should be searched and all response variables, their standard errors, and all explanatory variables should be recorded. 3) Data should be evaluated for transcription errors and outliers. 4) Missing standard errors should be estimated by error propagation, where possible. 5) Standard errors from fixed-effect regression and mixed-effect regression should be standardized to remove statistical analysis effects, and weights should be calculated from these standardized standard errors. 6) Backward, stepwise regression should be performed, using fixed effects for all explanatory variables of interest, and random effects for study, laboratory, or location, as needed. 7) After a model is identified where all variables included are below a significance cutoff defined by the researchers, the parameters removed from the model should be iteratively re-tested for significance in the final model. This step helps ensure variables were removed for non-significance rather than accidently removed due to model instability. 8) Parameter estimate correlation should be evaluated using variance inflation factors. Variance inflation factors above 10 are acceptable for parameters correlated by calculation but all other parameters should have variance inflation factors below 10. 9) Researchers should iterate through steps 6 to 8 until a model is identified where all parameters are statistically significant and have acceptable covariation. Although this procedure might require adjustment for some applications, it provides a general framework for performing meta-regression analysis of animal nutrition literature. The workshop associated with this abstract will walk through this process using an example data set.

Key Words: meta-analysis, regression, methods
How to teach and how to learn effectively: A review of the recent literature. M. A. Wattiaux1, A. Faciola2, and C. C. Williams3, 1University of Wisconsin-Madison, Madison, WI, 2University of Nevada, Reno, NV, 3Louisiana State University, Baton Rouge, LA.

Our objective was to review factors influencing students’ learning, the maximization of which is the ultimate goal of any college classroom. The instructor (I), the students (S), and the course content (C) are the 3 fundamental parts of a college classroom. Thus one could conceivably predict learning (L) as a multiple regression including these 3 factors and their interactions: $L = I + S + C + I\times S + I\times C + S\times C + I\times S\times C + \text{error}$. Arguably, instructional effectiveness (I, I×S, I×C, and I×S×C in the equation) can be measured with tools meant to determine students’ performance relative to stated learning goals. Grades and failure rates have been used as metrics of effectiveness in large enrollment classes; however other non-graded assessments might also shed light on students’ perception of learning. Allegedly a more subjective (and controversial) mode of evaluating instructional effectiveness is the end-of-semester course evaluation. It is incumbent to administrative units to determine whether the instrument used is valid and reliable. Contribution of students to their learning (S in the equation) can be found in the literature on motivation, diversity, and achievement gaps. A recent review of learning techniques (S×C in the equation) has indicated high utility for practice testing (self-testing or taking practice tests) and distributed practice (scheduling study activities over time) and moderate utility for elaborative interrogation (generating an explanation), self-explanation (connecting to known information, or explaining steps in problem solving), and interleaved practices (mixing different kinds of materials or problems, within a single study session). The most effective modes of teaching within a discipline or a profession (C in the equation) have been captured in the research on “pedagogical content knowledge” (e.g., nutrition and genetics are taught and learned differently) and “signature pedagogy” (e.g., future lawyers and medical doctors are taught from distinct professional paradigms). Careful and deliberate planning of the interactions among the 3 fundamental parts of a college classroom may be paramount to maximize the learning of each student.

Key Words: undergraduate education

Dr. Dale E. Bauman is recognized internationally for his significant contributions to the understanding of the biology of the dairy cow. His research began with reciprocal activities of enzymes in mammary and adipose tissues with stage of lactation. He introduced the term homeorhesis which described the coordination of actions of organs and glands for utilization of nutrients for maintenance and productive functions. Work with somatotropin and lipids opened the window to understanding milk and milk fat synthesis. He was the first member of ADSA to be elected to the National Academy of Science. Perhaps Dr. Bauman’s greatest contribution has been in the training of graduate students who have gone into highly productive careers in dairy science. Our 3 speakers, all of whom trained with Dale, will review his scientific contributions from the late 1960s to date.

Key Words: Bauman

14 Dale Bauman Symposium—The early years at the University of Illinois. J. P. McNamara*, McNamara Research in Agriculture Firm, Pullman, WA.

From the ending of the “age of pathway biochemistry” to the beginnings of “nutritional physiology,” the 1970s was a dynamic and fertile time for biochemistry, nutrition, physiology, and animal sciences research. Likely even more so, in the animal and dairy sciences there was a well-recognized need for basic biology research on all facets of animal metabolism to help explain and improve the practical farm situation and food production. The young scientists of the day, including Ransom Baldwin V, Allen Tucker, Don Beitz, Don Palmquist, Dale Bauman, Jerry Young (to name a few), concentrated on unravelling the metabolic and physiological pathways of the rumen and the body organs and their nutritional and endocrine control in support of lactation. A natural and sequential timeline starting with discovery of bio-hydrogenation of fatty acids in the rumen and their (predicted) effect on milk fat synthesis; the discovery of variations in pathways of fatty acid biosynthesis in the rumen, adipose, liver and mammary glands; continuing on to the definition of control of enzyme transcription and translation in metabolism and low milk fat syndrome; induced lactation and the ideal combination of steroid and protein hormones in the control of mammary development and ‘ending’ with the foundational discovery of reciprocal control of ana-bolic and catabolic pathways in the mammary gland and adipose tissue during late pregnancy and parturition. The theme of the research was on defining not just the pathways but the complex control on enzymes, pathways and organs leading to support of the dominant physiology state, a now fully integrated concept defined as homeorhesis, to close the decade. Essential and inseparable from the outstanding science was the human respect, collegiality and downright fun that research was in that time and place.

Key Words: homeorhesis, nutrient partitioning, adaptations

15 Homeorhesis and nutrient partitioning. R. Collier*, University of Arizona, Tucson, AZ.

Dale Bauman’s journey toward defining and delineating homeorhesis and the concepts around control of nutrient partitioning began with his doctoral studies at the University of Illinois on the genetic differences in the ATP citrate lyase pathway between ruminants and non-ruminants. He was the first to demonstrate the glucose sparing mechanism ruminants evolved to reduce the flow of glucose carbons into fatty acid synthesis sparing glucose for lactose synthesis. As a new assistant professor at the University of Illinois, he and his graduate students produced classic publications on the metabolic adaptations required for onset and maintenance of lactation in ruminant and non-ruminant animals. This work stimulated his thinking on how these metabolic adaptations were coordinated. His work at the University of Illinois also included the role of prolactin in the initiation of lactation in cattle. He continued his work on the biology of prolactin and somatotropin while on sabbatical leave with Allen Tucker at Michigan State University where he began thinking of the role of these hormones in coordinating metabolism with onset of lactation. Subsequently, he moved to Cornell University where he fully developed the concept of homeorhesis and published the complete concept with Bruce Currie; their much cited paper on this subject was published in 1980. Dale went on to demonstrate how homeorhetic regulation was involved in widely varying physiological states including hibernation, pregnancy, starvation, stress, lactation and growth to name a few. In the late 70s recombinant bovine somatotropin became available and Dale worked with multiple forms of this molecule both native and recombinant to study somatotropin as a key homeorhetic regulator. He and his students and colleagues published hundreds of papers, abstracts and reviews over the next quarter century. For these and other contributions to science, Dale was nominated to the National Academy of Science in 1988.

Key Words: homeorhesis, nutrient partitioning

16 Dr. Dale E. Bauman: Training graduate students and solving the riddle of milk fat depression (MFD). L. Baumgard*, Iowa State University, Ames, IA.

Over his career, Dale Bauman mentored 40 graduate students receiving MS/PhD degrees, and 20 post-docs and visiting scholars. His mentees have become faculty members at universities around the world or key industry researchers. These individuals represent the next generation of educators, scientists, and industry influencers and many have already become leaders in agriculture science. This was the basis for his selection as the ASN Dannon Award for Mentoring. Bauman was also faculty advisor to undergraduates (~20 annually) and over the last 10 years before retirement he directed 43 undergraduate independent research projects and supervised 14 senior honors thesis projects. Bauman’s loyalty and allegiance to his former students and his willingness to be a “lifelong” mentor is a tribute to his character and passion for his “teaching” trade. By the mid-1990s, Dale had inarguably become the world’s thought leader in multiple fields (biochemical pathways of ruminant fatty acid synthesis, homeorhesis, rbST, and nutrient partitioning). During the 1990s and 2000s, Bauman became the global authority in 2 more scientific areas: milk fat depression (MFD) and conjugated linoleic acid (CLA) synthesis. He discovered the role of nutrition and management practices on milk composition and the application of this knowledge to address diet-induced MFD, a problem that had perplexed dairy producers and baffled scientists for almost 150 yr. Bauman and associates first identified that CLA isomers inhibited mammary fat synthesis and proposed the “biohydrogenation theory” to explain MFD. An important component of Bauman’s research focused on “functional foods” to improve the healthfulness of ruminant-derived...
foods. His group’s original contributions include developing analytical methods, identifying biochemical pathways of CLA synthesis in the rumen and via endogenous synthesis, and demonstrating nutrition and management practices that influence milk fat CLA content. Bauman and collaborators demonstrated that the major CLA isomer in milk fat has anti-carcinogenic and anti-diabetic effects in biomedical studies and they were the first to show that CLA effectively reduces mammary tumors when fed as dairy products.

Key Words: Bauman, homeorhesis, CLA


As a 9 year old at the 4-H County Fair, I informed the newspaper reporter that my goal was to become a researcher in dairy nutrition. Over a half-century later, I can look back at a dream come true. The National Academy of Sciences publication titled “On Being a Scientist” (https://www.nap.edu/catalog/12192/on-being-a-scientist-a-guide-to-responsible-conduct-in) is a must read for all in the scientific community. The report lists many of the challenges and issues faced by scientists in the 21st Century and concludes that researchers have 3 sets of obligations. First, an obligation to honor the trust of colleagues by producing results that stand the test of time. Mentors and colleagues made a difference, and of special importance are graduates, undergraduates, and post-doctorates that represent the heart and soul of our research programs. Second, an obligation to themselves. Scientific knowledge is cumulative, with new discoveries building on past results. The same ethical values that apply in everyday life apply to science as researchers seek to be productive while maintaining high professional standards and personal integrity. Third, an obligation to act in ways that serve the public. Research involves the use of public funds for public good, and communication of results to consumers, producers, and other scientists is essential. My career has presented interesting challenges, exciting opportunities, and many satisfying experiences; the presentation will relate some of these to the 3 NAS obligations.

Key Words: career, symposium, Bauman
M1 Protein biopolymer molecular structure determined protein supply during gastrointestinal digestion. N. Xu1,2, J. Liu2, and P. Yu1, 1Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada, 2Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, China.

Three new warm-seasoned corn lines (LM10, LM01 and LD999) were used in this study to reveal mechanism with which protein molecular structure determined protein rumen and intestinal digestion characteristics. Protein molecular structure features were determined by using attenuated total reflectance Fourier-transform vibrational molecular spectroscopy; then revealed by OMNIC in 2 major peaks regions: amide I (ca. 1720–1575 cm$^{-1}$) and amide II (ca. 1575–1489 cm$^{-1}$). Protein structural α-helix (centered at ca. 1650 cm$^{-1}$) and β-sheet (centered at ca. 1640 cm$^{-1}$) were also derived from Amide I regions. Standard in situ method and 3-step in vitro procedure were applied to evaluate ruminal and intestinal digestion characteristics. PROC MIXED and PROC CORR (SAS 9.4) were applied to analysis of digestibility and molecular spectral features; and Statistica 8.0 was performed to multivariate analyses of molecular spectral data. Molecular spectral intensities of amides I and II and structural α-helix and β-sheet were highest in LD999, and lowest in LM01. Spectral peak height and area ratio of amide I to amide II were both greater in LM01 than the other 2 lines of corn ($P < 0.01$). Agglomerative hierarchical cluster analysis and principal component analysis results showed that 3 lines of corn could be distinguished from each other in protein molecular spectral region, indicating that they differed in protein molecular features and conformation. Line LD999 had greater crude protein (CP), truly digestible CP and rumen undegradable protein than LM10 and LM01, but rumen degradable protein was greater in LM01 than in LD999. No difference was observed in digestibility of rumen undegradable protein among 3 lines. Total digestible CP was greater in LM10 and LM01 than LD999 ($P < 0.01$). Correlation analysis showed that protein structural spectral intensity was positively correlated with rumen undegradable protein, but negatively correlated with rumen degradable protein and total digestible CP. Hence, protein molecular structure in warm-seasoned corns apparently influenced protein gastrointestinal digestion characteristics in ruminant animals.

Key Words: warm-seasoned corn, protein molecular structure, gastrointestinal digestibility

M2 Preparation of milk protein concentrates by ultrafiltration and continuous diafiltration: Effect of process design on overall efficiency. C. Gavazzi-April1, S. Benoit1, M. Britten2, and Y. Pouliot1, 1STELA Dairy Research Center, Institute of Nutrition and Functional Foods (INAF), Department of Food Science, Université Laval, Québec, Québec, Canada, 2Food Research and Development Center (FDRC), Agriculture and Agri-Food Canada, St-Hyacinthe, Québec, Canada.

High-milk-protein concentrates (>80%) are typically produced by ultrafiltration (UF) with constant-volume diafiltration (CVD). Polymeric spiral-wound (SW) UF membranes with a molecular weight cut-off (MWCO) of 10,000 Da are mostly used in dairy plants to maximize protein retention. Flux decline and membrane fouling during UF have been studied extensively and the selection of an optimal UF-CVD sequence is expected to have a considerable impact on both the process efficiency and the generated volumes of by-products. The objective of this work was to characterize performances of UF-CVD process in terms of permeate flux decline, fouling resistance, energy consumption and retentate composition as a function of MWCO (10,000 and 50,000 Da) and UF-CVD sequence (3.5×–2 diavolumes (DV) and 5×–0.8DV). UF-CVD experiments were performed on pasteurized skim milk by means of a pilot-scale filtration system (GEA NIRO) operated at 50°C and under a constant transmembrane pressure (TMP) of 465 kPa. Energy consumption was measured in situ for each UF-CVD sequence and was expressed as energy required to produce 1 kg of protein. Results showed that MWCO had no impact ($P > 0.05$) on permeate flux for a same UF-CVD sequence. However, permeate flux values were significantly higher during CVD for the 3.5×–2DV sequence whatever MWCO ($P < 0.05$), which could be explained by lower concentration polarization of milk components at the membrane surface when a larger DF volume was used. Regardless the MWCO, the 5×–0.8DV sequence showed a significant increase ($P < 0.05$) in energy consumption. The 3.5×–2DV sequence resulted in a higher permeate flux and lower energy consumption but required higher volume of water for CVD and increased volumes of permeate, which could lead to greater environmental impacts.

A comparative life cycle assessment is currently underway to determine the best UF-CVD sequence in a sustainable development perspective.

Key Words: ultrafiltration, milk concentrate, process efficiency

M3 Influence of Bacillus spp. on microstructure, graininess, lipolysis and sensory properties of sour cream. D. Mehta1, L. Metzger1, A. Hassan2, and B. Nelson3, 1Dairy and Food Science Department, South Dakota State University, Brookings, SD, 2Daisy Brand, Garland, TX.

The objective of this study was to investigate the influence of proteolytic and lipolytic Bacillus spp. isolated from raw milk at a dairy processing facility on microstructure, graininess, lipolysis and sensory properties of sour cream. B. subtilis (a proteolytic Bacillus strain) and B. licheniformis (a proteolytic and lipolytic Bacillus strain) were spiked at 10³ cfu/mL individually and together in sour cream blend and fermented at 26°C until a pH value of 4.6 ± 0.05 was attained. Sour cream was evaluated for microstructure, graininess, sensory, phospholipids and free fatty acids at the end of 30 d of storage at 4°C and compared with a control, without added Bacillus. Cryo-scanning electron microscopy observations revealed a tighter protein network in all Bacillus containing samples with extensive crosslinking that was not seen in a control. The proteolytic activities of all Bacillus treated samples may induced inter and intra molecular aggregation that could be responsible for crosslinking and dense appearance of the network. Graininess of sour cream was visualized under a stereomicroscope and grains with perimeter >1 mm were enumerated. We observed 272, 171, and 185 grains/g of sour cream treated with B. subtilis, B. licheniformis, and both Bacillus species, respectively. All Bacillus treated sour creams showed significantly ($P < 0.05$) higher numbers of grains/g of sour cream compared with control (23 grains/g of sour cream). Sensory evaluation also indicated increased graininess in sour cream containing Bacillus subtilis. No Bacillus induced flavor defects ($P > 0.05$) were observed by sensory analysis in all Bacillus spiked sour creams compared with control. Sour cream was evaluated for phospholipids and free fatty acids contents to indicate lipolysis. The level of phospholipids and free fatty acids did not differ ($P > 0.05$) between sour cream containing a lipolytic B. licheniformis individually, combined Bacillus treated sour cream and control. In con-
Preliminary studies on the effect of cooling rate on lactose crystallization characteristics in deproteinized whey (DPW). K. Pandalanen* and J. Amamcharla, *Kansas State University, Manhattan, KS.

Crystallization of lactose in supersaturated solution is influenced by factors like cooling rate, the presence of impurities, pH, the degree of supersaturation, and agitator speed. This study was focused on the influence of different cooling rates on crystallization of lactose in supersaturated deproteinized whey (DPW). DPW powder composed of 78.3% lactose, 10% minerals, and 6.3% proteins was reconstituted to 60% (wt/wt) total solids at 80°C for 2 h under constant stirring to ensure complete solubilization of lactose. Supersaturated DPW was then transferred to double jacketed crystallizer connected to a programmable water bath and cooled from 80°C to 60°C in 40 min followed by one of the cooling rates. Three cooling rates 0.04 (slow), 0.06 (medium), and 0.08 (fast) °C/minute from 60°C to 20°C were studied as part of the experimental design and were all done in duplicate. The effect of cooling rates on the quality of the lactose crystals in terms of lactose yield, protein, and mineral percent in dried lactose, and mean particle chord lengths were studied. Lactose yield was measured by weighing dried lactose obtained from crystal slurry that was centrifuged, washed thrice and dried at 60°C for 14 h. Lactose yield for slow, medium, and fast cooling rates were 74 ± 0, 71.5 ± 3.54, and 72.5 ± 0.71%. The amount of the proteins in dried lactose crystals were 0.77 ± 0.06, 0.72 ± 0.04, and 0.68 ± 0.06%; the and amount of the minerals were 1.32 ± 0.02, 1.38 ± 0.03, and 1.46 ± 0.18% for slow, medium and fast cooling rates respectively. Mean chord lengths measured at the end of crystallization using focused beam reflectance measurement (FBRM) for slow, medium and fast cooling rates were 33.22 ± 2.43, 30.28 ± 5.13, 27.05 ± 3.04 µm, respectively. Lactose yield, protein and mineral percent, and mean particle chord lengths showed no significant difference (P > 0.05) between the 3 cooling rates studied. This study successfully investigated the effect of cooling rate on crystallization to reduce its time.

Key Words: deproteinized whey, lactose crystallization, cooling rate

M5 Preliminary studies on monitoring storage changes in milk protein concentrates using front-face fluorescence spectroscopy and chemometrics. K. S. Babu* and J. Amamcharla, *Kansas State University, Manhattan, KS.

The functional properties of milk protein concentrates (MPCs) are influenced by composition, processing conditions, and storage conditions. The objective of the study was to determine if front-face fluorescence spectroscopy (FFFS) could be used as a tool to understand the changes in MPCs during storage. Twenty MPCs with 4 different protein contents (70, 80, 85, and 90%) were collected from 4 different manufacturers and were stored at 2 temperatures (20 and 45°C) for 1, 2, 4, 8, and 12 wk. Three scans were performed on each sample to record the fluorescence spectra of tryptophan [excitation (Ex) 290/emission (Em) 305 to 450 nm], Maillard products (Ex 360/Em 380 to 480 nm), and riboflavin (Ex 380/Em 400 to 590 nm). Subsequently, the spectra were averaged and normalized by reducing the area under each curve to unity. The spectral data were then analyzed using principal component analysis (PCA). Multivariate statistical methods were applied to identify the variations during storage. Colorimetric values (L*, a*, and b*) were also determined. For each of the spectral data obtained, differences in the MPC samples stored at 45°C and 20°C were observed using PCA. After storage, there was a decrease in L* value and an increase in a* and b* values. MPCs exhibited a tryptophan fluorescence emission peak at 335 nm, Maillard emission peak at 432 nm, and riboflavin emission peak around 436. After storing the powders for 12 wk at 45°C, a decrease in peak intensities were observed in the tryptophan emission spectra and an increase in Maillard products emission spectra between 420 and 450 nm. Of the total variability, the first principal component (PC-1) took into account 99% (tryptophan data), 96% (Maillard products data), and 99% (riboflavin data) characteristics of the data. The results indicated that the FFFS, coupled with chemometrics, could be applied as a rapid and nondestructive method to monitor storage changes in MPCs.

Key Words: milk protein concentrate, fluorescence spectroscopy, chemometrics

M6 Sensory characteristics of Cheddar-type caprine milk cheeses supplemented with microencapsulated and normal ferrous sulfate. A. Siddique* and Y. W. Park, Fort Valley State University, Fort Valley, GA.

Because iron deficiency anemia is widespread epidemics around the world, iron supplementation in dairy foods would be desirable. Although iron fortification on qualities of bovine milk and dairy products has been studied, such research on caprine milk counterparts is almost non-existent. The objective of this study was to compare sensory characteristics of non-fortified caprine control cheese (CC) with those of iron fortified corresponding cheeses by addition of regular ferrous sulfate (RFS) and large microencapsulated ferrous sulfate (LMFS) salts. Three batches of Cheddar-type caprine milk cheeses were manufactured at the Georgia Small Ruminant Research and Extension Center, Fort Valley State University (Fort Valley, GA). For each batch, the cheeses were subdivided in 3 groups as CC, RFS, and LMFS, vacuum packed and stored at 4 and −18°C for 0, 2 and 4 mo. Iron was fortified in RFS and LMFS cheeses by adding 8.23 and 9.03 g of Fe per 9 kg of cheese, respectively, at milling step, formulating 16% Fe in both forms of ferrous sulfate. Sensory evaluation was performed for all cheese samples in duplicates by 8 panelists according to the USDA judging and scoring methods for dairy products. Results showed that CC, RFSm and LMFS cheeses contained 0.0162, 0.822, 0.932 mg Fe/g cheese, respectively, showing substantial increases in Fe in both fortified cheeses. In sensory properties, cheese type had significant (P < 0.05 or P < 0.01) differences in rancid, high acid, oxidized, gummy, soggy, weak body, and color characteristics between the 3 type of goat cheeses. Temperature effect did not influence on sensory properties of all 3 cheeses, while storage period had an effect only on oxidized flavor. None of the 2-way or 3-way interactions affected the sensory properties except for cheese type × storage interaction on crumbly and soggy taste. We concluded that LMFS cheese showed lower defect scores than control and RFS counterparts, suggesting that oxidoreductive effect of Fe in cheese matrices might have been delayed by microencapsulation of iron salt during storage.

Key Words: goat cheese, iron fortification, sensory property
The objective of this study was to investigate the role of casein as a percentage of true protein and total protein content on the physical and sensory properties of skim milk beverages. Pasteurized fluid skim milk was subjected to ceramic microfiltration and diafiltration to produce 95% serum protein reduced fresh liquid micellar casein concentrate (MCC) as retentate with about 8.4% protein. Microfiltration permeate from skim milk was ultrafiltered and diafiltered to produce liquid serum protein isolate (SPI) at about 24% protein. MCC, SPI, lactose monohydrate, cream and deionized water were formulated into 20 skim milk beverages (0.2% fat) with 5 casein: true protein ratios (5, 25, 50, 75, and 80%) and 4 protein levels (3.00, 3.67, 4.34, and 5.00%), with constant lactose (4.65% anhydrous lactose). The experiment was replicated twice. Hunter color and relative viscosity were measured at 4°C, 20°C, and 50°C. A trained panel evaluated flavor, appearance and texture attributes. As true protein levels increased, the milks became more white (higher L value), less green (lower negative a value) and more yellow (higher b value) \((P < 0.05)\). As casein as a percentage of true protein increased, the milks were more white and green \((P < 0.05)\). Milks were more white at 50°C compared with 4°C \((P < 0.05)\). Following pasteurization, milks were generally more white, less green and more yellow \((P < 0.05)\). Relative viscosity increased with increasing protein levels and casein as a percentage of true protein and decreasing temperature \((P < 0.05)\). Pasteurization increased sensory opacity and whiteness \((P < 0.05)\) as did casein as a percentage of true protein and protein content. Cooked/sulfur and cardboard flavors, viscosity and throat clench increased with protein content \((P < 0.05)\) while increased casein as a percentage of true protein decreased aroma intensity, cardboard flavor and astringency \((P < 0.05)\) and increased cooked/milky, cooked/sulfur and throat clench \((P < 0.05)\). These results demonstrate that membrane fractionation can be applied to optimize physical and sensory properties of milk beverages.

**Key Words:** microfiltration, casein, serum protein

**M8 Components of procream and cream improve the viability of yogurt and probiotic bacteria.** B. Chinnasamy*, K. Choquette, and S. Clark, Iowa State University, Ames, IA.

Ingredients (hydrolyzed caseins, whey proteins, fats and fatty acids, ascorbic acid, cysteine) have been supplemented to growth media to understand their influence on the growth and to enhance the viability of commonly used yogurt cultures and probiotic cultures. The objective of the current study was to determine the influence of protein, phospholipids and fat extracted from cream or whey protein phospholipid concentrate (procream) on yogurt \([Streptococcus salivarius\ ssp. thermophilus (ST-M5) and Lactobacillus delbrueckii ssp. bulgaricus (LB-12)]\), and probiotic cultures \([Lactobacillus acidophilus\ (LA), Bifidobacterium lactis\ (BL)]\). Control broths and broths containing ST-M5, LB-12, LA, and BL, in equal concentrations, were supplemented with procream or with fat extracted either from cream (FC) or from procream (FP), and incubated at 42°C for 4 h to mimic yogurt fermentation. The total fat was 1% in all media broths. The viability of yogurt and probiotic bacteria was selectively enumerated after 2, 4, and 6 wk of refrigerated storage. Viability of ST-M5 did not significantly differ across shelf life. In contrast, LB-12 and LA had significantly better viability in broths supplemented with procream; FC and FP did not influence viability. Viability of BL was particularly enhanced by dairy ingredients; at the end of 6 wk, significantly higher viability was observed in broth supplemented with procream than FP. Viability in broth with FP was significantly higher than FC, and viability in broth with FC was significantly higher than control. The higher viability observed in broth supplemented with FC and FP is attributed to assimilation of needed phospholipids and fatty acids by BL. Improvement in the viability of yogurt and probiotic bacteria in broths supplemented with procream is attributed to the synergistic impact of protein substrates, phospholipids and fat.

**Key Words:** yogurt and probiotic bacteria, whey protein phospholipid concentrate (procream)

**M9 Effect of pectin on digestion properties and β-carotene delivery of whey protein-stabilized emulsions.** Y. Tang* and B. Vardhanabhuti, University of Missouri-Columbia, Columbia, MO.

Research has shown that increasing emulsion stability during digestion could improve nutrient encapsulation and delivery. Pectin can alter protein digestion but its effect on the digestion of protein-stabilized emulsions is not well understood. This study investigated the effect of pectin on digestion properties and encapsulation of β-carotene of whey protein-stabilized emulsion. Unheated and heated whey protein-pectin mixtures (UH-Mix and H-Mix, respectively) and biopolymer ratios were studied. H-Mix of whey protein isolate (WPI) and pectin were prepared by heating the mixed solutions (1–5% protein and 0.1 or 0.2 pectin to protein weight ratio) at pH 7 and 85°C for 30 min. Emulsions (5% oil, 1–3% protein, and 0–0.2% pectin) were obtained by homogenizing mixed solutions with oil. Digestion was carried out in an in vitro gastric model. The mean droplet size, zeta potential, as well as β-carotene release were determined. Confocal laser scanning microscopy was used to characterize the structure of the emulsions during digestion. Results showed pectin led to a drastic increase in mean droplet sizes during digestion. At similar pectin concentration, the largest increase was from UH-Mix followed by H-Mix. Overall, droplet sizes increased when pectin increased. Zeta potential results showed no significant differences among samples. Confocal images revealed that emulsions stabilized by unheated or heated WPI had the highest degree of coalescence. Extensive flocculation but less degree of coalescence was observed in emulsions containing pectin. Emulsions stabilized by H-Mix were composed of smaller and well-defined droplets compared. Coincided with the microscopy results, H-Mix systems also had the lowest β-carotene release at the end of digestion (e.g., 6.4% release compared with 17% release in systems without pectin). Furthermore, when the emulsions contained higher protein β-carotene release decreased. We concluded that heating, biopolymer concentration, and protein concentration in the emulsions played the major roles in stabilizing the emulsion and β-carotene during digestion. Results can be applied to improve delivery properties of WPI-stabilized emulsions.

**Key Words:** whey protein, emulsion, in vitro digestion

**M10 The effect of different solids concentration on the drying kinetics of whey protein concentrate.** H. N. Vora*, L. E. Metzger1, C. Selomulya2, M. W. Woo3, and A. Putranto2, 1Dairy and Food Science Department, South Dakota State University, Brookings, SD, 2Department of Chemical Engineering, Monash University, Clayton, VIC, Australia.

Spray drying of whey protein concentrate (WPC) is routinely performed in the dairy and food industry to increase storage life and reduce transportation costs. Single droplet drying (SDD) is an innovative technology that can be used to optimize drying conditions on a small scale. The SDD approach involves a single droplet suspended on the tip of a glass filament, where changes in droplet diameter, mass, and temperature can be measured during drying. The aim of this study was to develop a predictive model generated using SDD, which can be used as a tool to optimize the drying conditions and reduce costly plant trials when developing new ingredients with unique functional properties. In this

**Key Words:** yogurt and probiotic bacteria, whey protein phospholipid concentrate (procream)
study, 2 ± 0.05 µL droplets of WPC80 were dried using SDD at 3 different levels of total solids viz. 10%, 20%, and 30% at 90°C with hot air at a velocity of 0.8 m/s. Droplet diameter and mass change data were collected and processed using Adobe After Effects 7.0 to enable the extraction of images. The same WPC80 at 3 different levels of total solids was also spray dried using a NIRO single stage pilot-scale dryer fitted with a spray nozzle. The inlet and outlet temperatures were maintained at 190°C and 90°C, respectively. The mean particle sizes and bulk density data obtained on the pilot-scale spray dryer were used as an input for REA modeling. The change in average diameter data obtained from SDD followed a similar pattern for all 3 total solids level i.e., a drop in the initial diameter (falling rate drying period) followed by a linear change in the remaining moisture content during the constant rate drying period. The curves of average mass change obtained from SDD were plotted against time. It was observed that as the total solids level increases the drying time increases, this is mainly due to the formation of crust on the particle and subsequent slower moisture migration to the surface of the particle with higher total solids level. The data obtained from SDD was then used as an input for the reaction engineering approach (REA) for modeling of drying kinetics of WPC80.

**Key Words:** single droplet drying, drying kinetics, whey protein concentrate

**M11** See T298 on page 279

**M12** Feasibility of soluble soybean polysaccharide for enhancing lactose crystallization during lactose manufacture. V. Sunkesula*, L. E. Metzger, and S. L. Beckman, Midwest Dairy Foods Research Center, South Dakota State University, Brookings, SD.

Previous research has established that soluble soybean polysaccharide (SSPS) can enhance lactose crystallization in pure lactose solutions. However, commercial lactose is typically manufactured by crystallization of concentrated permeate (CP). The objective of this study was to determine the feasibility of using SSPS to improve lactose yield during manufacturing of lactose from CP. A laboratory scale crystallization set up with parallel crystallizers was utilized to conduct control and treatment (with SSPS) experiments simultaneously. CP (total solids from 58 to 60% and 48 to 49% lactose) obtained from a lactose manufacturer was used in the experiments. CP was heated to 80°C to dissolve lactose before transferring to the crystallization tanks. The CP solution in the tanks was cooled from 80°C to 18°C (rate, −0.0479°C /min) using an automatic temperature controlled water bath. Constant agitation of 120 rpm was applied during the cooling cycle. Both the control and treatment solutions were seeded with lactose crystals (0.027 g/100 g of solution) and 0.1% SSPS was added to treatment solution. After completion of crystallization, chilled water (at 4°C, 15 g per 100 g of solution) was added to the crystallized solution and centrifuged at 10,000 × g for 20 min at 4°C. The supernatant was decanted, weighed, and an equal quantity of deionized water (4°C) was added to wash the crystals. A total of 4 washing cycles were applied to purify the lactose crystals. The mass of the washed lactose crystals (corrected for total solids) was used to calculate lactose yield. The collected supernatant from each washing was freeze-dried and analyzed for SSPS. Lactose yield with 0.1% SSPS addition was significantly (P < 0.05) higher (76.1%) as compared with the control (71.5%). Out of the total SSPS added to the treatment solution, 79.7% was recovered in to the wash water. The findings of this study suggest potential feasibility of SSPS for enhancing lactose crystallization during lactose manufacture from concentrated permeate.

**Key Words:** lactose, crystallization, soluble soybean polysaccharide (SSPS)

**M13** Moved to Dairy Foods I: Chemistry (page 45)

**M14** Improving emulsification properties of whey protein isolate by heating with pectin at near neutral pH. Y. Wang* and B. Vardhanabhatti, Food Science Department, University of Missouri, Columbia, MO.

Interactions between protein and polysaccharides could lead to improved protein functional properties including emulsification properties. Most studies focus on complex coacervates which are formed at pH < pl. Much less attention has been given to interactions at pH > pl, especially when the mixtures are heated. The objective of this study was to investigate the emulsification properties of heated whey protein isolate (WPI) and pectin complexes formed at near neutral pH. The effect of heating pH and pectin concentration were studied. Heated WPI-pectin complexes (CPX) were formed by heating mixed WPI (3% protein) and pectin (0 to 6.6%) at pH 6, 6.5, or 7 at 85°C for 30 min. Emulsions (5% oil and 0.5% protein) were obtained by homogenizing oil and aqueous solution, followed by ultrasonic processing. The emulsions were slowly acidified to pH 5.5. Droplet size, zeta-potential, and rheological properties of emulsions were measured. Stability against creaming was determined for 14 d. Unheated or heated WPI-stabilized emulsions were unstable and separated into 2 layers within a few hours. Regardless of heating pH, all CPX formed more stable emulsions with significantly smaller droplet size and higher negative charge (P < 0.05). At similar pectin concentration, emulsions stabilized by CPX formed at pH 7 were the most stable. At optimum heating pH (pH 7), increasing pectin concentration up to 0.3% led to a decrease in mean droplet size from 2.6 µm to 491 nm and an increase in negative charge from −24 to −31.7 mV. Increasing pectin concentration above 0.3% did not affect mean droplet size or zeta potential (P > 0.05); however, the emulsions were more stable. Maximum stability (>30 d) was achieved with emulsion stabilized by CPX formed with 0.6% pectin at pH 7. Rheological results suggest that, in addition to increased charge potential, an increase in viscosity could also contribute to improved stability. This study demonstrates that heat complexation of whey protein with pectin at near neutral pH could improve emulsification properties at pH near pl of the protein. Heated WPI-pectin complexes could be utilized as clean-label ingredients in foods and beverages.

**Key Words:** whey protein, complex, emulsification properties

**M15** Level of Listeria cross contamination in ice cream mix can serve as a predictor of its overall risk from injured cells. N. Neha*, R. Suliman1, S. Anand1, G. Djira2, B. Kraus3, and S. Sutariya4, 1Midwest Dairy Foods Research Center, Brookings, SD, 2Department of Dairy and Food Science, South Dakota State University, Brookings, SD, 3Department of Mathematics and Statistics, South Dakota State University, Brookings, SD, 4Wells Enterprises Inc., Le Mars, IA.

Listeriosis is a life-threatening infection caused by eating foods contaminated with Listeria monocytogenes. Some major ice cream recalls
in recent years reaffirm the ability of this food-borne pathogen to survive in diverse dairy processing environments and cause cross contamination. Inspection reports revealed lapses in implementing adequate hygienic practices for Listeria persistence in the processing environment leading to cross contamination of ice cream. The level of cross contamination can thus serve as a predictor to establish the overall Listeria risk, which is the aim of this study. To conduct the dose-response challenge studies, ice cream mixes of different total solid levels (36, 40, 42, and 45%) were spiked at 1, 2, 3, and 4 log cfu/g levels of Listeria innocua (an established surrogate). The dose levels were based on the potential risk of environmental cross contamination. The spiked samples were pasteurized at 80°C for 25 s, and the survivors, including injured cells, were enumerated using standard protocols. A binary logistic regression model was fitted for the severity of risk. The impact of total solids, water activity, and pH variability was also studied for Listeria survival. Based on direct plating on MOX and RLM, no survival was detected at any of the total solid levels for the dose levels tested. However, the enrichment protocol revealed the presence of injured cells at the highest dose level of 4 logs, indicating the risk from injured cells, which showed a non-significant trend with the level of total solids. This was also confirmed with the logistic model, which resulted in quasi-complete separation, indicating dose as a strong predictor of risk. The statistical modeling thus indicates it to be a case for further developing the risk model based on response surface using some additional inquiry points. On the other hand, recovery of injured cells in the actual ice cream mix during holding at 7°C for 72 h was found to be zero, even at 4 logs contamination, suggesting a much lower risk from injured cells during the normal handling of mix.

Key Words: *Listeria*, injured cells, ice cream

**M16 Reduction of Zygosaccharomyces parabailii in dairy-based salad dressings using different combinations of acidulants.**
A. Meldrum* and H. Joyner, University of Idaho, Moscow, ID.

Dairy-based salad dressings are susceptible to *Zygosaccharomyces parabailii* (Zygo) growth, which causes spoilage by producing off-flavors. Zygo growth also damages packaging containers due to gas production during fermentation. Zygo, a yeast similar to *Saccharomyces cerevisiae*, is tolerant of low pH and can survive in high-acid dairy-based salad dressings, shortening the dressing’s shelf life. Therefore, the goal of this research was to evaluate the effectiveness of different organic acids to slow or stop Zygo growth over a 45-d period. Lactic, acetic, and gluconic acids were used alone and in different combination in a reduced-fat ranch dressing formulation. Ten different formulations of dressing were acidified to a pH of 4.1. A pre-enriched culture of Zygo was added to each sample until a final concentration of 10⁴ cfu/mL of Zygo was achieved. The dressing was stored in incubators at 4°C, 10°C, and 25°C for 45 d and evaluated at d 0 and every 5 d during the storage period for microbial growth using tryptone glucose yeast extract agar (TGYE) with 0.5% acetic acid for selectivity. The different acid combinations had varying effects based on storage temperature. At 4°C, no Zygo growth was observed; Zygo concentration was dependent on the acid’s ability to reduce the initial inoculated load. Gluconic acid had the most significant effect on initial Zygo death in all samples at 4°C, so samples prepared with gluconic acid had the least amount of growth over the incubation period. At 10°C, samples containing combinations of acids and gluconic acid by itself were less effective at preventing growth. Acetic acid and lactic acid by themselves were the most effective at reducing growth, but did not prevent it. Samples stored at 25°C generally had 1–2 log growth. At 25°C, the type of acids used played a less significant role as the cfu/mL reached 10⁷ after 10 d. These results highlight the importance of storage temperature compared with acid type or combination on Zygo growth. This information may be used by food manufacturers to extend the shelf life of dairy-based products that are susceptible to Zygo contamination.

**Key Words:** *Zygosaccharomyces parabailii*, shelf-life

**M17 Maintaining high level of intact casein in Cheddar cheese during aging.**
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As Cheddar cheese ripens, intact casein is broken down. High levels of intact casein are desirable if the cheese is to be used for processed cheese. Our goal was to minimize ripening changes using 4 strategies: (1) use of milk containing higher casein, (2) minimizing the amount of rennet used by up to 50%, (3) using camel chymosin as the rennet, as it is less proteolytic than the calf chymosin currently used, (4) use of high pressure processing (HPP) applied to the cheese shortly after manufacture. Cheddar cheese was made from ultrafiltered (UF) milk. The cheesemilk protein and casein contents of were ~5.15% and 4.30%, respectively. Three types of cheeses were made each using different levels of rennet: (a) control, which had 2.0μL rennet/50g milk, (b) 25% reduced, and (c) 50% reduced. To determine the optimal amount of rennet for the control, coagulation tests were run on the UF milk using small strain dynamic rheology. The targeted coagulation time for all treatments was 20–30 min. To decrease coagulation time as rennet levels decreased, we added calcium chloride and varied coagulation temperatures. We determined the coagulation temperatures to be 32.4°C, 35.2°C, and 37.8°C for the control rennet, 25% reduced and 50% reduced samples, respectively. During cheesemaking, a licensed cheesemaker used these coagulation temperatures and maintained a high draining pH. Cheeses had similar moisture contents (~37%). Four days after the cheese was made, half of the samples from each vat were saved as natural cheese base and the other half underwent HPP at 600 MPa for 3 min. Hardness was measured by texture profile analysis and loss tangent (LT) values were measured by heating cheese during small strain oscillatory rheology. At 4 d, there was a decrease in hardness with a decrease in rennet level, but no impact of HPP treatment on hardness. For the max LT value, HPP treatment resulted in an increase in the LT max, but decreasing rennet level had little impact. Cheeses will be aged for 8 mo and will be made into processed cheese at 2 week, 2, 4, 6, and 8 mo time points to determine changes in the performance of the cheese base over time.

**Key Words:** intact casein, high-pressure processing, cheese ripening
M18 Effect of delaying colostrum feeding on passive transfer and intestinal bacterial colonization in neonatal male Holstein calves. A. Fischer¹, Y. Song, Z. He, L. Guan, and M. Steele, University of Alberta, Edmonton, AB, Canada.

Dairy calves are born without an active immune system, and therefore rely on good-quality, adequate volumes of colostrum to ensure the passive transfer of IgG. Despite this knowledge, poor colostrum management still occurs on farm, with one of the main reasons for failure of passive transfer being due to feeding colostrum more than 6h after birth. The objective of this study was to investigate how delaying the first colostrum feeding can impact the passive transfer of IgG, as well as bacterial colonization in the intestine of neonatal dairy calves. Twenty-seven male Holstein calves were randomly assigned to 1 of 3 treatments at birth: calves were fed colostrum before 1 h after birth (0 h, n = 9), at 6 h after birth (6 h, n = 9), or at 12 h after birth (12 h, n = 9). Calves were fed pooled colostrum at their respective feeding times at 7.5% of birth body weight, and fed milk replacer at 2.5% every 6 h thereafter. Blood samples were taken every 3 h using a jugular catheter. At 51 h of life, calves were euthanized and tissue and digesta of the distal jejunum, ileum, and colon were collected. QRT-PCR was performed using DNA extracted from tissue and digesta samples and the prevalence (% of total bacteria) of bacterial groups was determined. Calves fed colostrum at 0 h had significantly higher (P < 0.001) serum IgG concentrations (g/L; 24.77 ± 1.91) compared with 6-h (17.13 ± 0.91) or 12-h calves (16.88 ± 1.50), while no differences existed between 6-h and 12-h calves. In addition, 0h calves had a greater prevalence (P < 0.10) of Bifidobacteria (1.24 ± 0.64) and Lactobacillus (0.26 ± 0.08) attached to colon tissue compared with those fed at 12 h (0.12 ± 0.02 and 0.07 ± 0.02, respectively). In contrast, there were no differences (P > 0.05) in E. coli, Clostridium, and Fecalibacterium colonization among treatments in the digesta or tissue of the distal intestine. These findings suggest that feeding dairy calves colostrum immediately after birth can increase the passive transfer of IgG and the colonization of beneficial bacteria in the colon; both of which are hypothesized to assist in protecting the calf from enteric infections during the pre-weaning period.

Key Words: passive transfer, immunoglobulin G, bacterial colonization

M19 The effect of dietary supplementation of monobutyrin on growth and intestinal morphophysiology of preweaning Holstein calves. L. K. Hilligsøe¹,², J. E. Mendez¹, A. M. Ehrlich², R. Sygall³, H. Raybould¹, and P. Ji¹, University of Copenhagen, Copenhagen, Denmark, ²University of California, Davis, Davis, CA, ³Perstorp Feed & Food, Malmö, Sweden.

Butyric acid, naturally present in cow milk, serves as energy source for GIT epithelial cells. We hypothesize that supplementing butyric acid in form of its glycerol ester in milk may enhance its intestinal delivery and stimulate epithelial development in preweaning calves. Twenty-two Holstein bull calves (<4 d old) were stratified by arriving BW and serum total protein and randomly assigned to treatments. Calves were fed milk replacer that was supplemented with 0 (CON), 0.4 (LOW) or 0.8% (HIGH) of monobutyrin (MB, solid basis of milk replacer) until 8 wk of age. Milk replacer containing 27.6% CP and 14.4% crude fat (DM basis) was fed twice daily at 1.5% (solid basis) of BW which was updated weekly. Starter grain and water were provided for ad libitum consumption during the study. Scores for health (respiration, diarrhea, alertness) were assigned once daily. BW was measured at arrival and weekly, and body frame parameters were measured at arrival and on wk 4, 6 and 8. Calves were weaned and euthanized on wk 8. Tissue, mucosa, and digesta samples from GIT were harvested. The jejunal epithelial permeability was measured through an Ussing chamber immediately after collection. The data were subject to ANOVA using mixed procedure of SAS. Preliminary results showed that BW was numerically higher in LOW than HIGH and CON, whereas wither height and body length were greater (P < 0.05) in calves of LOW than those in CON group on wk 6 and 8. Supplementation of MB tended to increase (P = 0.08) hip height. Despite lack of significance (P = 0.17), villus height of jejunal epithelium was the highest in LOW followed by HIGH and CON. The crypt depth was not affected by treatment. However, the ratio of villus height to crypt depth was significantly higher (P = 0.04) in LOW than that in CON. MB increased (P < 0.05) mRNA of tight junction proteins (CLDN1 and OCLN) in jejunal mucosa. Para- and transcellular permeability were not affected by treatment. Collectively, low dose of MB supplementation moderately improved growth and jejunal epithelial development in preweaning calves.

Key Words: monobutyrin, calf, preweaning


Heat stress during the dry period (DP) impairs milk yield in the subsequent lactation. Our objective was to examine if exposure to heat stress during the DP alters milk and colostrum quality and tight junction (TJ) permeability during the subsequent lactation. Holstein cows, selected based on parity and mature equivalent milk production, were enrolled into 2 groups: heat stress (HT, access to shade, n = 12) or cooled (CL, access to shade, fans, and soakers, n = 12) during the entire DP (~46 d, temperature-humidity index ≥68). After calving, all cows were managed together and had access to shade, fans and soakers. Colostrum and milk volume and mastitis events were recorded daily until 84 d in milk (DIM, AfiFarm System). Milk samples were collected at 0, 1, 2, 7, 14, 21, 49, and 84 DIM and analyzed for somatic cell count (SCC) by flow cytometry. Blood was collected at these time points and at −7, −2, −1 d relative to calving and assessed for lactose concentrations. Mammary gland biopsies (n = 6 per treatment) were collected at 14, 42, and 84 DIM. Total RNA was extracted to analyze the expression of genes related to TJ formation (ZO1, 2, 3, and OCLN) by real-time PCR. Data were analyzed by general linear mixed models with DIM as a repeated measure. Cooled cows had greater colostrum and milk yield compared with HT cows (7.79 vs. 3.72 ± 0.79 kg; 39.93 vs. 34.27 ± 0.80 kg/d; for colostrum and milk respectively; P < 0.01). Colostrum SCC was lower for CL compared with HT cows (839 vs. 2,729 ± 518 × 1,000 cells/mL, respectively; P < 0.01), and CL cows tended to have lower milk SCC (230 vs. 441 ± 95 × 1,000 cells/mL; P = 0.11). Cooled cows had fewer cases of mastitis relative to HT cows (1 vs. 4 cases, respectively). There was no difference in blood lactose concentrations. Expression of most TJ genes in the mammary gland did not differ between CL and HT cows, however, ZO3 expression was upregulated at 14 DIM in HT cows (P = 0.04). Cows exposed to heat stress during the DP produced less colostrum and milk with higher SCC. However,
blood lactose and genes related to TJ formation were not substantially impacted by DP heat stress.

**Key Words:** heat stress, mammary gland, tight junction

**M21** Effect of prepartum dietary calcium and DCAD concentration oncolostrum quality and newborn calf blood mineral and gas concentration. A. L. Diehl*1, J. K. Bernard1, S. Tao1, T. N. Smith1, T. Marins1, D. J. Kirk2, D. J. McLean2, and J. D. Chapman2,

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Eighty-two multiparous Holstein cows were fed diets differing in dietary cation-anion difference (DCAD) and calcium concentrations, in a randomized block design experiment with a 2 × 2 factorial arrangement of treatments, beginning 4 wk before anticipated calving to determine the effects on colostrum yield and quality and acid-base balance of calves. Diets were formulated to provide 2 DCAD [−22 mEq/100 g DM (NEG) or −3 mEq/100 g DM (NEU)] and 2 Ca concentrations (1.0 or 1.5% of DM). Calves were fed a commercial colostrum replacer to provide 200 g of IgG within 4 h of birth. Blood samples were collected before and 24 to 36 h after the initial colostrum replacer feeding for analysis of plasma minerals and blood gases. Cows were milked within 2 to 8 h after calving and colostrum yield recorded. A sample of colostrum was collected for Brix analysis by refractometer immediately after sample collections. Birth weight and dystocia score were not different (P > 0.10) among treatments and averaged 42.7 kg and 1.12, respectively. No differences (P > 0.10) were observed in colostrum yield among treatments, which averaged 8.75 kg. Colostrum quality, as measured using a Brix refractometer, was not affected by prepartum DCAD but was higher (P = 0.0442) for 1.0% compared with 1.5% Ca: 21.58% and 19.87%, respectively. No differences (P > 0.10) were observed in plasma concentrations of Ca, P, K, Cl, anion gap, or whole blood pH, pO2, pCO2, or SO2 of calves due to treatment. Plasma Mg (P = 0.0391) and lactate (P = 0.0591) were higher for calves born to cows fed 1.0% compared with 1.5% Ca. Interactions of DCAD and Ca were observed for plasma Na (P = 0.0232), plasma Cl (P = 0.0619) and whole blood HCO3 (P = 0.0515) due to higher concentrations observed with NEG and 1.0% Ca compared with NEG and 1.5% Ca. Feeding prepartum diets with 1.5% compared with 1.0% Ca concentrations reduced plasma Mg and lactate concentrations in calves immediately after birth and reduced Brix value of colostrum. Results of this trial indicate that feeding −22 mEq/100 g DM prepartum does not alter blood mineral or gas concentrations of calves compared with feeding a −3 mEq/100 g DM diet.

**Key Words:** DCAD, calcium, colostrum

**M22** Effect of automatically recorded body condition scorecondition score at calving on subclinical hyperketonemia. C. Truman*, I. Mullins, M. Falk, and J. Bewley, University of Kentucky, Lexington, KY.

Body condition score (BCS) at calving is a time of interest when evaluating risk of future transition disease outcomes. An observational study was conducted comparing calving BCS to β-hydroxybutyrate (BHB) concentrations. The DeLaval Automated BCS Camera (DeLaval, Tumba, Sweden) was installed at a Southern Illinois farm to access BCS at calving of Holstein cows, all lactations. The automated scores were reported in 0.01 increments on a 1 to 5 scale. Blood samples were taken once per cow within 0 to 7 DIM. Concentrations of whole blood BHB were determined using BHBCheck (PortaCheck, Moorestown, NJ). Distributions of BCS were described using the FREQ procedure SAS (version 9.3 SAS Institute, Inc., Cary, NC). Classification of BCS <3.0 and >4.0 accounted for 2.54% and 0.28% of the recorded scores, respectively. The percentage of scores 3.25 ≤ BCS <3.75 was high in the herd (77.12%). Data from 354 cows were evaluated for effects of BCS at calving (BCS ≤ SD; 3.48, 1.23 ± 0.53) and BHB concentrations (BHB ≤ SD; 0.78, 0.48 ± 1.32), using the GLM procedure of. No statistically significant relationship was found between calving BCS and BHB concentrations (P = 0.10). Concentrations of BHB were described as either a positive (BHB ≥1.2 mmol/L) or negative (BHB < 1.2 mmol/L) hyperketonemia. The GLM procedure was used to evaluate the relationship between calving BCS and hyperketonemia case outcome and no statistically significant relationship was found (P = 0.47). The results of this study do not agree with previous studies reporting BCS at calving can be related to hyperketonemia. Although the BCS at calving did not have an effect in this study, managing BCS throughout lactation and the dry period may help to reduce other metabolic disease incidences in high-risk cows.

**Key Words:** body condition score, hyperketonemia, BHB

**M23** Immunological and metabolic responses of lactating dairy cows fed diets supplemented with exogenous β-mannanase enzyme (CTCzyme). B. M. Roque*1, G. C. Reyes1, J. A. D. R.N. Appuhamy1, T.A. Tewoldebrhan1, J. J. Lee2, S. Seo3, and E. Kebreab1,

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Exogenous fibrolytic enzymes have been used to improve feed efficiency by releasing nutrients bound in complex feed matrices such as hemicellulose. β-Mannanase, an exogenous fibrolytic enzyme, is known to hydrolize mannan structures found in plant hemicellulose matrices. β-Mannanase acts in 3 main ways; reduction of feed viscosity, improvement of energy metabolism, and decreased immune stimulation. Objectives of this study were to determine the effects of β-mannanase supplementation on immunological and metabolic responses in lactating dairy cows. Two weeks after calving, 20 Holstein cows (10 multiparous and 10 primiparous), were blocked by parity and assigned to one of 2 diets for 182 d. All cows were housed in the same environment and fed the same basal diet. The basal diet of the treatment group was supplemented with β-mannanase (CTCzyme) at 0.1% of concentrate DM. Haptoglobin (Hp), Immunoglobulin G (IgG) and somatic cell counts (SCC) were analyzed for immune responses and nonesterified fatty acids (NEFA) were analyzed to explore metabolic responses. Blood samples were taken once weekly, starting 14 d after calving, and were analyzed for immune and metabolic markers. Milk samples were collected twice daily and analyzed separately for SCC. There was a significant reduction in Hp levels (P = 0.01) in supplemented multiparous cows, compared with control multiparous cows, indicating that β-mannanase was associated with decreased systemic inflammation. Primiparous cows fed β-mannanase showed tendencies for reduced Hp levels (P = 0.06), when compared with control primiparous cows. Multiparous cows are considered to be more susceptible to acute infections and inflammation, so the enzyme may have a better effect in multiparous cows. NEFA levels tended to be lower in cows fed β-mannanase (P = 0.08), regardless of parity, suggesting that β-mannanase was associated with improved energy balance during early to mid-lactation. IgG and SCC were not affected by β-mannanase supplementation, regardless of parity. Results suggest supplementation of β-mannanase could aid in reduced instances of metritis and mastitis, through reduced systemic inflammation, and improved energy balance in lactating cows.

**Key Words:** β-mannanase, immune response, lactating cow
M24 Evaluation of commonly used atmospheric carbon dioxide concentrations for the culture of bovine Mycoplasma spp. J. L. Lowe*1, B. D. Enger2, L. K. Fox3, A. Adams Progar1, and J. M. Gay1, 1Washington State University, Pullman, WA, 2Virginia Polytechnic Institute and State University, Blacksburg, VA.

Mycoplasma spp. are difficult to culture because of their fastidious growth requirements such as atmospheric carbon dioxide (CO2) levels exceeding 3%. Recommendations for culture CO2 concentrations are varied and not empirically derived. The objective was to determine if bovine mycoplasma isolates differ in growth measures when incubated in CO2 concentrations of 10% and 5% or in candle jars (2.7 ± 0.2% CO2). Growth at these CO2 concentrations was evaluated using isolates of the 3 most common mycoplasma mastitis pathogens, M. bovigenitalium (n = 5), M. bovis (n = 19), and M. californicum (n = 18). Isolates were standardized to OD540 of 0.20, roughly 10^8 cfu/mL, and serially diluted in pasteurized whole milk to achieve final concentrations of 10^5 and 10^6 cfu/mL. One hundred microliters of each dilution was spread onto the surface of a modified Hayflick’s agar plate to enumerate colony growth on d 3, 5, and 7 of incubation. SAS 9.3 GLM was used to evaluate fixed effects of CO2 treatment (2.7, 5, or 10% CO2), species, and day (3, 5, or 7 d) and their interactions on recorded colony counts. Carbon dioxide concentration and day were not associated with overall mycoplasma growth differences but species differences were identified (P < 0.0001). Species GLM models assessed fixed effects of CO2 treatment, isolate, and day and their interactions on recorded colony counts isolate growth differences. For M. bovis, CO2 concentration and day did not significantly affect growth, but growth differences among isolates were identified (P < 0.0001). A significant interaction between isolate and CO2 concentration was found for M. californicum and M. bovigenitalium (P < 0.0001 and P = 0.007, respectively). Growth after 7 d incubation was significantly greater for M. bovigenitalium and M. californicum (P < 0.0001). These findings are being confirmed on field isolates received from a commercial veterinary diagnostic laboratory. The results suggest that the range of suitable CO2 culture conditions and incubation times for the common mastitis-causing Mycoplasma spp. is broader than currently practiced.

Key Words: mastitis,enumeration

M25 Feed efficiency and reproductive performance are genomically independent in lactating Holstein cows. E. M. Bart*1, M. D. Hanigan2, D. M. Spurlock2, M. J. Vandenhaar2, and R. R. Cockrum1, 1Virginia Polytechnic Institute and State University, Blacksburg, VA, 2Iowa State University, Ames, IA, 3Michigan State University, East Lansing, MI.

For residual feed intake (RFI) or dry matter intake (DMI) to be used as indirect measures of feed efficiency for selection, they must not be unfavorably associated with reproduction. Previous research in other livestock species suggests there may be a phenotypic relationship between feed efficiency and reproduction; however, the underlying genomic relationship is unknown. Therefore, we hypothesized that associated variants will be shared between feed efficiency and reproductive traits. The objectives of this study were to (1) identify single nucleotide polymorphisms (SNP) associated with DMI, RFI, and reproductive traits, (2) determine concordant variants among these SNP, and (3) identify the underlying genes of any shared variants. Feed, milk, and reproductive data were collected on lactating Holstein cows (n = 1,513) from Virginia Tech (VT), Iowa State University (ISU), and Michigan State University (MSU). Measurements of feed intake, milk yield, milk composition, and BW were used to calculate RFI. Reproductive performance was measured using number of services (NS), days open (DO), and days to first calving (DFC). Genotypic data were available on 677 cows using the Illumina Bovine SNP50 Beadchip. Markers were filtered by call rate (<0.9), allele number (>2), and minor allele frequency (<0.05), which resulted in 54,734 SNP. Genome-wide association analyses were performed using a multi locus mixed model. Significant variants for RFI (n = 55), DMI (n = 54), DO (n = 65), NS (n = 59), and DFC (n = 54) were identified. There were 3 common variants between RFI and DMI on BTA2 (rs109734679 and rs2910223) and BTA17 (rs41845355). A protein coding gene within the intronic region of PDE1A underlied rs109734679. Previous association studies in beef cattle have also identified PDE1A as associated with RFI. There were no concordant SNP between RFI or DMI with any of the reproductive traits. Though weak, there was a genomic relationship between RFI and DMI. However, both RFI and DMI were genomically independent of reproduction. Overall, genomic selection for RFI or DMI would not unfavorably impact reproduction.

Key Words: genomic, feed efficiency, reproductive performance

M26 Differences in lying behavior between Jersey and Holstein dairy cattle during the transition period. K. L. Kutina*, O. C. Duner, Y. I. Ruiz, E. A. Whisler, and J. M. Huzzey, California Polytechnic State University, San Luis Obispo, CA.

Changes in lying behavior can help identify management needs for cows; for example, a spike in lying bouts on the day of calving emphasizes the need for adequate bedding. To date, research describing behavioral changes around calving has focused on Holsteins; however, Jerseys are becoming a more popular breed in the dairy industry and research on their behavior is lacking. The objective of this study was to compare lying behavior between Jersey and Holstein dairy cattle during the period around calving (the transition period). A total of 86 dairy cows (n = 48 Jersey cows and n = 42 Holstein cows) were monitored from 2 wk before until 2 wk after calving. Daily lying time and number of lying bouts were measured using a data logger (Hobo Pendant G Acceleration Data Logger, Onset, Bourne, MA) that recorded leg orientation at 1-min intervals. This data was used to summarize lying behavior for each cow, by day. Four periods were defined for the statistical analyses: wk -2 (d -14 to -8, relative to calving), wk -1 (d -7 to -2), wk +1 (d +2 to d +7) and wk +2 (d +8 to d +14). Data for the day of calving (d 0) was analyzed independently of the other experimental periods. Lying time and lying bouts were analyzed using a mixed model, which included the fixed effects of parity, postpartum body condition score, breed, week, and the interactions of breed by week and parity by breed. Holstein cows spent more time lying than Jerseys across all experimental periods (702 ± 16.3 vs 658 ± 12.5 min/day respectively; P < 0.003). There was also a significant effect of week, whereby lying times were lower after calving than before (P < 0.001). Lying bouts only differed between the 2 breeds on the day of calving; Jersey cows averaged 14 ± 0.8 bouts/d while Holstein cows averaged 11 ± 0.6 bouts/day (P = 0.008). Differences in the lying behavior of Jersey and Holstein dairy cattle around calving may necessitate unique management practices for these respective breeds in an effort to accommodate cow comfort during the transition period.

Key Words: Jersey, Holstein, lying behavior
M27  Assessment of microbiota and short-chain fatty acids profiles in the hindgut of pre-weaned dairy calves. Y. Song*1, N. Malmuthuge1,2, M. A. Steele1, and L. L. Guan1, 1Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, 2Vaccine and Infectious Disease Organization, International Vaccine Centre, University of Saskatchewan Saskatoon, Saskatchewan, SK, Canada.

Microbial colonization in the gut during early life plays important roles in the host immunity development, metabolism, growth, and health. However, the knowledge on hindgut microbial colonization in dairy calves during the pre-weaned period is very limited. In this study, amplicon sequencing was used to characterize mucosal and digesta associated microbiota in the hindgut (cecum, colon and rectum) of newborn (at birth, n = 6), d 7 (n = 6), d 21 (n = 6), and d 42 (n = 6) Holstein bull calves. The comparison of the relative abundance of bacteria was performed with the nonparametric Kruskal-Wallis test. In total, 14 phyla were identified with Firmicutes, Bacteroidetes and Proteobacteria being the dominant phyla. At genus level, Lactobacillus and Bacteroides were the 2 predominant genera for both mucosa and digesta associated microbiota. The age effect was detected for both mucosa and digesta associated bacterial communities, while no regional effect was observed for them. Among the detected bacterial genera, the relative abundance of mucosa-attached Escherichia and Pseudomonas decreased significantly with the increase of age (P < 0.01), suggesting the initial higher prevalence of these potential pathogenic bacteria during first week of life but the prevalence lowered in healthy calves with the growth. The concentrations of total short-chain fatty acids (SCFAs), acetate, propionate, and butyrate were significantly higher at d 21 compared with d 7 (P < 0.01); however, there was no significant difference observed between d 21 and d 42 except for acetate concentration on cecum. The changing pattern of SCFA concentration was similar with the changes in the relative abundance of SCFA-producing bacterial genera such as mucosa and digesta associated Prevotella, Blautia, Ruminococcus, and mucosa-attached Fecalibacterium. This study provided the information on hindgut microbial composition and their metabolite, which may play an important role in the hindgut fermentation and health of dairy calves during pre-weaned period.

Key Words: dairy calf, hindgut, pyrosequencing

M28  Role of galectins 3 and 9 in the immunity of periparturient dairy cows. E. Asiamah*1, S. Adjei-Fremah1, K. Ekwemalor1, M. Worku1, L. Sordillo2, and J. Gandy2, 1North Carolina A&T State University, Greensboro, NC, 2Michigan State University, East Lansing, MI.

The peripartal period in the dairy cow is a crucial time influencing future fertility and milk production. Postpartal diseases like bovine mastitis can result in major economic set back in the dairy industry. Based on the hypothesis that postpartal diseases are most likely due to immune dysfunction, there is a need for the identification of candidates involved in the immuno-modulation of the peripartal immuno-competence. Galectins are multipotent, evolutionarily conserved, carbohydrate-binding proteins that, by crosslinking cell surface glyco-conjugates, trigger a cascade of transmembrane signaling events such as cell activation, cytokine secretion, migration, and apoptosis. The objective of the study was to evaluate galectin 3 and galectin 9 levels in Holstein-Friesian cows in the periparturient period. Eight Holstein-Friesian periparturient cows were used for the study. Blood was taken 2 weeks close to calving (close up), and 7 d after calving(+7). Enzyme-linked immunosorbent assay (ELISA) was used to detect and determine the concentrations of galectins 3 and 9 in the plasma of the cows. Each sample was done in triplicate. The Student t-test in SAS 9.2 was used to analyze the data obtained. The results demonstrated that galectin 3 levels decreased significantly 7 d after calving (P = 0.0362). Galectin 9, on the other hand, was observed to increase 7 d after calving but the increase was not significant compared with 2 wk before calving (P = 0.6035). This indicates that galectin levels change during the periparturient period of cows, and are differentially regulated. A better understanding of the molecular and physiological properties of galectins could help to establish its therapeutic potential in inflammatory diseases especially mastitis and other postpartum diseases in the dairy industry

Key Words: peripartal, postpartal, galectin

M29  Effects of timing of C16:0 supplementation on production and metabolic responses of early lactation dairy cows. J. de Souza* and A. L. Lock, Michigan State University, East Lansing, MI.

Fifty-two multiparous cows were used in a randomized complete block design experiment and assigned to either a control diet containing no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving to 24 DIM (fresh period) or from 25 to 67 DIM (peak period). Fresh diets were formulated to contain (% DM) 17% CP, 30% NDF, 24% forage NDF, and 24% starch. Peak diets were formulated to contain (% DM) 17% CP, 29% NDF, 19% forage NDF, and 26% starch. The C16:0 supplement (85% C16:0), added at 1.5% of diet DM, replaced soyhulls in the CON diets. During the fresh period, PA did not affect DMI (21.5 vs. 21.6 kg/d, P = 0.92) or milk yield (47.7 vs. 46.3 kg/d, P = 0.38) compared with CON. In contrast, compared with CON, PA increased milk fat content (4.89 vs. 4.48%, P = 0.01) and yield (2.29 vs. 2.01 kg/d, P < 0.01), milk protein yield (1.60 vs. 1.53 kg/d, P = 0.03), and ECM (56.6 vs. 51.9 kg/d, P = 0.02). PA decreased BW (668 vs. 709 kg, P = 0.93) due to PA only decreasing these variables after 10 DIM compared with CON. During the peak period, compared with CON, PA did not affect DMI (29.9 vs. 30.2 kg/d, P = 0.68), but increased milk yield (58.0 vs. 54.6 kg/d, P = 0.01), milk fat content (3.88 vs. 3.67%, P < 0.01) and yield (2.27 vs. 2.06 kg/d, P < 0.01), milk protein yield (1.80 vs. 1.66 kg/d, P = 0.04), and ECM (62.3 vs. 57.8 kg/d, P < 0.01). Compared with CON, PA reduced plasma insulin concentration (0.25 vs. 0.32 µg/L, P = 0.05), but did not affect plasma NEFA concentration (0.35 vs. 0.32 mEq/L, P = 0.41) or BW (673 vs. 684 kg, P = 0.93). There were no interactions between feeding PA and the time that supplementation started for production variables. Our results demonstrate that supplementing C16:0 during early lactation increases ECM without changes in DMI. Feeding PA may increase BW loss during the fresh period, but not during the peak period.

Key Words: body condition, palmitic acid, postpartum

Excessive adipose tissue (AT) lipolysis increases serum free fatty acids (FFA) and triggers AT inflammation predisposing cows to disease. Fetuin-A (FETA) is a FFA carrier and an acute-phase protein (APP) that enhances lipid-induced inflammation in AT of monogastrics. Little is known about its role and potential use as a biomarker in transition cows. We hypothesized that serum and AT FETA content increases as the periparturient period progresses and is enhanced by high lipolysis rate. Blood and subcutaneous AT were collected from 10 multiparous cows through the transition period at far off (FO; −51 ± 3d) and close-up dry (CU; −14 ± 2d), and early lactation (EL; 7 ± 0.5d). FETA was analyzed by ELISA in serum, and by RT-qPCR and protein blotting in AT. Contrary to our hypothesis, serum FETA concentration and AT gene and protein expression were greatest at FO compared with EL (P ≤ 0.05) when FFA concentration was the least (P < 0.01). Serum FETA concentration was 1.11 ± 0.08, 1.08 ± 0.08, and 0.98 ± 0.08 mg/mL at FO, CU, and EL (P < 0.05), respectively, and was positively associated with serum albumin (r = 0.27; P = 0.03) and calcium (r = 0.32; P = 0.05). Circulating FETA was negatively associated with FFA (r = −0.25; P = 0.05) and BCS loss (r = −0.73; P < 0.001) over the transition period, and adverse health events at EL (r = −0.42; P = 0.05). AT FETA expression dynamics through FO, CU and EL was analogous to adipogenic and lipogenic genes PPARy, FASN, FABP4, and SCD1, and negatively correlated with AT inflammatory markers SPP1 (r = −0.46; P = 0.01) and CD68 (r = −0.38; P = 0.04). To test adipocyte inflammatory response to FETA in vitro, primary bovine adipocytes were treated with 0, 50, 100 or 200 µg/mL of FETA (8h), or LPS (25 ng/mL; 4h). Adipocytes treated with FETA had lower CCL2 expression than LPS (P = 0.03), and reduced adipocyte IL6 transcription (P = 0.01) when treated with 100 µg/mL of FETA compared with 0. These results indicate that FETA is a negative APP inversely linked to AT lipolysis and health events in transition cows. Contrary to monogastrics, FETA plays a beneficial role in AT inflammation in cows by modulating the expression of pro-inflammatory cytokines by adipocytes.

Key Words: adipose marker, inflammation, lipolysis

M31 Effects of oral administration of acetylsalicylic acid after parturition on activity patterns, prevalence of diseases, mortality and culling rates in dairy cows. A. A. Barragan*, L. M. Bauman1, J. Velez2, J. D. Rozo Gonzalez2, G. M. Schuennemann3, and S. Bas1, 1Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 2Department of Animal Sciences, The Ohio State University, Columbus, OH, 3Aurora Organic Farms, Boulder, CO.

Dystocia has been categorized as a painful event and has been associated with increased risk of cow morbidity, mortality and culling in dairy farms. Administration of NSAID drugs has been proposed to decrease postpartum discomfort. The objectives of this study were to assess the effects of oral administration of acetylsalicylic acid after calving on (1) daily activity patterns and (2) prevalence of diseases, mortality and culling rates in lactating dairy cows. Cows from 3 organic dairy herds were enrolled in the present study. Immediately after parturition, cows were blocked by parity and calving ease [eutocia (EUT); dystocia (DYS)] and were randomly assigned to 2 treatment groups: (1) ASP (n = 278): at approximately 12 h after parturition cows received 4 consecutive treatments with acetylsalicylic acid (100 mg/kg; 2 boluses) 12 h apart; or (2) placebo (PLC; n = 285): at approximately 12 h after parturition cows received 4 consecutive treatments with gelatin capsules (2 capsules) filled with water 12 h apart. Activity monitors were placed on the rear leg of a subset of cows (ASP = 48, PLC = 47) at enrolment, and were removed 7 d later. Overall, there was no difference in lying and standing time between ASP and PLC groups. Cows in the ASP group tended (P = 0.05) to have more steps compared with PLC (ASP = 3514 ± 129 steps/d; PLC = 3162 ± 129 steps/d). Furthermore, cows that experienced DYS spent more time lying (P < 0.05; DYS = 590 ± 17 min/d; EUT = 511 ± 17 min/d), less time standing (P < 0.05; DYS = 850 ± 17 min/d; EUT = 929 ± 17 min/d), and had less steps (P < 0.05; DYS = 3089 ± 126 steps/d; EUT = 3587 ± 133 steps/d) than EUT cows. Additionally, ASP cows that experienced EUT tended (P = 0.06) to spend less time lying and more time standing, and had more (P < 0.05) steps compared with PLC cows that experienced EUT. No difference was found on the incidence of health events, culling and mortality rates between groups. The results of this study suggest that activity patterns of cows that experience DYS are different from cows that experience EUT, and that administration of ASP after calving may increase activity of dairy cows.

Key Words: acetylsalicylic acid, activity, calving ease

M32 Preliminary evaluation of the DeLaval Cell Counter’s ability to quantify somatic cell counts in nonlactating bovine mammary secretions. B. D. Enger*, C. E. Crutchfield1, S. C. Nickerson2, C. L. M. Parsons1, and R. M. Akers1, 1Virginia Polytechnic Institute and State University, Blacksburg, VA, 2University of Georgia, Athens, GA.

Measurement of milk somatic cell count (SCC) is often used to screen lactating dairy cows for mastitis. Many methods have been developed to rapidly measure SCC in milk from lactating cows but few validated tools are available to quantify SCC in secretions from nonlactating mammary glands. The objective was to quantify SCC in nonlactating secretions by direct microscopic examination and determine if these measures correlate with the SCC produced by a commercial SCC counter designed for lactating cows, the DeLaval Cell Counter (DCC). Mammary secretions (n = 90) collected from 6 dry, non-pregnant, Holstein cows (1 to 3 lactations; 53 to 64 d dry) were diluted 1:4 in PBS containing 2.2% BSA and used to make duplicate secretion smears for microscopic quantification and measured in duplicate by the DCC. Each smear was enumerated by 2 independent counters. Duplicate secretion smears produced a within-sample coefficient of variation of 12.8%. Average values were used for further evaluation. Mean microscopic SCC ranged from 1.56 to 131.0 × 106 cells/mL (mean = 20.9 × 106 cells/mL; SD = 22.4 × 106 cells/mL). Secretion SCC enumerated by counter 1 (mean = 19.8 × 106 cells/mL) were lower (P = 0.0045) than those produced by counter 2 (mean = 20.5 × 106 cells/mL); but SCC were highly correlated (R² = 0.970). Duplicate DCC SCC measures produced a within-sample coefficient of variation of 15.8% and mean DCC SCC ranged from 0.926 to 16.0 × 106 cells/mL (mean = 6.16 × 106 cells/mL). When average DCC SCC were compared with counts produced by counter 1, counter 2, and their average using PROC CORR in SAS, respective Pearson correlation coefficients of 0.342, 0.321, and 0.334 resulted. Overall, a weak relationship existed between the microscopic SCC and those produced by the DCC which is not surprising because the DCC was designed and calibrated to quantify SCC in milk rather than secretions containing extremely high concentrations of cells. Modifications will be necessary for the DCC to better mirror values obtained by direct microscopic examination.

Key Words: mastitis, SCC, dry cow
M33  Bovine mammary epithelial cell (MAC-T) phenotype impacts TNFα-mediated MAPK signaling and inflammation. L. G. Silva⁠,*1, B. S. Ferguson⁠,1 L. Hernandez2, and A. P. Faciola3,1 University of Nevada, Reno, NV, United States of America.

The objective was to determine if MAC-T phenotype would impact inflammatory signaling and inflammatory gene expression. MAC-T cells were cultured under basal (DMEM 10% fetal bovine serum and 10 µg/mL of insulin) or lactogenic conditions (basal media + 1 µg/mL ovine prolactin, 0.5 µg/mL hydrocortisone, and 10 mM sodium acetate) and mitogen-activated protein kinase (MAPK; ERK, JNK, and p38) phosphorylation and pro-inflammatory gene expression examined in response to tumor necrosis factor α (TNFα). Statistical analysis was assessed via ANOVA and Tukey’s post-hoc analysis at P ≤ 0.05. MAC-T cells were co-stimulated with increasing concentrations of TNFα (0, 10, 30, 100, 300, 1000 µM). Cell lysates were harvested 15 min post-TNFα stimulation and assessed for MAPK phosphorylation via immunoblotting. JNK and p38 phosphorylation increased in a dose-dependent manner; yet the magnitude of JNK and p38 signaling was greater under basal compared with lactogenic conditions. Cells were next stimulated in parallel with TNFα (300 pM) and lysates harvested over time (0, 15, 30, 100, 120, 180 min). JNK and p38 phosphorylation were robust and transient in MAC-T cells stimulated with TNFα over time. Similar to dose-response experiments, JNK and p38 signaling were significantly more robust in MAC-T cells under basal conditions. We next examined inflammatory gene expression in MAC-T cells cultured under basal or lactogenic conditions and co-stimulated in the presence or absence of TNFα (300 pM). RNA was isolated and PCR array performed to evaluate the expression of 83 inflammatory genes. Pro-inflammatory gene expression was increased in MAC-T cells in response to TNFα. Consistent with enhanced MAPK signaling; pro-inflammatory gene expression was significantly increased in MAC-T cells under basal compared with lactogenic conditions. Real-time qPCR was used to validate PCR array findings. Collectively, our data demonstrate that MAC-T cells cultured under basal conditions are more responsive to TNFα. These findings suggest that investigators consider the importance of MAC-T phenotype when designing future inflammation-related studies.

Key Words: gene expression, mammary, transition period

M34  Prediction algorithms for early detection of clinical mastitis caused by gram-positive and gram-negative pathogens. N. M. Steele¹,2,3, A. Tholen¹, A. De Vries², S. J. Lacy-Hulbert², R. R. White³, and C. S. Petersson-Wolfe¹, ¹Department of Dairy Science, Virginia Tech, Blacksburg, VA, ²Department of Animal Science, University of Florida, Gainesville, FL, ³DairyNZ Ltd., Private Bag 3221, Hamilton, New Zealand.

Producers now have many technologies available for monitoring daily changes in milk composition and cow behavior to assist in disease detection. This study aimed to develop algorithms for identifying gram-negative (GN) and gram-positive (GP) mastitis using a combination of milk and activity measures. Milk yield, quality (electrical conductivity and SCC) and composition (lactose, protein, and fat percentage) were collected using an in-line milk analyzer (Afilab, S.A.E. Afikim, Israel) at Virginia Tech (VT) and University of Florida (UF) Dairy for 14 d before and following a clinical mastitis (CM) event (n = 268). Activity measures included daily steps at UF (Afip Pedometer), as well as number of rest bouts, total resting time and rest bout duration at VT (Afip PedometerPlus). A quarter milk sample was collected for bacteriology upon detection of CM. Data were also retained for control animals matched to each clinical case (n = 268) based on breed, lactation number and DIM. Rather than using the absolute value of variables as the primary explanatory variable, slopes of each variable were estimated using linear regression over the days before CM detection. Slopes were calculated between d 7 and 5, 4, 3, 2 or 1 before infection to better understand how early these metrics could be used to detect CM. Infection was treated as a binomial response and backward stepwise elimination mixed-effect regression was used to relate infection to explanatory variable slopes, with the algorithms suggesting activity and milk data may be potentially useful for early detection of CM.

Key Words: sensor data, milk component, activity


Most rumen bacteria are uncultured, making their niche hard to identify. Fluorescent substrates could potentially identify the substrate preferences and the niche of these uncultured bacteria, but uptake of these substrates has not been tested with mixed rumen bacteria. Our objective was to determine if a fluorescent analog of glucose (2-NBDG) would be taken up by mixed bacteria from the rumen. A second objective was to determine if we could separate cells taking up 2-NBDG by using fluorescence-activated cell sorting. We prepared mixed bacteria from rumen fluid by centrifugation, incubated them in 2-NBDG (0 to 100 µM) for up to 60 s, and monitored uptake of 2-NBDG with fluorimetry. We found mixed bacteria took up 2-NBDG, and they did so with a maximum velocity (V_max) of 0.180 (0.05 SEM) nmol mg protein⁻¹ min⁻¹ and Michaelis constant (K_m) of 6.37 (SEM 4.86) µM (n = 3). We confirmed that cells took up 2-NBDG by using flow cytometry, which revealed that up to 18.5% cells were positive after incubating them in 100 µM 2-NBDG for 5 min. Positive cells could be separated with fluorescence-activated cell sorting, and post-sort analysis revealed 94% of sorted positive cells were in fact positive. Work is ongoing to (1) optimize cell sorting, (2) sequence sorted cells for identification, and (3) synthesize and test uptake of other glucose analogs. Our work supports that 2-NBDG, in combination with other analogs, could be used to identify which bacteria consume which substrates, helping define what role uncultured bacteria play in the host.

Key Words: 2-NBDG, rumen bacteria, cell sorting

M36  Effects of replacing soybean meal with canola meals in vitro. H. F. Monteiro*, E. M. Paula¹, J. L. P. Daniel², P. D. B. Benedetti³, R. Bittner¹, L. G. Silva¹, T. Shenkoru¹, and A. P. Faciola¹, ¹University of Nevada, Reno, Reno, NV, ²State University of Maringá, Maringá, PR, Brazil, ³Federal University of Viçosa, Viçosa, MG, Brazil.

This study aimed to evaluate the effects of replacing soybean meal (SBM) with canola meals (CM) differing in rumen-undegraded protein (RUP; % of CP) content (38% RUP, LCM or 50% RUP, HCM) on in
vitro ruminal fermentation, including total gas and CH$_4$ production. Two in vitro experiments were conducted. Both experiments had 3-48 h incubations, totaling 18 observations per treatment. Experiment 1 tested 3 protein supplements as the sole ingredient (SBM, LCM, and HCM). Experiment 2 tested 3 TMR containing the protein supplements from experiment 1. Measurements were: pH, gas production (GP), degradability kinetics, metabolizable energy (ME), in vitro true organic matter digestibility (i-v-tOMd), CH$_4$ and volatile fatty acids (VFA) production. Mixed linear models were used to analyze the data. Degradability kinetics were fitted using nonlinear regression. Means were compared by orthogonal contrasts: SBM vs. (LCM + HCM) and LCM vs. HCM. Partial data are presented in Table 1. The SBM ingredient had greater i-v-tOMd, VFA production, ME, total GP$_{48}$ and CH$_4$ (mM) when compared with both CM. When comparing both CM ingredients, HCM had shorter lag phase and lower branched-chain VFA production. However, ingredients did not differ in CH$_4$ (g/kg dOM). In experiment 2, SBM diet had a trend to increase total CH$_4$ production (mM), but diets did not differ in CH$_4$ (g/kg dOM). The SBM diet also had a trend to increase valerate and iso-valerate concentration which may indicate more proline and leucine deamination. When comparing both CM diets, HCM increased final pH and had a trend to lower ME, while decreased total GP$_{48}$.

**Key Words:** degradability kinetics, metabolizable energy, methane

**M37 Evaluation of the NRC predictions in response to changes in dietary rumen degradable and undegraded protein on dairy cows exposed to warm climates.** J. D. Kaufman* and A. G. Rius, The University of Tennessee, Knoxville, TN.

A study was conducted to evaluate the prediction accuracy of the National Research Council (2001) model for metabolizable protein (MP) allowable for milk production. Thirty multiparous Holstein cows were used in a completely randomized design with a 2 × 2 factorial arrangement of treatments. Dietary treatments comprised 2 levels of rumen degradable protein (RDP; 10 and 8%) and 2 levels of rumen undegradable protein (RUP; 8 and 6%) as follows: 10RDP:8RUP, 8RDP:8RUP, and 8RDP:6RUP. The 10RDP:8RUP diet was fed from d 1 to 21 followed by respective treatments from d 22 to 42. Cows were exposed to the warm climates of July and August in Tennessee without supplemental cooling. Least squares means of dry matter intake, milk, and body weight from each treatment were input into the model, and tabular crude protein and fiber contents were adjusted to reflect chemically derived values. Treatments did not affect feed intake. The RDP treatment did not affect milk yield but, at 6% RUP, milk yield declined compared with the 10RDP:8RUP diet (RDP x RUP interaction; P < 0.01). The NRC model overpredicted a decline in milk yield (1.9 kg) in response to lowering RDP at 8% RUP (10RDP:8RUP vs. 8RDP:8RUP). At 6% RUP, the model predicted a decline in milk yield (2.0 kg) in response to lowering RDP (10RDP:6RUP vs. 8RDP:6RUP); however, milk yield increased by 2.3 kg. At 10% RDP, the model overpredicted a decline in milk yield (5.1 kg) in response to lowering RUP (10RDP:8RUP vs. 10RDP:6RUP). At 8% RDP, the model overpredicted a decline in milk yield (7.5 kg) in response to lowering RUP (8RDP:8RUP vs. 8RDP:6RUP). Reduction of dietary RDP decreased predicted RDP supply and increased RUP requirements. Reduction of dietary RUP decreased predicted RUP supply but did not affect RUP requirements. In summary, the NRC model underestimated RDP supply and overestimated RUP requirements in response to low dietary RDP. The model underestimated the RUP supply in response to low dietary RUP. An improvement in predictions of MP allowable for milk production should increase the accuracy of the NRC model.

**Key Words:** National Research Council, rumen degradable protein, rumen undegradable protein

**M38 Relationship between ano-genital distance and fertility in Holstein cows.** M. Gobikrushanth*1, T. C. Bruinjé1, M. G. Colazo2, and D. J. Ambrose1,2, 1Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, 2Live-stock Research Section, Alberta Agriculture and Forestry, Edmonton, AB, Canada.

The ano-genital distance (AGD) has been studied as a marker for genital development and fertility in rodents and primates. An inverse relationship between AGD (distance from anus to clitoris) and fertility in dairy cows was first reported in a preliminary study. Current objectives were to determine associations between (1) AGD and height at hip, and (2) categories of AGD and reproductive outcomes (pregnancy at 1st AI (P/AI), cumulative pregnancy at 120 DIM (P120), times bred, days

<table>
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<td>LCM</td>
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<td>Experiment 1 (sole ingredients)</td>
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open] in a larger population. AGD and height were measured in 573 cows (1st, 2nd and 3rd lactation [lac]; n = 203, 155 and 215) at 165 ± 4.3 DIM from 3 herds. The overall mean (=SEM) AGD and height were 132.3 ± 0.5mm (range, 96.0–170.0) and 149.6 ± 0.2cm (range, 138.0–161.0). Mean AGD and height, respectively, were shorter (P < 0.05) for 1st (125.8 ± 0.7mm and 149.2 ± 0.2cm) than 2nd (128.4 ± 0.7mm and 150.5 ± 0.2cm) and 3rd+ lac (128.6 ± 0.6mm and 150.6 ± 0.2cm). The overall correlation between AGD and height was very weak (r² = 0.08; P < 0.01). The threshold AGD that predicted probability of P/AI (Receiver operating characteristic curve analysis) for 1st and 2nd lac cows were 126.0 (Sensitivity:61.9 and Specificity:66.4%), and 135.0 (Sensitivity:69.8 and Specificity:75.5%) mm, but AGD did not predict fertility in 3rd+ lac cows. Therefore, only 1st and 2nd lac cows were categorized into SHORT or LONG AGD (< or > threshold for each lac). First lac cows of SHORT AGD (119.2 ± 0.7mm) group had higher (P < 0.05) P/AI (56.7 vs. 28.8%) and P120 (81.5 vs. 56.7%), fewer times bred (1.6 ± 0.1 vs. 2.5 ± 0.1) and days open (95.4 ± 5.9 vs. 125.7 ± 5.5d) than those in LONG AGD (136.2 ± 7.4mm). Similarly, 2nd lac SHORT AGD (125.6 ± 0.8mm) cows had greater (P < 0.05) P/AI (41.6 vs. 24.2%) and P120 (60.7 vs. 51.5%), fewer times bred (2.2 ± 0.2 vs. 2.6 ± 0.2) and days open (116.9 ± 6.3 vs. 147.7 ± 7.8d) than LONG AGD (144.9 ± 1.0mm). Height did not differ between SHORT and LONG AGD cows in 1st and 2nd lac. In summary, AGD was very weakly associated with height, highly variable even among cows of similar lactation, and cows with shorter AGD were more fertile than those with longer AGD. The factors determining AGD in dairy cows remain to be explored.

Key Words: ano-genital distance, height, fertility

M39 The effect of body condition loss on hepatic and ovarian tissue function in dairy cattle, Y. Schuermann1, A. St. Yves2, N. Dicks1, V. Higginson1, R. Bohrer1, M. Taibi1, E. Madogwe1, A. Mustafa1, V. Bordignon1, B. Baurhoo1,2, and R. Duggavathi1, 1McGill University, Montreal, QC, Canada, 2Belisle Nutrition Solutions Inc., Saint-Mathias-sur-Richelieu, QC, Canada.

The transition period is a stressful adaptation time for high-producing dairy cows. The inability to meet her energy requirements leads to a state of negative energy balance, often accompanied by increased lipid mobilization from adipose tissue. Body condition scoring (BCS) provides a visual appraisal of lipid mobilization. Our aim was to investigate the association of body condition loss on hepatic and ovarian tissue function in dairy cows during the transition period until onset of breeding. Holstein cows were studied as of 4 weeks pre-calving until 8 weeks post-calving and retrospectively grouped by changes in BCS. Group 1 (n = 9) consisted of cows that lost ≤0.75 BCS and Group 2 (n = 8) cows that lost ≥0.75 BCS during the sampling period. Cows were subjected to blood collection and liver biopsies at 3 weeks, approximate day of calving and +7 weeks. The last liver biopsy was accompanied by follicular aspiration of the dominant follicle and retrieval of granulosa cells (GC) and follicular fluid. We evaluated levels of circulating metabolic indicators, and hepatocyte and GC mRNA. Circulating levels of β-hydroxybutyric acid, glucose and haptoglobin used to assess liver and metabolic stress were unchanged between groups. The proportion of nonesterified fatty acids (NEFAs) were assessed by gas chromatography in both follicular fluid and blood samples collected at +7 wk, where linoleic acid was the predominant NEFA, representing 52% of all NEFAs, yet no differences were observed between fluids and groups. Reproductive competence was evaluated through mRNA abundance of genes required for follicle development (FSHR and LRP8) in granulosa cells, but remained constant between groups. In hepatocytes, CYP7A1 transcripts gradually increased (P < 0.05) in Group 2 from −3 wk to +7 wk, but remained constant in Group 1. Other transcripts involved in lipid metabolism and inflammation (LDLR, ACAT1, and IL1B) remained unaltered between groups at all time points. Thus, cows experiencing elevated BCS loss lead to a gradual increase in hepatic CYP7A1, an enzyme involved in bile acid synthesis from cholesterol, suggesting that Group 2 experienced a greater need for cholesterol removal from the body.

Key Words: BCS, liver, ovary

M40 Fungal treatment of lower part of corn stem does not improve its nutritional value, Y. He1, J. Dijkstra1, A. S. M. Sonnengeberg2, T. M. B. Mouthier3, M. A. Kabel3, W. H. Hendriks1, and J. W. Cone1, 1Animal Nutrition Group, Wageningen University & Research, De Elst 1, Wageningen, the Netherlands, 2Plant Breeding, Wageningen University & Research, Droevendaalsesteeg 1, Wageningen, the Netherlands, 3Food Chemistry, Wageningen University & Research, Borne Wellandien 9, Wageningen, the Netherlands.

The aim of this study was to evaluate the effects of fungal treatment on chemical composition, lignin composition and in vitro rumen degradability of lower parts of corn stem (LCS, internodes 6 and 7). Two lignin degrading fungal species (Lentinula edodes and Pleurotus eryngii) and 2 corn cultivars (LG30211 and MZF8057), which differed in lignin content and rumen degradability were used. Autoclaved LCS was inoculated at 24°C and a relative humidity of 70% in an air-conditioned chamber for 3, 6, and 9 wk and autoclaved LCS was used as control. All treatments were tested in triplicate. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) content were determined. Lignin composition was analyzed by pyrolysis GC-MS and S:G ratio (ratio of syringyl units to guaiacyl units being lignin building blocks) was determined. An in vitro gas production (IVGP) technique was used as a measure of rate and extent of organic matter degradability (OMD). Data on chemical composition, S:G ratio, and IVGP were analyzed using a generalized linear model in SAS (v9.3) with corn cultivar, fungal treatments. Compared with untreated LCS, incubation with fungi increased NDF, ADF, and ADL content and S:G ratio of LCS significantly (P < 0.05) for both corn cultivars. Only numerical differences of NDF, ADF, and ADL content and S:G ratio were found among LCS treated with fungi for 3, 6, and 9 wk. IVGP within 72 h, which represents OMD, was 263 mL/g OM in control LCS, decreased to the lowest value (178 mL/g OM) after 3 weeks treatment with fungi (P < 0.05) and slightly increased (203 mL/g OM) afterward. In conclusion, treatment of LCS with L. edodes and P. eryngii did not improve rumen degradability of LCS within 9 weeks treatment.

Key Words: rumen degradation, corn stem, fungal treatment

M41 Evaluation of two adsorbents after an aflatoxin challenge in Holstein cows, M. E. Weatherly1, R. T. Pate1, G. E. Rottinghaus2, F. de Oliveira Roberti Filho3, and F. C. Cardoso1, 1Department of Animal Sciences, University of Illinois, Urbana, IL, 2Veterinary Medical Diagnostic Lab, University of Missouri, Columbia, MO, 3Bioriginal, São Paulo, Brazil.

The objective of this study was to determine the effects of 2 different adsorbents composed of yeast fractions and bentonite in different proportions (AD1 and AD2) during an aflatoxin (AF) challenge. Lactating Holstein cows [(n = 76); BW (mean ± SD) = 698 ± 72 kg; DIM = 153 ± 83 d] were assigned to 1 of 5 treatments in a randomized complete block design. Period was 28 d with measurement phase on d 22 to 24.
From d 22 to 24, cows received an AF challenge (100 μg of AFB1/kg of diet DM administered orally). The appearance and disappearance of AF excreted in milk was tested at each milking from d 22 to 28 using SNAP tests (SNP; IDEXX, Inc.). Blood was sampled on d 1, 22, and 26 (n = 3 per cow) of wk 1 and 4 for each period and analyzed for superoxide dismutase (SOD) content. Milk samples from d 22 to 26 were analyzed for AFM1 concentrations by HPLC. Treatments were: control (CON), no AD or AF; positive control (POS), no AD plus AF challenge; 30 g per cow per day of AD1 30 and AF challenge (AD30); 60 g per cow per day of AD1 60 and AF challenge (AD60); and 60 g per cow per day of AD2 and AF challenge. Statistical analysis was performed using the MIXED procedure of SAS. Cows in CON had no positive SNP tests, while cows in POS had 7.85 ± 0.27 positive SNP tests (P < 0.001). Plasma SOD concentrations were greater for POS than CON at 2.77 and 1.96 ± 0.05 U/mL, respectively (P < 0.001). A quadratic treatment effect was observed for plasma SOD concentrations at 2.77, 1.99, and 1.97 ± 0.05 U/mL for POS, AD30, and AD60 treatments, respectively. Aflatoxin M1 transfer (1.14 and 0.00 ± 0.16%), excretion (29.52 and 0.00 ± 4.58 µg/d), and concentrations in milk (0.76 and 0.00 ± 0.16 µg/kg) were greater for the POS treatment than the CON, respectively (P < 0.0001) but no differences were observed among other treatments. Oral supplementation of yeast and bentonite clay based AD during AF challenge resulted in quadratic changes in plasma SOD, and fecal AFB1 concentrations. In conclusion, yeast cell wall and bentonite based AD may be beneficial in reducing inflammation during AF challenge.

Key Words: aflatoxin, adsorbent, milk

M42 Producer perception of precision dairy monitoring technology health alerts. E. Eckelkamp* and J. Bewley, University of Kentucky, Lexington, KY.

The objective of this study was to assess how producers categorized alerts from a daily generated alert list designed to identify sick or injured animals. Data from 4 commercial farms in Kentucky were collected from October 2015 to 2016. Each cow was equipped with a CowWatch leg tag (Alta Genetics Inc., Watertown, WI) measuring steps (steps/d) and lying time (h/d), and a neck tag measuring eating time (h/d). Dairy producers evaluated a technology-generated daily herd health report where alerts were generated based on a cow level threshold of ≥30% decrease in steps, lying time, or eating time compared with each cow’s 10-d moving average. Producers evaluated alerts within overall categories: alert accepted to be true and cow checked (A), alert accepted to be true and cow not checked (B), and alert not accepted to be true (C). A total of 25,027 cow alerts occurred, with producers evaluating 15,644 cow alerts (62%). The FREQ procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) with a Chi-squared analysis was used to assess category distribution. Overall, more alerts were categorized as A (5,129) and B (8,424) compared with C (2,091; P < 0.01). Producer categorization by farm (Table 1) indicated most of evaluated alerts were accepted to be true (75 to 99%, total A and B). However, 27 to 88% of alerts were categorized as B, indicating although behavior changes were real, the producer did not check the cow. Reasons for not checking cows included: changes from normal not enough for producer to worry, no time for producers to check alerts, changes in ambient temperature, and hoof trimming or veterinary checks. The frequency of A and B alerts indicated that producers believed the behaviors changes identified were real. The frequency of cows in category B indicated alert generation should be refined to identify only sick or injured cows.

<table>
<thead>
<tr>
<th>Item</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
<th>Farm 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category A</td>
<td>105 (2)</td>
<td>429 (7)</td>
<td>1,599 (28)</td>
<td>2,996 (44)</td>
</tr>
<tr>
<td>Category B</td>
<td>2,802 (42)</td>
<td>2,708 (45)</td>
<td>1,778 (32)</td>
<td>1,136 (17)</td>
</tr>
<tr>
<td>Category C</td>
<td>292 (4)</td>
<td>609 (10)</td>
<td>1,151 (20)</td>
<td>39 (1)</td>
</tr>
<tr>
<td>Missing</td>
<td>3,427 (52)</td>
<td>2,282 (38)</td>
<td>1,100 (20)</td>
<td>2,574 (38)</td>
</tr>
</tbody>
</table>

Key Words: precision dairy technology, producer assessment, health monitoring
M43 The effects of vanilla flavoring in calf starter on calf starter intake. A. Tomei* and S. Kehoe, University of Wisconsin-River Falls, River Falls, WI.

Calf starter is necessary for the proper development of the rumen in young calves. Calves are born as pre-ruminants. In the beginning of their lives, they will depend on milk to meet their nutrient demands. Calf starter is the next step in preparing the calf for consumption of solid feed. The physical form of calf starter and the nutrition provided in the calf starter both have an impact on the development of a calf. The objective of this experiment was to increase the palatability of calf starter through a flavor additive such as vanilla, and evaluate whether there would be a concurrent increase in consumption of calf starter. From the University of Wisconsin-River Falls, 9 calves were utilized on the dairy operation. Calf starter intake was measured for 5 d in all calves, then the treatment was changed to add vanilla to the calf starter and measure intake for 5 d. This repeated from d 20–45 of life, making each calf its own control. The treatments were vanilla added or no vanilla added and alternated every 5 d. Vanilla was added to the calf starter at 5% of calf starter weight. Data was analyzed using the Mixed procedure of SAS (2014) using calf as a random measure and day as repeated measure. Over the entire trial period, calf starter intake least squares means for vanilla were 250.18g and 202.60g without vanilla (SEM = 47.98, 52.42; P = 0.25). Least squares means of calf starter intake for day one through 5 for vanilla treatment were 240.72, 233.43, 211.54, 226.82, 396.12, respectively. Least squares means of calf starter intake for d 1 through 5 for no vanilla treatment were 265.09, 202.94, 228.33, 169.04, 162.53, respectively. The addition of vanilla extract to calf starter did not significantly affect starter intake. However, a numerical increase was seen in starter intake that contained vanilla flavoring. More research is needed with an increased number of calves.

Key Words: calf, starter, palatability

M44 Economic analysis of feeding costs for diets including corn silage or sorghum silage as the main forage source. E. S. Richardson* and G. Ferreira, Department of Dairy Science, Virginia Tech, Blacksburg, VA.

The objective of this study was to evaluate the cost of diets for high-producing cows including either corn silage (CS) or sorghum silage (SS) as the main forage source. A database was generated for the nutritional composition of SS, and included: dry matter (DM; n = 22), ash (n = 16), crude protein (CP; n = 23), ether extract (EE; n = 13), neutral detergent fiber (NDF; n = 25), acid detergent fiber (ADF; n = 21), acid detergent lignin (ADL; n = 18), starch (n = 11), and in vitro dry matter digestibility (IVDMD; n = 5). The nutritional composition of CS was obtained from the dairy NRC (2001). Diets were formulated with CPM Dairy Ration Analyzer using least cost optimization. Diets were formulated for a 635-kg Holstein cow producing 40 kg of milk (3.5% fat, 3.1% protein). Formulation constraints included 100% of predicted DM intake, 100 to 110% of metabolizable energy requirement, 95 to 103% of metabolizable protein requirement, 28 to 33% dietary NDF, 30 to 40% dietary non-fiber carbohydrates, and 50 to 60% dietary forage. Ration formulation was performed under 7 scenarios (Table 1): very low, low, middle, and high grain prices, and considering the price of SS to be either 85, 70, or 55% of the price of CS. When the price of SS was 85% of that of corn, it was cheaper to include CS in the diets, likely explained by the greater inclusion of expensive grain in diets including SS. When the price of SS was 70% of that of CS, marginal differences in diets costs were observed between CS and SS. When the price of SS was 55% of that of corn, it was more expensive to include CS in the diets. In conclusion, SS had to be 30% cheaper than CS to obtain diets of similar composition and cost.

Key Words: corn silage, sorghum silage, economic analysis

M45 Comparison of two housing systems and dairy calf physiological responses during hot weather. H. A. Young*, A. Adams Progar, and A. Lopez Ayala, Washington State University, Pullman, WA.

Dairy calves are susceptible to heat stress when environmental temperatures exceed 68°F. Heat stress conditions cause decreases in feed intake and calf health. The objective of this study was to compare the behavior and well-being of Holstein heifer calves housed in 2 different housing systems during hot weather. This study was conducted over the course of 2 summers (Trial 1 and Trial 2). At the age of 24-48 h, calves were assigned to one of 2 treatments: 1) housed in stalls in a barn (S; n = 14) or 2) housed in hutches placed outside (H; n = 8). Each calf was observed until weaning at 42 d of age. Temperature and relative humidity within the housing systems were recorded at 1-h intervals using data loggers (HOBO) and used to calculate the temperature humidity index (THI). Calf body temperatures were measured hourly using temperature recording devices (iButton). Calf BW were measured weekly and ADG was calculated. Blood samples were collected at 7, 24, and 42 d of age via jugular venipuncture and analyzed for thyroxine concentrations using enzyme-linked immunosorbent assays. Data were analyzed using Pearson correlations and mixed model ANOVAs with repeated measures. No differences between trials were detected so data were combined. The THI was significantly lower in hutches (63.79 ± 0.20) than in stalls (66.44 ± 0.16; P < 0.0001). Calves housed in hutches had higher body temperatures (S: 38.59 ± 0.008°C; H: 38.92 ± 0.01°C; P < 0.0001), ADG (S: 0.6 ± 0.04 kg/d; H: 1.1 ± 0.06 kg/d; P < 0.0001), and

Table 1 (abstract M44). Daily costs of diets including corn silage (CS) or sorghum silage (SS) as affected by corn grain prices and the relative silage prices

<table>
<thead>
<tr>
<th>Corn grain</th>
<th>CS price</th>
<th>Diet cost</th>
<th>SS price</th>
<th>Diet cost</th>
<th>SS price</th>
<th>Diet cost</th>
<th>SS price</th>
<th>Diet cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2.7/bu</td>
<td>$27/ton</td>
<td>$3.87/d</td>
<td>$23/ton</td>
<td>$3.99/d</td>
<td>$19/ton</td>
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<tr>
<td>$3.7/bu</td>
<td>$37/ton</td>
<td>$5.36/d</td>
<td>$31/ton</td>
<td>$5.50/d</td>
<td>$26/ton</td>
<td>$5.39/d</td>
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<td>$5.24/d</td>
</tr>
<tr>
<td>$5.8/bu</td>
<td>$58/ton</td>
<td>$8.31/d</td>
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<td>$41/ton</td>
<td>$8.37/d</td>
<td>$32/ton</td>
<td>$8.16/d</td>
</tr>
</tbody>
</table>
tended to have higher plasma thyroxine concentrations (S: 15.83 µg/dL; H: 16.37 ± 0.22 µg/dL; \( P = 0.08 \)) than calves housed in stalls. Although the THI was lower in hutches than in stalls, calves housed in hutches had higher body temperatures, but these effects did not negatively impact calf growth. Future studies may investigate how calf behavior is affected by hot weather in these housing systems.

Key Words: calf body temperature, heat stress, thyroxine


Negative energy balance, subsequent rapid mobilization of triglycerides (TG), and accumulation of excess TG within the liver are characteristic of the transition to lactation period in dairy cattle. The objective of this study was to examine the coordinated response of hepatic lipolysis-associated proteins during the transition to lactation. Liver biopsies were collected at −14, +1, and +14 d relative to calving (DRTC) from multiparous cows. Liver TG were quantified and used to retrospectively assign cows to either a high (>20% liver lipids, dry matter; n = 5) or low (<20% liver lipids, dry matter; n = 3) treatment based on the maximal liver TG concentration. Protein abundance of hepatic abhydrolase domain containing 5 (ABHD5), hormone sensitive lipase (HSL), lipase A and C (LIPA, LIPC), lipoprotein lipase (LPL), perilipin 1 (PLIN), patatin-like phospholipase domain containing 2 and 3 (PNPLA2, PNPLA3) were determined through Western blot analysis and normalized to total lane protein. For analysis, each lipase was expressed relative to −14 DRTC and transformed as log(relative abundance +1) because data were not normally distributed. Data were analyzed for main effects of treatment, DRTC, and treatment × DRTC using PROC MIXED (SAS 9.4). Differences were declared at \( P < 0.1 \) and tendencies at \( P < 0.15 \). Cows with low liver TG had greater ABHD5 (\( P = 0.05 \)) and HSL (\( P = 0.049 \)), and tended to have greater LIPA (\( P = 0.146 \)), LPL (\( P = 0.11 \)), and PHSL (\( P = 0.12 \)) abundance compared with cows with high liver lipids. Abundance of ABHD5 tended to be greater (\( P = 0.13 \)) and PLIN was greater (\( P = 0.06 \)) at +14 compared with −14 and +1 DRTC. Abundances of LIPA (\( P = 0.0006 \)) and PNPLA3 (\( P = 0.04 \)) were decreased at +1 and then increased by 3.3 and 1.8 times by +14 DRTC, respectively. These data indicate that cows with lower liver TG postpartum had a greater abundance of some hepatic lipases while other hepatic lipases are increased postpartum regardless of liver TG concentration. This suggests that there may be a coordinated response of several hepatic lipases to mediate both liver TG accumulation and subsequent remobilization during fatty liver recovery.

Key Words: lipolysis, transition cow, fatty liver syndrome

M47  Production responses to supplementation with rumen-protected lysine and two sources of rumen-protected methionine in Holstein cows. C. R. Seely*, S. E. LaCount1, C. M. Ryan1, K. E. Griswold2, and T. R. Overton1, 1Cornell University, Ithaca, NY, 2Kemin Industries, Des Moines, IA.

Multiparous Holstein cows (n = 57) averaging 124 DIM at the start of the experiment were used to determine production responses to supplementation with rumen-protected Lys and 2 forms of rumen-protected Met. Following a 2-wk covariate period, cows were assigned to one of 3 treatments in a completely randomized design. Treatments were formulated using the Cornell Net Carbohydrate and Protein System (version 6.5.5) and consisted of control [C; Met at 2.30% of metabolizable protein (MP) supply and 0.98 g MP-Met/Mcal of metabolizable energy (ME); Lys at 6.68% of MP and 2.85 g MP-Lys/Mcal of ME]; AA-S [Met at 2.66% of MP and 1.14 g MP-Met/Mcal of ME; Lys at 7.16% of MP and 3.08 g MP-Lys/Mcal of ME]; and AA-M [Met at 2.66% of MP and 1.14 g MP-Met/Mcal of ME; Lys at 7.16% of MP and 3.07 g MP-Lys/Mcal of ME]. Lysine was supplemented to AA-S and AA-M using USA Lysine (Kemin Industries, Des Moines, IA). Methionine was supplemented to AA-S using Smartamine-M (Adisseo, Alpharetta, GA) and to AA-M using MetiPEARL (Kemin Industries, Des Moines, IA). Data were analyzed using the MIXED procedure of SAS (SAS Institute, Inc. Cary, NC) with repeated measures. Contrasts consisted of AA (control vs. both AA treatments) and Met (AA-S vs. AA-M). Dry matter intake (26.0, 25.8, and 25.5 kg/d for C, AA-S, and AA-M, respectively; \( P = 0.54 \)) and milk yield (45.0, 44.5, and 44.9 kg/d; \( P = 0.84 \)) were not different among treatments. Milk true protein percentage (2.92, 2.92, and 2.98%) was increased (\( P = 0.01 \)) for cows fed AA-S and AA-M compared with controls, but was not different between AA-S and AA-M (\( P = 0.81 \)). Yield of milk protein (1.32, 1.32, and 1.35 kg/d; \( P = 0.58 \)) was not different among treatments. Percentages (3.46, 3.56, and 3.51%; \( P = 0.54 \)) and yields (1.56, 1.57, and 1.59 kg/d; \( P = 0.82 \)) of milk fat and yields of energy-corrected milk yield (45.3, 45.0, and 45.7 kg/d; \( P = 0.75 \)) were not different among treatments. Supplementation of AA increased milk true protein percentage but did not affect yields of milk and milk components; responses were the same between the 2 forms of rumen-protected Met used in this experiment.

Key Words: methionine, lysine, milk protein

M48  Formation and characterizations of heated whey protein isolate and alginate complexes. S. Khumsangkha* and B. Vardhanabuthi, University of Missouri Columbia, Columbia, MO.

With increasing interest for clean-label products, there is a need to develop protein ingredients with improved functional properties. Forming protein-polysaccharide electrostatic complexes has been found to improve protein functionalities. Most studies focus on complexation at pH < pI and low biopolymer concentrations. The purpose of this study was to develop and characterize heated whey protein-alginic complexes (H-CPX) formed by heating mixed solutions of 1–5% whey protein isolate (WPI) and alginate (0–1%) at pH 6.0 at 85°C for 30 min. Characterizations of H-CPX included measuring particle size, zeta potential and rheological properties as well as turbidity observation. Results showed that, across all WPI concentrations, average mean diameters of H-CPX significantly decreased (\( P < 0.05 \)) with increasing alginate concentration until reaching a minimum and then increased. This corresponded to the change in turbidity of the H-CPX. Zeta potential results revealed that increasing alginate concentration led to the formation of H-CPX with higher negative charge, suggesting that more alginate was incorporated into the electrostatic complexes. At similar alginate concentrations, higher WPI concentration resulted in complexes with larger aggregate sizes but lower negative charge than those of H-CPX formed at lower WPI concentration. This indicated a limited degree of complexation. H-CPX formed at 5% protein became highly pseudoplastic and more viscous. With increasing interest for clean-label products, there is a need to improve protein functionalities. Most studies focus on complexation at pH < pI and low biopolymer concentrations. The purpose of this study was to develop and characterize heated whey protein-alginic complexes (H-CPX) formed by heating mixed solutions of 1–5% whey protein isolate (WPI) and alginate (0–1%) at pH 6.0 at 85°C for 30 min. Characterizations of H-CPX included measuring particle size, zeta potential and rheological properties as well as turbidity observation. Results showed that, across all WPI concentrations, average mean diameters of H-CPX significantly decreased (\( P < 0.05 \)) with increasing alginate concentration until reaching a minimum and then increased. This corresponded to the change in turbidity of the H-CPX. Zeta potential results revealed that increasing alginate concentration led to the formation of H-CPX with higher negative charge, suggesting that more alginate was incorporated into the electrostatic complexes. At similar alginate concentrations, higher WPI concentration resulted in complexes with larger aggregate sizes but lower negative charge than those of H-CPX formed at lower WPI concentration. This indicated a limited degree of complexation. H-CPX formed at 5% protein became highly pseudoplastic and more viscous. It can be concluded that, with decreased particle size and increased negative charge, heated WPI-alginic complexes could potentially be utilized as natural ingredients with improved heat stability and interfacial properties.

Key Words: whey protein isolate, alginate, electrostatic complexes

Heated whey protein and pectin complexes formed at near neutral pH and high protein ratio are being developed in our laboratory. These complexes have higher negative charge and can potentially be used as emulsifier and stabilizer in clean-label applications. The objective of this study was to assess the emulsification properties of heated whey protein and pectin complex (HCPX) in O/W emulsions at pH 3.5–7 based on a wide pH range of food products. HCPX was prepared by heating mixed solution of 3% whey protein isolate and 0.3% low methoxy pectin at pH 6.2 and at 85°C for 15 min. Emulsions (containing 5% oil and 2% protein) were prepared by homogenizing the aqueous solutions with oil, followed by ultrasonic processing. The pH of the emulsions was adjusted to 4.0–7.0 and the droplet size, zeta potential, and rheological properties were measured. Emulsion stability was determined by measuring creaming on Day 1 and 7. For pH 3.5, emulsions were formed by adjusting the pH of the aqueous solution to 3.5 before oil addition and emulsification. Emulsification properties of HCPX were compared with heated WPI without pectin (CONTROL). Results showed that, CONTROL formed stable emulsions without creaming at pH 4.0, 6.0 and 7.0; however, emulsions at pH 4.5, 5.0, and 5.5 separated into 2 layers on Day 1. HCPX formed stable emulsions across pH range of 4.0 to 7.0 and no creaming was observed on Day 7. At pH 4.5, 5.0 and 5.5, emulsions stabilized by HCPX had significantly smaller droplet sizes and larger zeta potential (P < 0.05) compared with those stabilized by CONTROL. Since pH 3.5 is below the pl of the whey protein emulsions were formed by adjusting the pH of the aqueous solution before emulsification. This approach also led to stable emulsion (e.g., no creaming on Day 7) when HCPX was used. Rheological results revealed that improved stability of HCPX-stabilized emulsions could be partly due to an increase in viscosity. It can be concluded that HCPX could be used to emulsify and stabilize the emulsions in a wide pH range. The major benefit of the HCPX is the significant improvement in emulsification properties at pH near pl. HCPX can be developed as WPI-based emulsifier and stabilizer for clean-label applications.

Key Words: whey protein isolate, pectin, emulsification

M50 Evaluating teat skin condition in response to phenoxyethanol as a post-milking teat disinfectant on lactating dairy cows. S. K. Reeves*, M. R. Borchers, and J. M. Bewley, Department of Animal and Food Sciences, University of Kentucky, Lexington, KY.

Alternatives to iodine-based teat dips may decrease risk of iodine residues in milk. The objective of this study was to evaluate how a post-milking teat dip containing phenoxyethanol as the active ingredient and a 5% emollient (treatment) affected teat end condition compared with a 1% iodine solution (control). A 9-wk, split-udder, non-inferiority study was conducted on 111 lactating Holstein dairy cows at the University of Kentucky Coldstream Research Dairy. The treatment (left side) and control (right side) were applied using a non-return dip cup. Teats were scored once a week, in accordance with NMC guidelines, for 9 weeks on a 1 (desirable) to 3 (undesirable) scale for teat skin chapping, teat skin dryness, and teat skin color. A 1 (desirable) to 3 (undesirable) scale was used for teat end condition. Data analyses were conducted with SAS Version 9.3 (SAS Institute Inc., Cary, NC). The FREQ procedure generated frequency distributions and the MIXED procedure generated a mixed linear model evaluating the effect of teat, week, and their interaction on teat end condition, teat skin color, teat skin chapping, and teat skin dryness. Teat skin chapping and teat skin color remained at a score of 1 throughout all 9 weeks of the study and were not analyzed further. For teat end condition, no significant differences in scores between treatment teats and control teats. For teat dryness, scores for treatment front teats were significantly greater than control front teats (1.06 ± 0.01 vs. 1.06 ± 0.01; P ≤ 0.001). Temporary increases in dryness scores during study wk 4, 5, 6, and 9 (P ≤ 0.01) on the treatment side occurred during expected teat exfoliation. The scores comparing treatment and control teats are significantly different, but are minimal enough that they do not suggest a biological difference. Results indicate phenoxyethanol may serve as an alternative to iodine-based teat dips, which raise a risk of milk iodine residues.

Key Words: post-dip, teat condition, split udder dairy model


Group calving pens are becoming more common in dairy management practices, especially in larger herds. This is beneficial since less area is required compared with individual calving pens. The latest USDA NAHMS survey of US dairy farms reported that 58.7% of herds have at least some cows calving in a group pen. It is predicted that multiple cows in a maternity pen may be disruptive to the cow in labor and therefore negatively affect partitioning. The objective of this research was to describe the behavior of Holstein and Jersey dairy cows in a group maternity pen at the time of calving. Cows were moved to the maternity pen 3 weeks before their due date. A total of 17 cows (n = 9, Jersey and n = 8, Holstein cows) were monitored from the time of calving through the following hour. Holstein and Jersey cows were grouped together but multiparous and primiparous cows were in separate pens. Observations obtained from video recordings included, 1) time of calving, 2) stocking density at the time of calving (# cows / pen), 3) time to first interaction of the community cows with both mother and calf, 4) degree of calving difficulty (assisted vs. no assistance). There was no difference in the proportion of Jersey cows calving in the PM hours compared with Holstein cows (78% vs. 62%, respectively; P = 0.27). The highest stocking density recorded was 15 cows/pen. No correlation was detected between stocking density and time to first interaction with cow or calf (r = 0.34, y = 0.196+9.177; P = 0.33). Only 2 events of dystocia occurred in this sample, one at a low stocking density (4 cows/pen) and one at a high stocking density (14 cows/pen); more data is required to investigate the link between stocking density in maternity pens and dystocia. This research will give insight on whether group pens are disruptive to the calving event and if dairy practices should be changed to allow for less stressful calving for both the mothers and calves.

Key Words: stocking density, calving, group pens

M52 Relationship of body condition changes during the first 30 d of lactation and pregnancy rate per AI at 75 to 81 DIM. E. L. Middleton*, and J. R. Pursley, Michigan State University, East Lansing, MI.

The objective of this experiment was to determine the relationship between body condition changes during the first 30 d of lactation and the chances of pregnancy following 1st AI in lactating dairy cows. All cows were assigned a body condition score (BCS) value on a 1 to 5 scale (in tenths) within 1 week of calving and 30 d following calving. All cows received timed-AI using G66G/Ovsynch at 75 to 81 DIM. Cows were diagnosed for pregnancy using ultrasonography 35 d following 1st AI. Body condition loss during the first 30 d in lactation was greater for 1st
and 3rd compared with 2nd parity cows (−0.26 ± 0.01 and −0.26 ± 0.02 vs −0.15 ± 0.01; n = 736). A greater % of 2nd parity cows maintained or gained BCS during the measurement period compared with 1st and 3rd+ parity cows (32 vs. 14 and 19%; n = 736). In 1st and 2nd parity cows there was a significant negative relationship between BCS loss in the 30 d period following calving and chances of pregnancy later in lactation. Cows with the least loss had the greatest pregnancies per AI. There was no relationship between BCS loss and chances for pregnancy in 3rd + parity cows. One of the best predictors for fertility was previous calving interval. Quartiles of cows with a previous calving interval between 334 and 362 and 363 to 407 DIM had less mean ± SEM BCS loss compared with cows that had 408 to 433 and 434 to 619 DIM in previous calving interval (−0.14 ± 0.01, −0.19 ± 0.02, −0.22 ± 0.02 and −0.30 ± 0.03; n = 736), a greater % of cows that maintained or gained during the 30d period (34 vs. 22 vs. 17 vs. 14%; n = 736) and a greater chance for pregnancy at 1st AI (41, 46, 33, and 31%; n = 316). In summary, cows with the least amount of BCS loss between calving and 30 d post-calving had greater fertility following timed-AI at 75 to 81 DIM. Previous calving interval was predictive of BCS loss and future level of fertility.

Key Words: body condition loss, fertility, dairy cow

M53 Flaxseed containing lipid supplement increases linearly omega-3 content in milk without compromising production parameters. S. Akers*,1, R. Wilson1, K. Swanson1, M. Keller1, L. Goddick1, G. Cherian1, R. Day2, and G. Bobe1, 1Oregon State University, Corvallis, OR, 2N3Feed, Tualatin, OR.

Health and nutrition professionals advise consumers to limit consumption of saturated FAs and increase consumption of foods rich in omega-3 FAs. We showed that feeding 3 kg of the flaxseed containing lipid supplement 12BT40 (N3Feed LLC; Tualatin, OR) increases omega-3 content in milk compared with its ground, unprocessed ingredients by protecting omega-3 FAs from ruminal biohydrogenation. To determine the optimal supplementation rate of 12BT40 for improving milk fatty acid profile without adversely affecting production parameters, we fed 6 mid- to late-lactation, pregnant Holstein cows (1 block each for primiparous and multiparous cows) for 6 weeks consecutively (Control; 1 week), 0.91 (1 week), 1.81 (2 weeks), and 2.72 kg/d (2 weeks) 12BT40 as top-dressing. Milk and serum samples were collected at the end of each treatment period and analyzed for fatty acid profile and metabolic indicators, respectively. Individual feed intake and milk yield and components were measured to evaluate the effect of 12BT40 on production characteristics. Data were analyzed using PROC MIXED in SAS version 9.4. Fixed effects were supplementation rate (linear, quadratic and cubic) and parity; cow was the random effect. 12BT40 supplementation rates increased linearly ($P_{\text{Linear}} < 0.0001$) the omega-3 proportion in milk FAs (0.48, 1.92, 2.53, and 3.67 ± 0.30wt% for 0, 0.91, 1.81, and 2.72 kg/d of 12BT40) whereas the proportion of artherogenic 12:0, 14:0 and 16:0 was decreased (51.2, 41.1, 38.6, and 34.1 ± 1.0wt% for 0, 0.91, 1.81, and 2.72 kg/d of 12BT40; $P_{\text{Linear}} < 0.0001$). 12BT40 supplementation rates increased linearly ($P_{\text{Linear}} = 0.009$) milk yield (20.7, 20.9, 21.4, and 22.8 ± 1.3 kg/d for 0, 0.91, 1.81, and 2.72 kg/d of 12BT40) and had curvilinear relations with DMI (18.4, 17.5, 16.5, and 20.9 ± 0.6 kg/d for 0, 0.91, 1.81, and 2.72 kg/d of 12BT40) and milk fat content (3.96, 4.11, 4.69, and 4.06 ± 0.23% for 0, 0.91, 1.81, and 2.72 kg/d of 12BT40). Based on these results, we conclude that feeding up to 2.72 kg/d 12BT40 may improve milk fatty acid profiles without compromising production parameters.

Key Words: flaxseed, lipid supplement, omega-3
M54 Sampling strategies for dairy cow welfare assessments. J. Van Os1,2, C. Winckler1, J. Trieb1, S. Matarazzo3, T. Lelenbauer3, J. Champagn4, and C. Tucker1. 1Department of Animal Science, University of California, Davis, CA; 2Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada; 3Division of Livestock Sciences, University of Natural Resources and Life Sciences, Vienna, Austria; 4Department of Agricultural and Environmental Sciences, Santa Cruz State University, Ilh6as, Brazil; 5Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA.

Our objective was to evaluate how prevalence estimates for health conditions are affected by the number of cows sampled and the selection method used. On 10 California dairy farms, we assessed all cows in the high-producing pen (DIM < 100; range 81–241 cows) using measures from the Welfare Quality Protocol for Dairy Cattle. Cows were evaluated for body condition, hygiene, skin alterations (hairless patches, lesions, or swelling), discharge (ocular, nasal, vulvar), diarrhea, and impaired respiration while restrained in headlocks. Lameness was scored upon release from the feed bunk. Prevalence for each condition was calculated as a percentage of cows in the pen. The most common conditions were dirty hindquarters (33.5 ± 10.7%, mean ± SD) and lesions or swelling on the knee (34.4 ± 17.0%) and hock (26.4 ± 16.7%). Diarrhea (8.0 ± 5.8%), lameness (mild: 7.3 ± 4.7%, severe: 2.2 ± 2.2%), and neck (5.8 ± 12.6%), flank (4.5 ± 5.0%), or hindquarter alterations (5.5 ± 3.9%) were less common. Very fat cows, vulvar discharge, and impaired respiration were excluded from further analysis (prevalence ≤ 1%). To evaluate how many cows are needed to accurately estimate prevalence, 7 subsets of data were created by selecting every 10th, 5th, 4th, 3rd, 2nd, 2 of 3, or 3 of 4 cows using their position at the bunk. In addition, 7 matching proportions of the pen were randomly computer-selected (14 subsets total). Estimates were compared with true values using regression analysis and were considered accurate if they met 3 criteria: R² > 0.9 and the slope and intercept did not significantly differ (P > 0.05) from 1 and 0, respectively. All estimates met the slope and intercept criteria, whereas R² increased when more cows were sampled. Regardless of how many cows were used, both selection methods were accurate for ocular discharge (22.2 ± 27.4%), neck alterations (5.8 ± 12.6%), and knee hairless patches (14.1 ± 17.4%). For the other conditions, all estimates based on feed bunk position were accurate when ≥ 2/3 of the pen was used, and all but severe lameness were accurately estimated when sampling randomly. Selecting cows using feed bunk position did not differ systematically from computer-selecting random ear tag numbers, suggesting the former may be an appropriate method for welfare assessments.

Key Words: cow welfare indicators, farm profitability, productivity

M55 Associations between on-farm animal welfare indicators, farm productivity, and profitability on Canadian freestall dairies. M. V. Robichaud1, J. Rushe2, A. M. de Passillé3, E. Vasseur3, D. Haley4, K. Orsel5, and D. Pellerin1. 1Université Laval, Québec, QC, Canada; 2University of British Columbia, Agassiz, BC, Canada; 3McGill University, Ste-Anne-de-Bellevue, QC, Canada; 4University of Guelph, Guelph, ON, Canada; 5University of Calgary, Calgary, AB, Canada.

Even though good animal welfare has always been an important preoccupation for dairy farmers, it is often thought to convey more costs than financial gains to the farm. The aim of this study was to evaluate the associations between farm productivity and profitability indicators and animal welfare indicators using information collected on 130 Canadian free-stall dairy farms of which 20 had automated milking systems. The animal welfare elements evaluated included animal-, environmental-, and management-based measures. The profitability indicators were retrieved from the dairy herd improvement program and included milk production, reproduction and cow longevity elements. Margin over replacement costs per cow (MORC) were also calculated for each farm using milk and culling revenues minus replacement and dead animals’ disposal costs. Univariable and multivariable linear regression models were used to analyze the associations between welfare and the farm profitability indicators. Average yearly milk production decreased with increasing percentage of obviously lame cows and this decrease was steeper with increasing dry period length for farms with more than 5% of obviously lame cows. Higher percentages of cows with low BCS, knee lesions, and dirty flanks were also associated with lower milk production. Farms with higher percentage of lame cows and cows with neck lesions had significantly longer average calving intervals. The farms with higher average MORC per cow (higher benefits) had significantly lower percentages of animals with low BCS ($47% per $), knee lesions ($4 per $), dirty flanks ($21% per $), and a lower percentage of animals that did not fit in the average stall length ($3 per $). The farm’s MORC per cow was also associated with the percentage of lame cows, which interacted with the dry period length. Management decisions are often interrelated with animal-based welfare indicators, resulting in better farm profitability. These results indicate that improving animal welfare on-farm with free-stalls is beneficial not only for the animals but also for the productivity and profitability of Canadian dairy farms.

Key Words: welfare assessment, sampling, validation


Lameness is now widely accepted as one of the most important animal welfare concerns facing the global dairy industry. Previous work has shown that the prevalence of lameness and related injuries vary regionally and among farms. The objective of the study was to describe the prevalence of lameness, and hock and knee injuries of high producing cows on freestall farms in the United States. Two hundred forty-eight farms were visited between 2012 and 2014. Farms were in California (CA; n = 38), the Midwest (IA, IL, IN, KS, MI, MN, ND, OH, SD, WI) (MW; n = 77) and the northeast (CT, MD, ME, NH, NY, PA, VT) (NE; n = 133). Evaluation of all cows in the “high-producing” group were performed as they exited the parlor for lameness (5-point numerical rating system (NRS), where 1 = sound and 5 = severely lame; categorized as clinical lameness (NRS ≥ 3) and severe lameness (NRS ≥ 4)), knee injuries (where 0 = sound and 1 = swollen and/or open wound) and hock injuries (where 0 = sound, 1 = loss of hair and swollen, 2 = excessively swollen and/or open wound). The prevalence of clinical lameness averaged 27.2 ± 11.9%, ranging from 5.5 to 70.1% between farms. This prevalence was 8–10% higher in the MW (27.4 ± 11.2%) and the NE (29.3 ± 11.7%) than in CA (19.5 ± 10.9%). The prevalence of the other ailments followed the same pattern. Severe lameness averaged 2.1 ± 2.0% in CA, 3.9 ± 4.2% in MW and 4.2 ± 4.4% in NE. Less than 1% of cows in CA had swollen knees, in comparison to approximately
3% of cows in both the MW and NE. Hock injury prevalence averaged 12.0 ± 7.8% in CA, 20.3 ± 22.5% in MW, and 33.3 ± 21.9% in NE. Severe hock lesions were almost nonexistent in CA (0.3 ± 0.6%), but higher in MW (1.6 ± 2.8%) and NE (2.6 ± 3.6%). These results indicate that lameness and leg injuries remain prevalent, and underscore the importance of efforts to reduce these problems.

**Key Words:** lesions, gait, cow comfort

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**M57 Changes in lying behavior in response to lameness.** A. J. Thompson1,2, J. A. Bran2, R. R. Daros1, M. J. Hötzel2

Changes in lying behavior in response to lameness.

**Key Words:** lesions, gait, cow comfort

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**M58 Early non-invasive clinical diagnosis of hoof ulcers by infrared thermographic images (IRT) in milking dairy cows.** S. Vázquez-Flores* and C. Lucio-Rodríguez, Tecnológico de Monterrey Campus Querétaro, Querétaro, Mexico.

Clinical assessment during hoof trimming implies restrictive methods and many hours of keeping cows waiting for their turn. The intention of this study was to characterize temperature emission in punctual areas of the hoof by capturing images with an infrared thermography camera (IRT) comparing them to other hoof conditions. The trial took place on a commercial dairy in Central México. Punctual temperature emission was used by tracking 14 specific areas in the sole. Mapping was related to the external/internal middle axis: Sp1/Sp9 (white line axial wall); Sp2/Sp10 (white line); Sp3/Sp11 (abaxial wall); Sp4/Sp12 (caudal claw); Sp5/Sp13 (cranial claw); Sp6/Sp14 (heel bulb); Sp7 (interdigital hind claw); Sp8 (interdigital medial claw). The study included a convenience sampling of 37 milking cows (<150 DIM), in an ambient temperature of 23°C. Cows were restrained in the hydraulic chute for hoof trimming, clinical analysis and IRT image capturing. Hoof analysis was characterized as sole ulcers, digital dermatitis, white line disease and normal cases. Two multiple statistical comparison tests (Hsu’s MCB test/Wilcoxon pairwise contrast) showed differences in temperature between heel bulb with ulceration and the other areas (JMP 11.1). The hind-left hoof showed an average temperature of 29.5°C in area Sp14 in cows with sole ulcer (P = 0.0003). For the hind-right hoof, sole ulcer cases were different from the normal in 3 instances: area Sp3 with a temperature of 22.9°C (P = 0.03); and areas Sp6 and 14 with average temperatures of 21.2 and 18.8°C respectively (P = 0.05; P = 0.0027). The most common cases overall were slight dermatitis in both hind limbs (49%); and sole ulcers in the left hoof (7.3%). Areas Sp6 and 14 are easily reached without causing distress in cows during milking, currently under investigation. The diagnostic approach is a non-invasive system that identifies elevated emission of temperature of the heel bulb related to ulcers when taking an IRT image.

**Key Words:** infrared thermography, sole ulcers, dairy cows

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**M59 Evaluation of the relationship between prepastoral ruminal and mammary gland temperature and calving day in dairy cows.** F. Batistela*, E. Gonzalez-Angulo, C. L. M. Garces, and J. J. Loor, University of Illinois at Urbana-Champaign, Urbana, IL.

Monitoring parturition is fundamental to avoid mortality of newborn calves. Physiological conditions such as pregnancy and lactation influence the diurnal temperature pattern of dairy cows. However, information on changes in body temperature and their association with calving time in dairy cows is lacking. Therefore, our objective was to evaluate the use of external ruminal and mammary gland temperature as predictors of calving day. Thirty Holstein cows were used and measurements were taken from 15 d before the expected calving day until the actual calving day. The ruminal temperature was recorded approximately 2 h post-feeding after an area of 0.4 × 0.4 m on the left side of the animal (including the paralumbar fossa) was shaved and cleaned. Measurements were made at a distance of 1.0 m using a hand-held portable infrared camera (E4 Box, FLIR Systems, Wilsonville, OR). The mammary gland temperature was assessed around 4 p.m. from the posterior side following the same approach used for rumen. The average temperature was calculated by tracing the shaved area using ThermalCAM Researcher Pro 2.7 software (FLIR Systems). The statistical model included the random effect of block and fixed effect of treatment, time and interactions. Ruminal temperature and DMI were not correlated (P = 0.08; r = 0.08). Ruminal temperature and calving day were negatively correlated (P < 0.01; r = 0.19). Compared with 5 d before parturition, ruminal temperature was 1.8°C (P < 0.01), 1.2°C (P = 0.02), 0.5°C (P = 0.15), 0.2°C (P = 0.22), and 0.0°C (P = 0.32) lower at calving, −1, −2, −3 and −4 d, respectively. Mammary gland temperature and calving day were positively correlated (P = 0.04; r = 0.11). Compared with 15 d before parturition, mammary temperature was 1.4°C (P = 0.02), 1.1°C (P = 0.03), 1.2°C (P = 0.02), 1.0°C (P = 0.05), 1.1°C (P = 0.04) and 0.7°C (P = 0.09) greater at calving, −1, −2, −3, −4 and −10 d, respectively. Overall, dairy cows exhibit a distinct change in ruminal and mammary gland temperature commencing approximately 8 and 1 d before parturi-
tion. Thus, monitoring the ruminal temperature externally could be a practical tool to predict calving.

**Key Words:** monitoring parturition, temperature

**M60** Effect of ketosis on behavioral activity in transition dairy cows. J. M. Piñeiro**,†**1, B. T. Menichetti1, A. A. Barragan1, W. P. Weiss2, S. Bas1, and G. M. Schuenemann1, 1Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 2Department of Animal Sciences, The Ohio State University, Wooster, OH.

The objective of this study was to assess the effect of ketosis status on behavioral activity in transition dairy cows. A behavioral activity index (BAI) was computed for every animal taking into account the number of steps (no./d), standing time (min/d), lying time (min/d), and lying bout (LB, no./d). A total of 387 Holstein dairy cows (110 primiparous and 277 multiparous) in 3 commercial dairy herds were enrolled at 7 d before calving until 14 d post-calving. Weekly, a cohort of 10 to 15 cows was enrolled at each farm and electronic data loggers (IceQube, IceRobotics, Edinburgh, UK) were fitted to the hind leg of individual cows to assess their behavioral activity. Postpartum cows were screened for ketosis (KET) at 7 and 14 DIM by measuring β-hydroxybutyrate in serum samples (Nova Vet Ketone Test Strips). A case of KET was recorded when lactating cows had serum concentration of β-hydroxybutyrate ≥ 1.2 mmol/L. Cases of metritis, retained placenta, lameness, milk fever, or mastitis during the study period were recorded and lactating cows were allocated into 1 of 4 groups: 1) non-disease (ND, n = 248; cows without KET and any other health conditions), 2) cows with only KET (n = 64), 2) sick cows experiencing ≥ 1 health conditions, but without KET (SICK, n = 59), or cows with KET plus at least 1 health condition (KET+H, n = 17). The BAI was computed for the first 7 DIM to assess differences among cow health groups. Data were analyzed using MIXED procedure of SAS. Primiparous cows (P < 0.05) had greater BAI compared with multiparous cows and as cows mature, they become less active early in lactation regardless of health status. Cows experiencing KET, SICK and KET+H had reduced BAI (93.1, 56.6 and 95.5, respectively) compared with ND cows (P < 0.05). These results suggest that monitoring a combination of behavioral metrics, such as lying time and BAI, could be used to predict stillborn calves in transition heifers and cows.

**Key Words:** stillbirth, behavior, dairy cattle


In dairy cattle, mammary biopsies are commonly used to study mammary development and function. The objective of this study was to investigate the behavioral changes following mammary biopsy. Pregnant, nonlactating Holstein dairy cows (n = 9/treatment; 20 d before expected calving date) were exposed to either: 1) a biopsy procedure, in which mammary tissue samples (60 × 4 mm in diameter) were obtained using a biopsy tool from the rear left quarter, following administration of a sedative (xylazine, 20 µg/kg of BW) and local anesthesia (3 mL lidocaine), or 2) a sham procedure, in which cows were removed from the pen and restrained for a similar duration of time as for the biopsy procedure. Cows were fitted with accelerometers to record daily standing time, standing bout frequency, and lying side. Dry matter intake (DMI) was recorded daily using the Calan gate system. Daily activity data and DMI were recorded for 1 wk following the biopsy or sham procedure, and analyzed in a general linear mixed model with day as a repeated measure. The biopsy procedure had no effect on DMI (11.2 kg/d; SE = 0.67; P = 0.88) or total daily standing time (10.0 h/d; SE = 0.44, P = 0.44). However, relative to control cows, biopsied cows had more frequent standing bouts (11.8 vs. 9.9 bouts/d; SE = 0.59; P = 0.04) of shorter duration (52.2 vs. 64.8 min/bout; SE = 4.4, P = 0.05) with no interaction of treatment and day (P > 0.33). On d 1 following the procedure, biopsied cows also showed greater laterality in their lying behavior (P = 0.021), having longer lying bouts on their right side than left (97.8 vs. 70.0 min/bout; SE = 8.6; P = 0.015) whereas control cows showed no lying side preference (P = 0.56). These results suggest that mammary biopsy does not affect overall activity and feeding behavior, but has subtle effects on activity, which may be indicative of increased restlessness or short-term discomfort in the biopsied quarter. These effects, however, dissipate rapidly following biopsy.

**Key Words:** dairy cow, mammary biopsy, lying behavior
Relationship between the clinical and behavioral response to a mastitis challenge with *Streptococcus uberis* from Holstein dairy cows. V. L. Couture*, P. D. Krawczel, G. M. Pighetti, R. A. Almeida, and S. P. Oliver, Department of Animal Science, The University of Tennessee, Knoxville, TN.

Treatment of mastitis may be improved by earlier detection through use of sickness behaviors. The objective was to determine the relationship between clinical signs of mastitis, induced by a *Streptococcus uberis* challenge, and resting behavior of early lactation dairy cows. Holstein cows in their second (n = 21) and third (n = 7) lactation were enrolled and intramammary challenges were conducted with *S. uberis* (10,560 ± 1,855 cfu) within 1.2 ± 0.7 d after calving. Somatic cell count, milk score (MS), and udder inflammation score (US) were collected 24 h and 0 h before challenge, every 12 h for 3 d post-challenge, and daily on d 4–7. MS and US were assessed from appearance of the milk and udder, respectively, with a score of 0 = normal and 3 = severe. Accelerometers attached to the hind leg recorded lying duration (h/d) and mean bout duration (min/bout). Data were summarized by 24-h period starting at 0900 h using the mean SCC, MS, and US for each d 0–3 d post-challenge and the recorded daily score from d 4–7. Data were analyzed using a regression model in SAS (v9.4) to determine the relationship between the independent variables of SCC, MS, and US and the dependent variables of daily lying duration and mean bout duration. MS was positively associated with mean bout duration \( (P = 0.005; R^2 = 0.04; y = 6.4x + 72.6) \), but SCC \( (P = 0.74) \) and US \( (P = 0.26) \) were not. SCC \( (P = 0.74) \), MS \( (P = 0.73) \), and US \( (P = 0.94) \) were not associated with daily lying time. Of the 3 severity measures, MS was the most sensitive (or most closely associated) with sickness-related behavior and offers the greatest potential to predict clinical mastitis caused by *S. uberis*. The positive relationship between MS and mean bout duration most likely is driven by the strength of the acute inflammatory response, which results in a more systemic response best represented by these 2 measures. Closer examination of the timing of these events relative to each other within and across cows can potentially improve the ability to predict future cases of clinical mastitis through changes in lying behavior.

**Key Words:** mastitis, behavior, *S. uberis*
M64  Effect of CalfAce on performance and health of Holstein dairy calves. M. Cooney1, D. Cooke2, and R. James3, 1phdR&D, Fort Atkinson WI, 2R&D Life Sciences, Menomonie, WI, 3Virginia Tech, Blacksburg, VA.

The objective of this study was to evaluate performance effects of CalfAce, a registered trademark of R&D LifeSciences LLC, compared with the antibiotic Neo-Terramycin, NT Concentrate, a registered trademark of Phibro Animal Health Corporation, and a non-treated control. Fifty-four female Holstein calves fed 0.4 to 0.9 kg of whole milk solids/day depending on age were assigned at random to 1 of 3 treatments upon birth, balanced by dam parity (n = 18). The experimental diets added to milk consisted of (1) CalfAce at 12mL/hd/d, administered 2×/d at 6mL/feeding for 21 d; (2) NT Concentrate at 46g/hd/d, administered 2×/d at 23g/feeding for 14 d; and (3) untreated control. Individual milk consumption along with grain consumption over 21 d was recorded. Serum total protein was measured 24 h post colostrum administration. Body weights were recorded at birth and the end of the experiment (d 21) to determine average daily gain. Treatment and weather conditions were recorded daily throughout the study. Data analyzed using the MIXED and FREQ procedures of SAS from calves that completed the entire duration of the study. Overall, supplementation with CalfAce led to a statistically greater number of days with 0.5 or less fecal score observed compared with control and NT Concentrate and a significantly lower number of days of treatment administration compared with control. A trend toward significance was seen for average 21-d fecal score and number of days scouring at 1.5 and 2.0 or greater in which CalfAce was the lowest. No treatment differences were observed in milk consumption, starter intake or ADG. Calf mortality due to intestinal health was equivalent between CalfAce and NT Concentrate treatments whereas control had 2 deaths.

Key Words: calf, health, antibiotic alternatives

M65  Effect of lameness on behavioral activity in transition dairy cows. J. M. Piñeiro*1, T. B. Menichetti1, A. A. Barragan1, W. P. Weiss2, S. Bas1, and G. M. Schuenemann*1, 1Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 2Department of Animal Sciences, The Ohio State University, Wooster, OH.

The objective of this study was to assess the effect of lameness on behavioral activity in transition dairy cows. A behavioral activity index (BAI) was computed (Titler et al., 2015 J. Dairy Sci. 98:5304–5312) for every animal taking into account the number of steps (no./d), standing time (min./d), lying time (min./d), and lying bouts (LB, no./d). A total of 387 Holstein dairy cows (110 primiparous and 277 multiparous) in 3 commercial dairy herds were enrolled at 7 d before calving until 14 d post-calving. Weekly, a cohort of 10 to 15 pre-partum heifers and cows were enrolled at each farm, and electronic data loggers (IceQube, IceRobotics, Edinburgh, UK) were fitted to the hind leg of individual animals to assess their behavioral activity. Pre-partum heifers and cows were moved into pre-partum pens 21 d before the expected calving date. All heifers and cows were housed in similar pre-partum free-stall barns and moved into a contiguous individual maternity pen for parturition. Animals were screened for lameness using a 3-point scoring system (LS: 1 = normal walk, 2 = moderately lame, and 3 = severely lame) at 7 d before calving and at 14 DIM. The BAI was computed for the first 7 DIM to assess differences among LS in primiparous and multiparous cows. Data were analyzed using MIXED procedure of SAS. Primiparous cows (P < 0.05) had greater BAI compared with multiparous cows, and as cows mature (multiparous), they become less active around the transition period. Regardless of parity, postpartum cows with LS of 3 spent more time lying (742 min/d) than cows with LS of 1 (654 min/d, P < 0.05). Postpartum cows with LS of 1 had a greater BAI (380; P < 0.05) compared with cows with LS of 3 (BAI of 278). These results suggest that monitoring a combination of behavioral metrics, such as lying time and BAI, could be used to consistently identify lame cows within herds.

Key Words: lameness, behavior, dairy cattle

M66  Pre- and postweaning performance and health of dairy calves fed milk replacers supplemented with various additives. D. Ziegler*1, H. Chester-Jones1, A. Geiger2, J. Olson2, B. Ziegler1, and D. Shimke3, 1University of Minnesota, Waseca, MN, 2Milk Products Inc., Chilton, WI, 3Hubbard Feeds Inc., Mankato, MN.

One hundred and nine (2 to 5 d old) individually fed Holstein heifer calves (39.2 ± 0.65 kg) from 3 commercial dairies were randomly assigned to 1 of 4 milk replacer (MR) treatments to evaluate pre- (d 1–42) and post weaning (d 43–56) calf performance and health when supplemented with various additives. The study was conducted between March and June 2016. Treatments included (1) all-milk protein, non-medicated MR 24% CP: 20% Fat fed at 0.34 kg in 2.38 L of water 2× daily from d 1 to d 35 and 1× daily from d 36 to d 42 (CON); (2) CON supplemented with neomycin sulfate and tetracycline (NT) at a rate of 22 mg/kg of body weight for 14 d (MRNT); (3) CON supplemented with 6 g of dried colostrum powder providing 3 g of IgG per feeding for 14 d (MRCP); and (4) CON supplemented with an additive blend of animal plasma, Bio-Mos, microalgae meal (All-G Rich, Schizochytrium limacium), essential oils (Apex) and multiple direct fed microbial strains including Lactobacillus and Bacillus from d 1–42 (MRAB). Calf starter (18% CP as fed) and water were fed free choice d 1–56. There were no differences in pre weaning gain (d 1–42; P > 0.05) averaging 0.57 kg/d. Post-weaning gains (d 43–56) did not differ and averaged 0.90 kg/d. Overall gain was similar (P > 0.05) and averaged 0.62, 0.68, 0.63 and 0.66 kg/day for CON, MRNT, MRCP, and MRAB, respectively. There was no difference in hip height gain, which averaged 11.4 cm for all treatments. There was no difference in MR solids intake, which averaged 27.7 kg for 42 d. Calf starter intake from d 1- 56 was similar (P > 0.05) for all treatments, averaging 37.1, 40.9, 35.6, and 40.9 kg for CON, MRNT, MRCP, and MRAB, respectively. There were no differences in daily fecal scores, scouring days, or treatment costs. Under conditions of this study, calves fed milk replacers with alternative additives for health and growth can perform similarly to calves fed NT.

Key Words: calf performance, milk replacer, supplemented additive

M67  Effects of non-digestible saccharides on passive immunoglobulin G transfer and serum immunoglobulin G concentration in newborn calves fed colostrum replacer. A. Huen*1, T. Sato2, and M. Hanada2, 1United Graduate School of Agricultural Sciences, Iwate University, Morioka, Iwate, Japan, 2Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.

It has been reported that non-digestible saccharides such as difructose anhydride III (DFA III), fructooligosaccharides (FOS), and maltitol (MAL) enhanced the intestinal calcium absorption by acting on intestinal
epithelium tight junctions. We have already shown that DFA III promoted apparent efficiency of absorption (AEA) of immunoglobulin G (IgG) and serum IgG concentration in newborn calves (Htn et al., J. Dairy Sci. 99:5701–5706), but effects of FOS and MAL on AEA and serum IgG concentration have not been investigated yet. This experiment was done to investigate the effects of FOS and MAL supplementation on AEA and serum IgG concentration in newborn calves. Eighteen newborn Holstein male calves were randomly assigned to receive no supplement (Control group, n = 6), or 18 g of FOS supplement (FOS group, n = 6), or 18 g of MAL supplement (MAL group, n = 6) in each feeding. All calves were fed 2 packages of colostrum replacer providing 120 g of IgG at 1 and 10 h after birth. After colostrum replacer feeding, 250 g/2 L of milk replacer was fed at 24 h, 36 h, and then twice daily for 7 d after birth. Blood samplings were done before feeding at 0 h, 10 h, 24 h, 36 h, 4 d and 7 d of age for age of for IgG analysis. One-way ANOVA followed by LSD multiple comparisons tests were used to compare individual parameters among the groups. Serum IgG concentration sharply increased from 0 h of age and reached a peak level at 24 h of age in all groups. Mean serum IgG concentration at 24 h of age in the MAL group (26.3 g/L) was higher than those in the control (20.0 g/L) and the FOS (19.1 g/L) groups (P < 0.05). Mean AEA at 24 h of age in the MAL group (42.3%) was higher than those in the control (31.4%) and the FOS (30.3%) groups (P < 0.05). In conclusion, MAL could improve both AEA and serum IgG concentration in newborn calves by feeding it with the colostrum replacer, but the effects of non-digestible saccharides on passive immune transfer in newborn calves differ with their types.

Key Words: non-digestible saccharide, immunoglobulin G, newborn calf

M68 Pre- and postweaning performance and health of dairy calves fed milk replacers supplemented with different strains of direct-fed microbials.
H. Chester-Jones*1, D. Ziegler1, E. Davis2, J. O’Neill2, and S. Hayes3, 1University of Minnesota, Waseca, MN, 2Agro Bio Sciences, Wauwatosa, WI, 3Day 1 Technology, Winona, MN.

One hundred and thirty-five (2 to 5 d old) individually fed Holstein heifer calves (38.8 ± 0.62 kg) from 3 commercial dairies were randomly assigned to 1 of 5 milk treatments to evaluate pre- (d 1–42) and post weaning (d 43–56) calf performance and health when fed milk replacers supplemented with a direct-fed microbial (DFM) or neomycin sulfate and oxytetracycline (NT). The study was conducted between June and September 2016. Treatments included 1) all-milk protein, non-medicated milk replacer (MR) 20% CP; 20% Fat fed at 0.28 kg in 2 L of water 2× daily from d 1 to d 35 and 1× daily from d 36 to weaning at d 42; 3) MR as in CON supplemented with NT at a rate of 22 mg/kg BW for 14 d, (MRNT); 4) MR as in CON supplemented with 5 g of DFM containing Bacillus subtilis strain 747 (1 × 109 cfu) per feeding for 42 d, (MRDFM); 4) MR as CON supplemented with 5 g of DFM containing B. subtilis strains 747+1781 (1 × 109 cfu) per feeding for 42 d, (MRDFM2); 5) MR as CON supplemented with 5 g DFM as in MRDFM2 plus Lactobacillus plantarum (5 × 108 cfu) per feeding for 42 d, (MRDFM3). Calf starter (18% CP as fed) and water were fed free choice from d 1 to 56. There were no differences in pre- or postweaning average daily gains (ADG) (average 0.42 kg/d and 0.88 kg/d respectively. Overall 56 d ADG tended to be greater (P = 0.06) for calves fed MRNT and MRDFM, 0.57 kg/d vs. those fed CON, 0.52 kg/d and MRDFM3, 0.50 kg/d with MRDFM2, 0.54 kg/d being intermediate. Calf starter and MR intake were similar (P > 0.05) across treatments averaging 35.9 and 20.8 kg total intake, respectively. There were no differences in daily fecal scores, scouring days or treatment costs. Under conditions of this study, calves fed milk replacers with MRDFM can perform as well as calves fed MRNT.*

Key Words: calf performance, milk replacer, direct-fed microbial

M69 Effects of Proteomace on the performance and small intestinal health of Jersey calves challenged with Salmonella enterica serotype Typhimurium at 7 day of life. Y. Liang*, R. Hudson, and M. Ballou, Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX.

The objective of this study was to determine the effects of Proteomace on the performance and health of Jersey calves challenged with Salmonella enterica serotype Typhimurium. Jersey bull calves within 1 d of birth were blocked by total serum protein and initial BW and randomly assigned to treatments in a 2 × 2 factorial arrangement with Proteomace (PM) and Salmonella enterica (ST) as the main effects (n = 8). Calves challenged with ST were administered 5.5 × 106 cfu in the AM milk replacer on d 7. Calves supplemented with PM had 8 mL added directly to the bottle before each feeding. All calves were fed 300 g of a 22% CP and 20% fat milk replacer twice daily. Calves had ad libitum access to a starter. Blood samples and body weight measurements were taken at d 0, 7, 10, 14, and 21. All calves were harvested on d 21, and duodenum and ileum samples collected for histomorphological measurements. Data were analyzed as a repeated measures with the fixed effects of PM, ST, time, and all possible interactions; block was included as an additional random effect. There was no treatment × time difference on ADG (P = 0.802); however, ST calves tended to decrease ADG (P = 0.054). No treatment × time difference was observed in rectal temperature (P = 0.883), but ST calves had greater rectal temperature than unchallenged calves at d 9 to 12 post challenge (P < 0.001). There was no treatment × time or treatment differences in fecal dry matter percentage either before or post challenge (P ≥ 0.261). There was no treatment × time difference in plasma haptoglobin concentration (P = 0.736); however, calves supplemented with PM had decreased concentrations (P = 0.016). The ST calves had a tendency (P = 0.082) for reduced villi:crypt in the duodenum, but PM calves had greater villi:crypt in the duodenum (P = 0.040). Additionally, the ST calves had a tendency (P = 0.072) for reduced villi:crypt in the ileum; however, there was no effect due to PM (P = 0.154). These data indicate that Proteomace reduced some inflammation indices of Jersey calves challenged with Salmonella enterica on d 7 of life.

Key Words: calf, health, nutrition
3 and 21 d in milk were assessed once for metritis based on vaginal discharge (n = 654; metritic = watery foul discharge 3 to 14 DIM or >50% pus and foul smell 14 to 21 DIM) and for RP (n = 661; >24 h to pass fetal membranes; reported by the farmer). Cow-level measurements included body condition score (BCS), parity, breed, dystocia, and RP. Farm-level variables included use of maternity pen, time before cow-calf separation (>or <12 h), and cleanliness of the holding area. Univariable models were used to select variables associated with RP and metritis (P < 0.2). Multivariable multilevel logistic regression models (farm as random effect) were reduced using manual backward elimination. The prevalence of RP and metritis were 13.9% and 11.2%, respectively. The odds of RP were higher if cows had dystocia (OR = 3; 95% CI: 1.6–5.4; P < 0.01) or were in parity ≥3 (OR = 2.3; 95% CI: 1.2–4.6; P = 0.01) and lower if cows were Jerseys (OR = 0.2; 95% CI: 0.1–0.8; P = 0.02), had access to a maternity pen (OR = 0.5; 95% CI: 0.3–1.0; P = 0.05) or were allowed >12 h before cow-calf separation (OR = 0.4; 95% CI: 0.2–0.8; P = 0.01). The odds of metritis were higher if cows had RP (OR = 16; 95% CI: 8.6–30.3; P < 0.01), if farms had a dirty holding area (OR = 2.2; 95% CI: 1.1–4.3; P = 0.03), and lower if cows had BSC between 3 and 3.5 (OR = 0.45; 95% CI: 0.2–0.7; P < 0.01). Management factors related to calving were associated with RP and environmental cleanliness was associated with metritis on pasture-based farms. Further research on the causal relationship between these management practices and their effects on disease could help in the design of improved disease prevention protocols.

Key Words: health, transition period, reproductive disease

M71 The determination of concentrations of tocopherol isoforms in whole tissues and mitochondria via high-performance liquid chromatography after short-term supplementation in dairy cows. Y. Qu1, T. H. Elsasser2, S. Kah1, M. Garcia4, C. M. Scholte1, E. E. Connor1, G. F. Schroeder2, and K. M. Moyes1, 1Department of Animal and Avian Science, University of Maryland, College Park, MD, 2Agricultural Research Service, Animal Biosciences and Biotechnology Laboratory, USDA, Beltsville, MD, 3Agricultural Research Service, Animal Genomics and Improvement Laboratory, USDA, Beltsville, MD, 4Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS, 5Cargill Animal Nutrition, Elk River, MN.

Mitochondria are cell organelles vulnerable to stress and may be damaged either by reactive oxygen or nitrogen species during the stress response. The antioxidant tocopherol may be useful in protecting mitochondrial health. The objective of our study was to determine the pattern of change in the concentrations of 4 isoforms of tocopherol (α-, β-, γ-, and δ-tocopherol) in whole tissue and mitochondrial fraction from bovine liver and mammary gland after short-term feeding of a vegetable-derived oil (Tmix) particularly enriched with γ- and δ-tocopherol (9% α-, 1% β-, 24% δ-, and 62% γ-tocopherol). Nine healthy, multiparous Holstein cows (≥90 d in milk) were assigned to dietary control (Notoc; n = 4) or tocopherol-fed treatment groups (n = 5; TOC; ~260 g Tmix/cow × d−1, top-dressed) and fed for 9 consecutive days. On d 10 of feeding, tissues were harvested at slaughter and mitochondria were isolated. Tocopherol isoform concentrations were determined by HPLC and data were analyzed as a complete randomized design. Significance was declared at P ≤ 0.05. Concentrations of γ-tocopherol increased (0.01 vs. 0.07 µg/g) in liver mitochondria from TOC cows compared with Notoc cows. In whole tissues and mitochondria, the α-isoform concentrations were higher than the γ-isoform. The accumulated portions of the α-isoform to the γ-isoform were similar for mitochondria and whole tissues regardless of tissue source differences. Regardless of tocopherol isoform or sample source (i.e., whole tissue vs. mitochondria), the liver accumulated higher total tocopherol concentration when compared with the mammary gland (8.2 vs. 2.7 µg/g, respectively). Limited concentrations of β- and δ-isoform were detected in whole tissues and mitochondria. In conclusion, the liver had higher tocopherol (α- and γ-isoforms) concentrations than the mammary gland suggesting that the liver may be preferred over the mammary gland for tocopherol accumulation. The α-isoform accumulated at higher concentrations than the γ-isoform in liver perhaps due to a higher affinity of tocopherol transport and binding proteins for α-tocopherol when compared with the γ-isoform.

Key Words: mitochondria, tocopherol, bioaccumulation

M72 Exploring lameness across a lactation through the eyes of a fatty pad. C. Stambuk*, H. Huson, and R. Bicalho, Cornell University, Ithaca, NY.

Lameness is a major animal welfare and economic issue for the dairy industry and is a challenge to overcome due to its multifaceted causes. Digital cushion thickness (DCT) is a strong predictor of lameness and is phenotypically associated with incidence of claw horn disruption lesions. The digital cushion is a complex structure composed of adipose and loose connective tissue located between the distal phalanx and the sole. It is important in dampening compression of the corium tissue beneath the cushion. The objective of this study was to characterize the change in DCT within the animal across lactation. Body condition score (BCS), visual locomotion score (VLS), DCT, and presence or absence of lesions were collected at 4 sample events: <40 d prepartum, 1 to 30 d in milk (DIM), 90 to 120 DIM, and ≥255 DIM for 124 commercial Holstein cows. Cow height was measured at the beginning and end of the study. Cows underwent digital sonographic examination for the measurement of DCT evaluated at the typical sole ulcer site for the right front and hind foot. Factors such as parity number and stage in lactation were obtained from the farm’s dairy management software (DairyCOMP 305). The prevalence of lameness (VLS ≥3) and lesions was greater in parity greater than 1 animals than parity equal to 1 animals. To evaluate the associations with DCT, a mixed linear model was built using MIXED procedure in SAS software. Compared with tall cows, DCT was significantly different by height; thinner for short cows and thicker for average cows. Those that are lame (VLS ≥3) and of average BCS group have significantly thicker digital cushions than those that are lame and of fat BCS group. Among fat BCS group animals, lame cows had significantly thinner digital cushions than cows that were not lame. Those with a lesion at 90 to 120 DIM had the thinnest digital cushion. The hind medial claw was the thinnest claw compared with the other claws. The average DCT of the measured claws at each sample event for parity greater than 1 appears to follow the BCS curve. The results indicate there is not a specific threshold of DCT where a dairy cow becomes lame or incurs a lesion.

Key Words: digital cushion, lameness, lesion

M73 Uterine microbiome, antibiotic resistance genes and virulence factors of metritic treated cows that cure or failed to cure from metritis. Z. Zhou*, M. S. Gomes, I. F. Canisso, E. F. Garrett, J. S. Stewart, and F. S. Lima, University of Illinois, Champaign-Urbana, IL.

Metritis is major postpartum disease in dairy cows causing reduced milk production, impaired fertility, and substantial economic losses. Although treatment with β-lactam antibiotics is the main therapeutic option for treating cows with metritis, ~35% of cows fail to respond
to treatment. Herein, we used whole-genome shotgun sequencing (WGS) to shed light of uterine microbiome, antimicrobial resistance genes (ARGs), and virulence factors genes (VFGs) profiles of cows that cured or failed to cure of metritis after treatment with ceftiofur or ampicillin. Uterine swabs were collected for each cow at the time of metritis diagnosis (D1) and 5 d later (D6) one day after treatments finished. Half of the cows (12/24) cured after the 5-d treatment (7 from ampicillin and 5 from ceftiofur). Our WGS revealed that over time (from d1 to d6) the mean relative abundance (MRA) of the genera Bacteroides, Prevotella, Alistipes, Fusobacteria, and Tannerella were reduced ($P < 0.01$), whereas Porphyromonas was increased ($P < 0.01$) independent of treatment ($P > 0.05$). For cows responding to treatment for metritis, only Streptococcus MRA was increased when compared with counterparts that did not cure of metritis. Beta-diversity decreased ($P < 0.01$) after treatment independent of treatment type ($P > 0.05$) and cure status ($P > 0.05$). Antibiotic treatment independent of type decreased VFGs abundance ($P < 0.01$), but increased ARGs ($P < 0.01$) abundance. Tetracycline resistance genes dominated the resistome of metritic cows, but β-lactam ARGs such as CMY-2 were not changed by treatment ($P > 0.05$) or time ($P > 0.05$). The ARGs TetT and TetW increased over time ($P < 0.01$) independent of treatment ($P > 0.05$) or cure status ($P > 0.05$). A higher MRA and presence of virulence factors for Streptococcus spp., Mycoplasma pneumoniae, and Vibrio cholerae were identified suggesting these bacteria and VFGs may be linked to metritis pathogenesis. In conclusion, antimicrobial treatment over time (from D1 to D6) independent of type and ability to cure metritis altered uterine microbiome, reduced VFG abundance and increased ARGs abundance.

**Key Words:** microbiome shift, metritis, metagenomics

M75 Economic comparison of ampicillin trihydrate and ceftiofur hydrochloride for treating metritis in dairy cows: A prospective cohort study. J. A. Snodgrass1, A. Vieira-Neto2, R. S. Bisinotto2, E. S. Ribeiro1, N. Martinez4, K. N. Galvao2, J. E. P. Santos1, and F. S. Lima*, 1University of Illinois, Champaign-Urbana, IL, 2University of Florida, Gainesville, FL, 3University of Guelph, Guelph, ON, Canada, 4Zoetis, Kalamazoo, MI.

Metritis is one of most prevalent and economic detrimental postpartum health disorders in dairy cows. However, there is lack of controlled prospective cohort studies evaluating its economic impact for dairy cows. The objective of this study was to perform an economic comparison of metritic treated cows using data from a previous prospective controlled cohort study that compared the efficacy of ampicillin trihydrate and ceftiofur hydrochloride. We hypothesized that an economic analysis considering differences in costs of antibiotics, labor, and feed, mean time to pregnancy, and milk production would determine the least costly treatment strategy for metritis. Cows diagnosed with metritis were blocked by parity and within each block allocated randomly to receive either ampicillin (n = 259), or ceftiofur (n = 269). A control group of cows without metritis matching parity and days in milk was also enrolled (n = 268) to be used as a baseline for comparison. Data on cows sold or dead, days open, and milk production (305 d) were used along with drug and commodity prices to create a per case cost of metritis. Mean time to pregnancy was analyzed using PROC PHREG and LIFETEST on SAS 9.4. Percent of dead or sold, feeding cost, milk production, and final cost analysis (with and without the value of feeding withdrawal milk to calves) were performed using PROC GLIMMIX. Cows without metritis had reduced mean time to pregnancy, reduced feeding cost, and increased milk production ($P < 0.01$) than counterpart diagnosed with metritis and these differences were accounted in the final cost analyses. There were no differences among treatments for mean time to pregnancy, percent of sold or dead, and feed costs ($P > 0.05$). Milk production tended to be greater ($P = 0.07$) in cows treated for ampicillin (9,078 kg) than cows treated with ceftiofur (8,732 kg). The final per case for treating metritis was higher ($P < 0.001$) than counterpart treated with metritic cows using data from a previous prospective controlled cohort study. The final cost per case for treating metritis was higher ($P < 0.001$) for cows treated with ceftiofur ($387.63$) than for ampicillin, either feeding milk from withdrawal period to calves ($294.83$ or not ($328.70)), indicating that ampicillin was the least costly treatment for metritis.

**Key Words:** ampicillin, ceftiofur, metritis

M76 Associations of gait score, lying behavior, hygiene, and body condition score between dairy cows with low and high somatic cell counts. A. Zambelis*, I. Robles, and T. J. DeVries, Dept. of Animal Biosciences, University of Guelph, Guelph, ON, Canada.

The objective of this study was to examine associations of gait score, lying behavior, hygiene, and body condition score (BCS) between cows with low and high SCC. Cows from 14 commercial free-stall dairy herds were enrolled in a cross-sectional study. Enrollment of herds was based on monthly participation in DHI milk testing. Each farm was visited for a total of 3 observation periods (at ~5-wk intervals) on 2 occasions
per period (7 d apart) until 3 DHI milk tests had been completed. Upon immediate receiving of the results of the DHI test, lactating Holstein calves were selected according to SCC. Cows with the highest 10% SCC in the herd (≥200,000 cells/mL) were first selected and matched for parity and DIM to cows with low SCC (≤100,000 cells/mL). Lying behavior was recorded for 6 d after each milk sampling using data loggers. On the visit where data loggers were attached, cows were scored for gait (1 = sound to 5 = lame) and hygiene of udder, lower legs, and upper legs/flank (1 = clean to 4 = dirty). On the visit where data loggers were removed 7 d later, BCS (1 = thin to 5 = fat) and hygiene were scored. Cows were then classified into each of the scoring categories for hygiene (dirty: ≤ 2, clean: ≥ 3), BCS (high: ≥ 4, normal: 3–3.5, low: ≤ 2.5), and gait (sound: ≤ 2, lame: ≥ 3). Association of cows being high (n = 352) and low (n = 362) in SCC with lying behavior, BCS, gait score, and hygiene score were tested in mixed-effect linear and logistic regression models. As compared with normal BCS cows, low BCS cows were found to be at greater odds of having a high SCC (OR = 1.57, 95% CI = 1.00–2.47, P = 0.049). As compared with normal BCS cows, low BCS cows were at a higher odds of having dirty lower legs (OR = 2.64, 95% CI = 1.08–6.46, P = 0.03), spent less time lying down (−27.2 ± 12.5 min/d, P = 0.03), and produced more milk (+2.90 ± 0.88 kg/d, P < 0.01). On average, cows with high SCC produced 2.2 ± 0.72 kg/d less milk (P < 0.01) than those with low SCC. These results suggest that cows with low BCS, which were at greater risk of having high SCC, were also the highest producing, had poorest lower leg hygiene, and spent the most time standing.

Key Words: mastitis, behavior, body condition


The effect of an accelerated-growth feeding protocol (AGFP), based on pasteurized waste milk, was evaluated in Holstein dairy calves under tropical conditions. Sixteen individually housed Holstein calves were used in the study. The control and AGFP groups consisted of 8 Holsteins (4 males and 4 females). The experimental period lasted 7 weeks. The control group was always fed 2 L of milk at 0600 h and 2 L of milk at 1800 h. The AGFP group was fed progressively more milk (divided equivalently in 2 feedings at 0600 h and 1800 h) with 4.0, 4.74, 5.68, 6.62, and 7.56 L from week 1 through 5, respectively. Then at wk 6, milk was reduced to 5.68 L and to 3.32 L at wk 7. Both groups were fed the same amount of calf starter that contained 18% CP; 2.5% fat; 8% CF; 14% ADF; 1.5% Ca; 0.5% P; 0.20 ppm Se; 2,273 IU/kg vitamin A; and 66 g/Tm of Lasalocid. The amount calf starter offered was progressively increased (divided equivalently in 2 feedings at 0600 h and 1800 h) with 0.23 kg (wk 1 and 2), 0.45 kg (wk 3), 0.68 kg (wk 4) and 0.91 kg (from wk 5 through 7). There was a treatment by sex interaction (P = 0.051). Although total average weight gain (wk 1 through 7) in females was not different (P = 0.999), in male calves total average weight gain was 14.25 vs 27.5 kg, for the control and AGFP groups, respectively (P = 0.048). Because all animals were exposed to identical experimental conditions, it is unclear why only male Holstein calves responded to the AGFP and further research is granted.

Key Words: accelerated growth, dairy calf, restricted growth


The primary goal of this project was to evaluate how pre-weaned calf housing, environment and management strategies impact calf health during periods of cold stress. This was an observational study in which calf facilities were evaluated on a single visit conducted between November 29, 2016, and January 4, 2017. Housing included hutches (n = 8), individual pens in a barn (n = 8) and group pens in a barn (n = 11). Facility, calf pen and animal evaluations included: wind speed, temperature, relative humidity, heat stress index, wind chill, bedding type, ammonia concentration, nesting score, calf health scoring, and number of calves/pen. Data were analyzed using SAS 9.3 to determine the impact of housing type, environmental and management variables on calf health score. A total of 27 facilities and 426 pre-weaned calves were evaluated. The mean outdoor temperature was 6.3°C (SD = 5.6; range −5.2 to 19.4). Mean respiratory score was 2.8 (SD = 1.64; range 0 to 9) with 14.5% of calves evaluated scoring >5, indicating they have a respiratory challenge and should be treated. Prevalence of respiratory illness among calves ranged from 0 to 46% on a farm basis (mean = 15.0%), with 8 farms having no respiratory illness, and 6 farms having 30 to 46% of evaluated calves exhibiting signs of respiratory illness. Health score was affected (P < 0.05) by housing, bedding, number of calves per pen, NH₃ concentration, temperature and wind chill (at calf level). Calves housed in hutches had greater (worse) health scores as compared with those in group pens (3.9 vs 2.2, respectively). Calves in individual pens did not differ in health scores from their counterparts (mean health score = 3.3). Risk of health score >5 increased if calves had a body condition score <2 (relative risk = 1.7; 95% CI 1.0, 2.8), if ammonia concentration in the pen was >1 PPM (relative risk = 1.9; 95% CI 1.2, 3.0) and if calves/pen was >5 (relative risk = 1.6; 95% CI 1.0, 2.6). Data collected from this study suggests that respiratory illness continues to be a challenge. However, factors that increased the risk of respiratory illness can be addressed by changes in management practices.

Key Words: calves, respiratory, housing

M79 Effects of the addition of electrolyzed water to a footbath solution on digital dermatitis incidence. H. K. Himmelmann*, B. W. Jones, and J. M. Bewley, University of Kentucky, Lexington, KY.

Digital dermatitis (DD) can cause lameness and pain in dairy cows. The objective of this 11-wk study, conducted at the University of Kentucky Coldstream Dairy Research Farm, was to test the effects of electrolyzed water, in a copper sulfate solution on DD. A split, plastic footbath was used to deliver 2 footbath solutions. The control solution, assigned to the left hooves of the cow, contained 79.5 L of water with 1.75 kg of copper sulfate, and 325 mL of acidifier. The treatment solution, assigned to the right hooves of the cows, contained the same solution as the control side with the addition of 7.5 L of electrolyzed water. The footbath solutions were made Monday thru Friday before morning milkings. Cows walked through the footbath while exiting the milking parlor once a day. The solutions were dumped after the completion of morning milkings. Holstein cows (n = 77) DD were scored biweekly in the milking parlor to determine active or inactive DD. Rear hooves were hosed off to remove debris before being evaluated. A headlamp was worn to provide clarity of hooves while scoring. The FREQ Procedure of SAS (SAS Institute Inc., Cary, NC) was used for a chi-squared analysis and a McNemar’s test was used to compare the number of hooves with active DD (scores
of M1 and M2) to the number of hooves with non-active DD (scores of M3 and M4). No significant differences in DD between the control and treatment groups existed ($P > 0.05$); however, over the course of the study, both footbath solutions improved DD overall (Table 1; $P < 0.01$). These results suggest that the addition of electrolyzed water in a footbath solution had no negative effect on DD.

Table 1 (abstract M79).

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**Key Words:** digital dermatitis, footbath, copper sulfate

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**M80 Management practices and prevalence of bovine respiratory disease in pre-weaned dairy calves in California.** B. M. Karle*1, G. Maier2, S. A. Dubrovsky3, W. J. Love4, D. R. Williams2, J. W. Stackhouse5, R. J. Anderson4, A. L. Van Eenennaam4, T. W. Lehenbauer6, and S. S. A. Aly7, 8, 9.1University of California Cooperative Extension, Orland, CA, 2UC Davis Veterinary Medicine Teaching and Research Center, Tulare, CA, 3Department of Animal Science, University of California, Davis, CA, 4University of California Cooperative Extension, Eureka, CA, 5California Department of Food and Agriculture, Animal Health Branch, Sacramento, CA, 6Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA.

The objective of this cross-sectional study was to estimate the prevalence of bovine respiratory disease (BRD) in California pre-weaned dairy calves and identify management practices that may be associated with BRD and their variations across the state. A convenience sample of 104 dairy herds in the 3 distinct dairy regions of CA were surveyed. Regions evaluated were Northern (NCA, San Francisco area and north, mean herd size 678, n = 33), Central (CCA, San Joaquin, Stanislaus, Merced counties, mean herd size 1,569, n = 36), and Greater Southern region (SCA, Fresno County and south, mean herd size 2,878, n = 35). A questionnaire on calf management practices and demographic information was administered via in-person interviews at each dairy and a random sample of pre-weaned calves evaluated using the CA BRD scoring system on the same day. Prevalence of BRD varied between the 3 dairy regions (NCA, 9.3% ± 0.89; CCA, 4.4% ± 0.70; SCA, 7.4% ± 0.92; $P = 0.005$). Calf breed was not associated with BRD prevalence at the statewide level (Holsteins 7.3% ± 0.82, Jerseys 5.4% ± 0.69, other and cross breeds 5.7% ± 2.68; $P = 0.4$). Differences in prevalence were observed between breeds across the regions with a higher prevalence in NCA for Jerseys (15.0% ± 1.83 NCA, 2.8% ± 1.01 CCA, 3.4% ± 0.96 SCA; $P < 0.001$) and in SCA for Holsteins (8.0% ± 1.1 in SCA, 4.7% ± 0.84 in CCA; $P = 0.045$) but not compared with NCA (5.9% ± 0.12; $P = 1.00$). Prevalence of BRD was 7.8% ± 1.0 in calves raised on organic dairies and 6.9% ± 0.71 on conventional dairies ($P = 0.4$). Group housed pre-weaned calves had a higher BRD prevalence than those individually housed for ages 21–40 d (group 14.36% ± 4.34, individual 4.8% ± 1.13; $P = 0.007$) and ages > 60 d (group 16.8% ± 2.62, individual 9.2% ± 1.64; $P = 0.015$). Proportion of dairies feeding pasteurized milk to calves varied by herd size (<250 cows 17.8% ± < 0.01; 250–999 cows 44.3% ± 0.11; 1,000–3,999 cows 86.3% ± 0.32; > 4,000 cows 100%; $P < 0.001$) and organic status (organic 27.6% ± 0.73; conventional 81.1% ± 0.18; $P < 0.001$). Management practices varied greatly across the state, likely contributing to the variation in BRD prevalence seen in the 3 regional evaluations.

**Key Words:** bovine respiratory disease, calf, pre-weaned

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**M81 Effect of calving stress on feed intake of dairy cows soon after calving.** M. R. Reshalaitihan*1, K. Matsuki2, T. Sato2, and M. Hanada3, 1United Graduate School of Agricultural Science, Iwate University, Morioka, Iwate, Japan, 2Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.

Several studies reported that dry matter intake (DMI) after calving was lower and the degree of calving difficulty was higher in primiparous cows compared with multiparous cows. This study was done to compare calving stress around calving between primiparous and multiparous cows and to investigate the effect of the stress on DMI in dairy cows soon after calving. Fifteen primiparous and 15 multiparous Holstein cows were used. The cows were offered a total mixed ration (TMR) restrictedly (80% of energy requirements) and hay ad-libitum before calving and were offered another TMR and hay ad-libitum after calving. DMI was measured from 1 to 6 d after calving. Blood was taken at −33, 0.5, 3 and 7 d after calving to measure metabolites. Urine was collected at −11, −8, −4, 0.25, 4, 8 and 13 d after calving to measure cortisol concentration. BW was measured once a week and milk yield was measured every day after calving. One-way ANOVA followed by LSD multiple comparisons tests were used to compare individual parameters among the groups. Average DMI for 6 d after calving was lower in primiparous cows (88 g/BW0.75/d) than in multiparous cows (112 g/BW0.75/d; $P < 0.01$). Highest urinary cortisol was observed at 0.25 d after calving in both cows and there was no significant difference between primiparous and multiparous cows ($P > 0.10$). However, urinary cortisol was higher in primiparous cows than in multiparous cows at 4 d after calving ($P < 0.01$). Pearson correlations were performed to investigate the relationship between the parameters. Average DMI for 6 d after calving was negatively related to the urinary cortisol at 4 d after calving ($P < 0.01$) and positively related to average milk yield for 6 d after calving ($P < 0.01$) and serum Ca at 3 d after calving ($P < 0.01$). To identify the effect of these 3 factors on the DMI, a multiple regression analysis was performed and a significant multiple regression equation was obtained ($r^2 = 0.67$, $P < 0.001$). The standardized partial regression coefficients of the equation were –0.43 for the urinary cortisol, 0.52 for the milk yield and 0.44 for the serum Ca.

**Key Words:** cortisol, intake, transition dairy cow

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**M82 Transgenerational effects of postpartum inflammatory diseases in dairy cows.** M. R. Carvalho*1, F. Pehagaranico, J. E. Santos2, T. J. DeVries1, B. McBride1, and E. S. Ribeiro1, 1Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, 2Department of Animal Sciences, University of Florida, Gainesville, FL.

Inflammatory diseases postpartum have long-lasting effects on reproduction of dairy cows and increase substantially the likelihood of pregnancy losses. The objective of this study was to investigate whether the lasting effects of inflammatory diseases extends into postnatal life in pregnancies that survive until term. Incidence of diseases (metritis, mastitis, lameness, respiratory and digestive problems) in 5,085 cows from a single herd in FL was recorded from calving until first breeding postpartum. Cows that became pregnant after first breeding were followed until calving. Born female calves were then followed up to 305
d in milk of their first lactation, and data related to morbidity, mortality, culling, reproduction and milk production were recorded. Data were analyzed by logistic regression or ANOVA using PROC GLIMMIX of SAS according to data distribution. A total of 1,211 cows calved from the first breeding. Out of those, 872 cows did not have any diseases postpartum in the previous lactation (H-DAM) and 339 cows had at least one disease postpartum in the previous lactation (D-DAM). Out of the 339 D-DAM, 300 had a single disease (SD-DAM) and 39 had multiple diseases (MD-DAM). The proportion of female calves born did not differ among groups and averaged 51.9%. Incidence of dystocia was greater in D-DAM compared with H-DAM (39.8 vs 30.2%; \( P < 0.01 \)). Rate of morbidity, mortality, and culling before and after first calving, age at first AI, pregnancy after first AI, age at first calving, and milk production in the first lactation did not differ between heifers born from H-DAM and those born from D-DAM. Nonetheless, the incidence of diseases before first calving was smaller for MD-DAM heifers compared with SD-DAM and H-DAM heifers (26.3 vs 62.2 vs 57.4%; \( P = 0.04 \)). The rate of morbidity was also lesser for MD-DAM compared with H-DAM (hazard ratio = 0.35; \( P = 0.01 \)) and S-DAM (hazard ratio = 0.34; \( P = 0.02 \)). The rate of morbidity was also lesser for MD-DAM compared with H-DAM (hazard ratio = 0.35; \( P = 0.01 \)) and S-DAM (hazard ratio = 0.34; \( P = 0.02 \)). The results indicate that transgenerational effects of postpartum inflammatory diseases were only present when multiple cases occurred and resulted in reduced susceptibility to diseases in heifers, but no differences in performance.

Key Words: inflammation, transgenerational effects, heifer

**M83** Colostrum mineral concentrations and their association with calcemic status at calving in Jersey cows. J. Chiozza-Logroño\(^*1\), A. Valdecabres\(^1\), A. Lago\(^3\), and N. Silva-del-Rio\(^1\), \(^1\)Veterinary Medicine Teaching and Research Center, University of California Davis, Tulare, CA, \(^2\)DairyExperts Inc., Tulare, CA.

The aim of the present study was to evaluate the association of postpartum calcemic status and colostrum concentration of Ca, P, Mg, K, Na, Fe, Zn and Cu on 131 multiparous Jersey cows. Colostrum samples were harvested at 9 h 36 min (±3 h 36 min) after calving and analyzed for mineral concentration by inductively coupled plasma–optical emission spectrometry. Final colostrum weigh was recorded at milking. Blood samples for serum Ca analyses were collected from the coccygeal vessels within 6 h after calving. Based on serum Ca concentration, cows were classified as hypocalcemic (SHC; Ca ≤8.5 mg/dL; \( n = 103 \)) and normocalcemic (NC; Ca >8.5 mg/dL; \( n = 28 \)). Descriptive statistics, including first (Q\(_1\)) second (Q\(_2\)) and third (Q\(_3\)) quartiles of colostrum mineral concentrations based on calcemic status at calving are shown in Table 1. Associations among calcemic status were analyzed using mixed models with MIXED procedures of SAS. There was a tendency (\( P = 0.07 \)) for higher colostrum weight on SCH cows (4.2 kg) than NC cows (3.2 kg). Cows with SCH had higher colostrum P concentration (1400.13 vs. 1140.43 mg/kg; \( P < 0.01 \)) Mg (338.88 vs. 299.52. mg/kg; \( P < 0.05 \)), K (1494.87 vs. 1302.73 mg/kg; \( P < 0.01 \)) and Zn (18.54 vs. 15.25 mg/kg; \( P < 0.05 \)) than NC cows, but lower Na (822.19 vs. 1003.73 mg/kg; \( P < 0.05 \)). Cows with SCH had higher colostrum excretion P (\( P < 0.05 \)) and Mg (\( P < 0.05 \)) than NC cows. Our results show that calcemic status tends to affect colostrum yield and is associated with mineral concentration at calving.

**Key Words:** colostrum minerals, hypocalcemia, Jersey cow

**Table 1 (abstract M83).** Quartile distribution (Q\(_1\) = 25th percentile, Q\(_2\) = 50th percentile, Q\(_3\) = 75th percentile) of colostrum mineral concentrations (mg/kg) at first milking

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>P</th>
<th>Mg</th>
<th>K</th>
<th>Na</th>
<th>Fe</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHC ( Q_1 )</td>
<td>2,000</td>
<td>1,100</td>
<td>280</td>
<td>1,287</td>
<td>619</td>
<td>0.51</td>
<td>13</td>
<td>0.16</td>
</tr>
<tr>
<td>( Q_2 )</td>
<td>2,200</td>
<td>1,400</td>
<td>330</td>
<td>1,428</td>
<td>759</td>
<td>0.64</td>
<td>18</td>
<td>0.21</td>
</tr>
<tr>
<td>( Q_3 )</td>
<td>2,600</td>
<td>1,600</td>
<td>380</td>
<td>1,677</td>
<td>980</td>
<td>0.79</td>
<td>23</td>
<td>0.26</td>
</tr>
<tr>
<td>NC ( Q_1 )</td>
<td>1,600</td>
<td>730</td>
<td>230</td>
<td>936</td>
<td>763</td>
<td>0.66</td>
<td>8.4</td>
<td>0.18</td>
</tr>
<tr>
<td>( Q_2 )</td>
<td>2,100</td>
<td>1,100</td>
<td>280</td>
<td>1,248</td>
<td>913</td>
<td>0.73</td>
<td>12</td>
<td>0.21</td>
</tr>
<tr>
<td>( Q_3 )</td>
<td>2,500</td>
<td>1,500</td>
<td>340</td>
<td>1,638</td>
<td>1,276</td>
<td>0.87</td>
<td>22</td>
<td>0.30</td>
</tr>
</tbody>
</table>

**M84** Association of colostrum Ca concentration at first and second milking with postpartum serum Ca concentration. J. Chiozza-Logroño\(^*1\), A. Valdecabres\(^1\), R. Rearte\(^2\), A. Lago\(^3\), and N. Silva-del-Rio\(^1\), \(^1\)Veterinary Medicine Teaching and Research Center, University of California Davis, Tulare, CA, \(^2\)Cátedra de Higiene, Epidemiología y Salud Pública, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (FCV–UNLP), La Plata, Argentina, \(^3\)DairyExperts Inc., Tulare, CA.

The objective of this study was to evaluate if the concentration or the amount of Ca excreted in colostrum harvested at first and second milking was associated with postpartum serum Ca concentration on multiparous Jersey cows. Colostrum samples and weights were collected at first (\( n = 134 \)) and second (\( n = 68 \)) milking at 9 h 36 min (±3 h 36 min) and 21 h 21 min (±3 h 14 min) relative to calving, respectively. Colostrum samples from first and second milkings were analyzed for Ca concentration (CC\(_{ac}\)). Blood samples for serum Ca concentration analyses were collected from coccygeal vessels within 6 h after calving before first milking (SC\(_{a1}\)), and 43 min (±28min) after second milking (SC\(_{a2}\)). Total Ca excreted in colostrum (CC\(_{ac}\)) was calculated as CC\(_{ac}\) × colostrum weight. The CORR procedure of SAS was used to evaluate the association among SC\(_{a1}\), SC\(_{a2}\), CC\(_{ac}\) and CC\(_{ag}\). To study explanatory variables of SC\(_{a2}\) a linear regression model with repeated measurements was fitted using the MIXED procedure of SAS including CC\(_{ac}\), milking time, and colostrum weight. There was not an association between SC\(_{a1}\) and SC\(_{a2}\) (\( r = 0.23 \); \( P = 0.06 \)), CC\(_{ac1}\) (\( r = 0.09 \); \( P = 0.45 \)), or CC\(_{ac2}\) (\( r = -0.05 \); \( P = 0.53 \)); nevertheless, SC\(_{a2}\) was associated with CC\(_{ag1}\) (\( r = -0.32 \); \( P = 0.007 \)), but not with CC\(_{ag2}\) (\( r = 0.02 \); \( P = 0.85 \)). At second milking, we observed a decrease in CC\(_{ag2}\) as CC\(_{ac}\) increased. No effect of colostrum weight was detected on SC\(_{a2}\). Our results indicate that postpartum calcemic status might be affected by concentration of Ca in colostrum.

**Key Words:** hypocalcemia, colostrum, Jersey cow
High-producing dairy herds where there is a predominant utilization of high-concentrate low-fiber diets can impair the buffering capacity of the rumen in dairy cows, and lead to a subacute ruminal acidosis (SARA). SARA is characterized by ruminal pH < 5.6 for extended hours. Decreased milk yield and milk efficiency, rumen epithelial damage, and laminitis are among several consequences of SARA. This study aimed to investigate the physiological adaptations during induced SARA in lactating Holstein cows. Eighteen cannulated cows were classified based on a retrospective analysis of pH after SARA induction, cows were grouped as non-SARA (n = 12) or SARA (n = 6) if ruminal pH was < 5.6 for ~4 h regardless of treatment. SARA induction at d 5 (challenge) of trial was performed by giving a wheat/barley pellet at 10% of previous day DMI. Blood samples were collected on d 2, 5, 6, 7, and on 8 of trial, and on d 5 at 0, 3, 6, and 12 h relative to the challenge. Data were analyzed using the PROC MIXED procedure of SAS, where group, hour, and day were fixed effects, while cow nested within group was the random effect. An interaction of Group × day (P = 0.01) was observed for haptoglobin with SARA group having greater concentration (0.23 vs 1.16 g/L) of this blood inflammatory biomarker on d 8 after challenge. Blood concentrations of creatinine and myeloperoxidase (P = 0.04) were also greater in the cows subjected to challenge. Similarly, an interaction of Group × hour (P = 0.04) was observed for blood creatinine where cows challenged had greater concentration 6 h after induction. Concentrations of BHB were greater (P < 0.01) during hours after SARA induction in SARA group. Overall, our results suggest that the metabolic and inflammatory profile in blood were more affected in cows induced to SARA regardless of treatment.

Key Words: SARA, immunometabolic profile, lactating cow

M85 Metabolic and inflammatory changes in blood of lactating Holstein cows induced to subacute ruminal acidosis. F. Rosa*, 1J. C. McCann2, E. Trevisi3, F. Cardoso2, J. J. Loor2, and J. S. Osorio1, 1South Dakota State University, Brookings, SD, US, 2University of Illinois, Champaign-Urbana, IL, US, 3Università Cattolica del Sacro Cuore, Piacenza, Italy.

Retained placenta (RP), a condition that fetal membranes failed to expel before calving and directly after calving serum samples in healthy cows were grouped as non-RP (n = 12) or RP (n = 6) if ruminal pH < 5.6 for extended hours. Decreased milk yield and milk efficiency, rumen epithelial damage, and laminitis are among several consequences of SARA. This study aimed to investigate the physiological adaptations during induced SARA in lactating Holstein cows. Eighteen cannulated cows were classified based on a retrospective analysis of pH after SARA induction, cows were grouped as non-SARA (n = 12) or SARA (n = 6) if ruminal pH was < 5.6 for ~4 h regardless of treatment. SARA induction at d 5 (challenge) of trial was performed by giving a wheat/barley pellet at 10% of previous day DMI. Blood samples were collected on d 2, 5, 6, 7, and on 8 of trial, and on d 5 at 0, 3, 6, and 12 h relative to the challenge. Data were analyzed using the PROC MIXED procedure of SAS, where group, hour, and day were fixed effects, while cow nested within group was the random effect. An interaction of Group × day (P = 0.01) was observed for haptoglobin with SARA group having greater concentration (0.23 vs 1.16 g/L) of this blood inflammatory biomarker on d 8 after challenge. Blood concentrations of creatinine and myeloperoxidase (P = 0.04) were also greater in the cows subjected to challenge. Similarly, an interaction of Group × hour (P = 0.04) was observed for blood creatinine where cows challenged had greater concentration 6 h after induction. Concentrations of BHB were greater (P < 0.01) during hours after SARA induction in SARA group. Overall, our results suggest that the metabolic and inflammatory profile in blood were more affected in cows induced to SARA regardless of treatment.

Key Words: SARA, immunometabolic profile, lactating cow

M86 Liquid chromatography-mass spectrometry metabolomic serum signatures indicate global and disease-specific challenges in protein and fatty acid metabolism precede retained placenta in dairy cows. F. Zandkarimi, C. Maier, and G. Bobe*, Oregon State University, Corvallis, OR.

Retained placenta (RP), a condition that fetal membranes failed to expel within 24 h of calving, is one of the most prevalent and costly diseases in dairy cows. Our objective was to discover molecular biomarkers and pathways, which can aid in screening, prediction and/or early diagnosis of RP. Using a nested case-control design, we collected 21, 14, 7, and 8 of trial, and on d 5 at 0, 3, 6, and 12 h relative to the challenge. Data were analyzed using the PROC MIXED procedure of SAS, where group, hour, and day were fixed effects, while cow nested within group was the random effect. An interaction of Group × day (P = 0.01) was observed for haptoglobin with SARA group having greater concentration (0.23 vs 1.16 g/L) of this blood inflammatory biomarker on d 8 after challenge. Blood concentrations of creatinine and myeloperoxidase (P = 0.04) were also greater in the cows subjected to challenge. Similarly, an interaction of Group × hour (P = 0.04) was observed for blood creatinine where cows challenged had greater concentration 6 h after induction. Concentrations of BHB were greater (P < 0.01) during hours after SARA induction in SARA group. Overall, our results suggest that the metabolic and inflammatory profile in blood were more affected in cows induced to SARA regardless of treatment.

Key Words: SARA, immunometabolic profile, lactating cow

M87 Polyphenolic extract from cowpea (Vigna unguiculata) modulates galectin 3 and 9 expression in bovine peripheral blood. S. Adjei-Fremah*, E. Asiamah, K. Ekewemator, and M. Worku, North Carolina A&T State University, Greensboro, NC.

Galectins are animal lectins that bind glycoconjugates containing β-galactose proteins. Galectin (gal)-3 and gal-9 expressions have been associated with innate and adaptive immunity, and inflammation response. Plant-derived polyphenols found in feed have a beneficial effect on animal health. The objective of the study was to determine the effect of polyphenol extract from cowpea (CPE) on gal-3 and gal-9 mRNA levels and protein expression in bovine peripheral blood. Blood collected from Holstein cows (n = 10) was treated with 10μg of CPE, controls were treated with either 100 ng/mL of lipopolysaccharide (LPS), or left untreated. Total RNA was extracted after treatment; reverse transcribed to cDNA and real-time PCR was performed using specific primers for bovine gal-3 and gal-9. Real-time PCR data analysis normalization was performed with the housekeeping gene GAPDH and fold change (FC) in gene expression was calculated using the 2−ΔΔCT method. Plasma concentration of gal-3 and gal-9 was measured using a commercial ELISA. One-way ANOVA was performed on gal_3 and gal_9 concentration data, a P-value of < 0.05 was considered significant. Results from the RT-PCR showed increased mRNA levels of gal_3 (FC = 48.90) and Gal_9 (FC = 147.41) after LPS treatment relative to the untreated controls. In the CPE-treated samples, the relative gene expression of gal-3 and gal-9 were 1.72 and 2.07 respectively. Also, the concentrations of gal-3 and 9 in plasma increased in LPS-treated samples but decreased in the CPE-treated group relative to the untreated control. The results from this study showed that polyphenols from cowpea modulate gal-3 and gal-9 mRNA and protein expression in bovine blood. Hence, the use of polyphenols extract from cowpea enriched feed supplements may have immunomodulatory properties for animal health, and aid in the design of galectin-based strategies to counteract LPS induced inflammation and morbidities.

Key Words: cowpea, galectin, polyphenol

M88 A statewide survey of colostrum management practices on organic dairy farms in Ohio. L. da Costa*1 and K. Bohland2, 1Department of Preventive Medicine, The Ohio State University, Columbus, OH, 2The Ohio State University, Columbus, OH.

Newborn calf survival is an important indicator of farm success. In order for calves to fully develop, ingestion of high quality colostrum is essential during the first hours of life. Proper intake of quality colostrum can determine the future health and milking performance of the calf. The aim of this study was to survey Ohio organic dairy farms regarding practices related to administration and management of colostrum. The survey was done by phone interviews based on questions of a questionnaire previ-
ous validated lasting approximately 10 min. Farm size were distributed as 68% between 30 to 50 cows, 26% between 51 to 100 animals, and the remaining 6% 101 or more cows. The range of farm size varies between 30 and 650 cows. We received response from 43 producers and 100% of them stated that they fed colostrum to their calves. A small percentage (6%) of producers indicated that calves stayed with their mothers in the first 12 h. The time that most calves receive colostrum varied between 1 and 3 h to 12–24 h. Over 90% of producers understand the importance of feeding colostrum as early as possible, with 16% offering within 1 h of birth, almost 40% giving it between 1 to 3 h and 32% between 3 to 6 h. Only 10% of producers offer colostrum to their calves between 6 to 12 h and 2.6% between 12 to 24 h after birth. Half of responders let calves drink colostrum ad libitum. Almost 25% of producers fed 4 L (or 4 quarts; liters and quart were used interchangeably) following by 12% offering 3 L and 12% offering 2 L. Calves were weaned at an age older than 8 weeks of age in 44% of responding herds, at 8 weeks in 46% and between 6 to 7 weeks of age in 10%. Out of 43 responders only 2 farms tested colostrum quality routinely and one farm tested it weekly. The remaining 40 farms (93%) never tested it. Testing was performed routinely in those farms with more than 101 cows. Based on the results it is possible to note that testing colostrum quality is not a routine practice used by small organic producers in Ohio. Furthering education and training practices could be very impactful to improve management practices regarding colostrum.

Key Words: survey, colostrum, organic


This study aimed to determine hair cortisol concentrations (HCC) in various sampling sites on the body of lactating Holstein cows and heifers. Forty-seven multiparous lactating Holstein cows (DIM = 110 ± 47, BW = 623 ± 75 kg) and 23 Holstein heifers (10–12 mo of age, BW = 258 ± 20 kg) were used in this experiment. To obtain serum, blood was collected by jugular venipuncture in vacutainer tubes at 12:00 h at the d 0, after one month and after 2 mo. Hair sampling sites included forehead, withers and rump sites. Statistical analysis was carried out using the ANOVA procedure of SAS. The intra-assay and inter-assay coefficients of variations were 3.15 and 10.05, respectively. Serum cortisol showed high fluctuations between sampling times (d 0, 1 mo and 2 mo) whereas first sampling values were the highest in both lactating cows and heifers (P < 0.05). No differences were found in HCC among the various sampling sites in both lactating cows and heifers (P > 0.05). It is concluded that the distribution of cortisol into the hair shaft does not depend on hair sampling sites, and so hair sampling for cortisol analysis can be collected from any of the 3 regions based on the ease of collections and the location’s facilities.

Key Words: hair cortisol, Holstein cows and heifers, various body sites

M90 | Upregulation of nitric oxide synthases and natriuretic peptides in healthy controls compared with pulmonary arterial hypertensive Holstein heifers exposed to chronic hypobaric hypoxia. S. Wang1, Y. Wang2, S. Li1, D. Han2, Q. Shi3, and S. Ji4*, 1College of Animal Science and Technology, China Agricultural University, Beijing, China, 2College of Veterinary Medicine, China Agricultural University, Beijing, China, 3Clinical Laboratory of General Hospital of Tibet Military Command, Lhasa, China,

Nitric oxide and natriuretic peptides are endogenous vasodilator that protect against pulmonary hypertension progression. We compared nitric oxide synthase (NOS) and natriuretic peptides (NPs) expression levels in Holstein heifers with brisket disease and healthy controls located at Lhasa for one year. Physiological parameters, blood pressure and blood gas status were measured. Plasma samples were analyzed for brain NP, C-type NP, adrenomedullin, endothelial NOS (eNOS), inducible NOS (iNOS), total NOS (TNOS) and NOx levels (n = 10/group). We performed histological analyses to detect remodeling of small pulmonary arteries. RT-PCR and Western blots were used to determine lung eNOS and endothelin-1 (ET-1) expression. Respiratory rates, oxygen saturation and blood velocity were significant higher in healthy controls. However, heart rates were higher in heifers with brisket disease. Peripheral arterial pressures were significantly higher in healthy controls than those in cattle with brisket disease. In healthy cattle, plasma NPs, eNOS, iNOS, TNOS and NOx levels were elevated relative to those in cattle with brisket disease. Moreover, eNOS mRNA and protein were highly expressed in healthy control lungs (P < 0.01, P < 0.01, respectively). Immunostaining revealed that eNOS was highly expressed in the intima of pulmonary arterioles. In addition, ET-1 mRNA and protein levels were reduced in healthy cattle compared with those of cattle with brisket disease (P < 0.05, P < 0.01, respectively). Cattle with brisket disease displayed small pulmonary arterial adventitial thickening, proliferation of smooth muscle cells and low eNOS expression in the intima. In conclusion, it is possible that highly expressed NO and NPs dilate vasculature, maintain blood flow and pressure and attenuate vascular remodeling to protect against pulmonary hypertension progression.

Key Words: Holstein heifer, pulmonary hypertension, nitric oxide


Objectives were to determine effects of an injectable formulation of calcitriol on Ca concentration, risk of subclinical hypocalcemia, and health in dairy cows. Cows were blocked by lactation number (1 vs. ≥2) and calving sequence, and within each block, randomly assigned to receive, within 6 h of calving, subcutaneously vehicle only (Control, n = 450), 200 μg of calcitriol (Cal200, n = 450), or 300 μg (Cal300, n = 450). Blood samples were collected before treatment administration, and on d 1, 2, 3, and 5. Samples were analyzed for blood ionized Ca, and total plasma Ca and Mg. Vaginal discharge (VD) was evaluated at 4, 6, and 8 DIM, and cows with VD reddish/brownish foul smell were diagnosed with metritis. Morbidity was evaluated until 60 DIM, and responses measured included metritis, mastitis, displaced abomasum, digestive and respiratory disorders. At 35 DIM, VD was scored for diagnosis of purulent vaginal discharge (PVD, VD > 2, mucopurulent discharge). Cyclicly was evaluated by presence of a corpus luteum (>20mm) in at least one of 2 ovarian ultrasound scans performed at 35 and 49 DIM. Data were analyzed using PROC MIXED and PROC GLIMMIX of SAS. Cows receiving calcitriol resulted in greater concentration of blood ionized Ca and plasma total Ca during the first 5 and 3 DIM, respectively, whereas concentration of plasma Mg were reduced during the first 3 DIM (Table 1). Treatment with calcitriol did not affect the incidence of metritis, puerperal metritis, morbidity by 60 DIM, PVD, and cyclicly (Table 1). Calcitriol treatment was effective
to improve Ca concentrations during the first 3 DIM, but was unable to improve health performance.

**Key Words:** calcitriol, hypocalcemia, transition period

**M92 Comparison of ionized calcium concentrations using an Abaxis Vetscan iSTAT with a Horiba LAQUAtwin ionized calcium meter in dairy cows fed DCAD rations with low, medium, or high concentrations of calcium and challenged with EGTA.** A. P. Prichard¹, C. E. Wimmler¹, L. A. Amunson¹, A. Cheng¹, S. R. Weaver¹, P. M. Crump¹, A. D. Rowson², S. S. Bascomb², D. E. Nuzback², K. P. Zanzalari², and L. L. Hernandez¹, ¹University of Wisconsin-Madison, Madison, WI, ²Phibro Animal Health Corporation, Teaneck, NJ.

The gold standard for assessing hypocalcemia is through measurement of blood ionized calcium (iCa) concentration. We assessed the use of a Horiba LAQUAtwin ionized Ca meter in comparison to the Abaxis Vetscan iSTAT as cow-side tools to measure iCa. Three groups of non-lactating, non-pregnant Holstein cows were fed negative DCAD rations with low, medium or high concentrations of total dietary Ca and then subjected to a controlled induction of hypocalcemia. Low Ca cows (n = 5) were fed 0.45% Ca, medium Ca cows (n = 6) were fed 1.13% Ca, and high Ca cows (n = 6) were fed 2.02% Ca. Average DCAD was -15.1 meq/100g DM for all cows resulting in urine pH values below 6.0. All cows were fed for 21 d before hypocalcemia was induced using intravenous infusion of 5% ethylene glycol tetraacetic acid (EGTA), a selective iCa chelator. Blood samples were collected every 15 min throughout infusion and analyzed for iCa by the Horiba LAQUAtwin and Abaxis Vetscan iSTAT until 60% of preinfusion iCa was achieved. Blood samples were taken post-infusion at 0, 2, 5, 10, 15, 30, and every 30 min thereafter and analyzed for iCa by both meters until 90% of preinfusion iCa was achieved. The LAQUAtwin was calibrated before every measurement, as this is critical to ensure proper function. We utilized a likelihood ratio to determine whether variance of the 2 groups were equal when measuring iCa concentrations during EGTA challenge and recovery period. Variance for the iSTAT was 0.02745, while variance for the Horiba meter was 0.14447. The chi-squared value was significant (P < 0.0001). These results indicate that the Horiba meter is more variable when measuring blood iCa concentration and produces values that are significantly higher than values obtained from the iSTAT (P < 0.001). The Horiba LAQUAtwin meter is less likely to accurately identify a cow with clinical or subclinical hypocalcemia than the Abaxis Vetscan iSTAT.

**Key Words:** ionized calcium, dairy cow, cow-side blood test

**M93 Effect of a single dose of an oral calcium bolus after parturition on plasma calcium concentration, milk production, and culling in Holstein dairy cows.** B. M. Leno¹, R. C. Neves², M. D. Curler³, M. J. Thomas³, T. R. Overton¹, and J. A. A. McArt³, ¹Department of Animal Science, Cornell University, Ithaca, NY, ²Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY, ³Dairy Health and Management Services LLC, Lowville, NY.

Our objectives were to determine (1) the effect of a single dose of an oral Ca bolus after parturition on blood Ca concentration, and (2) the effect of this supplemental Ca approach on milk production and culling. For our first objective, cows from 1 commercial dairy were enrolled within 19 h after parturition (mean ± SD = 8.3 ± 5.3 h) and randomized within parity group (1st, 2nd, and 3rd or greater) to control [CON (n = 25); no placebo] or bolus treatment [BOL (n = 25); 3 oral Ca boluses administered at once supplying 54 to 64 g of Ca (Quadrical, Biivet, Barneveld, WI)]. Blood samples were collected before group assignment and at 1, 2, 4, 8, 12, and 24 h thereafter. Plasma Ca concentration was analyzed by mixed effects repeated measures ANOVA. No preliminary associations between treatment and culling within 30 d in milk. Bolus administration did not affect mean plasma Ca concentration in the 24 h after bolus administration (CON = 2.00 ± 0.03 vs. BOL = 1.96 ± 0.03 mmol/L, P = 0.36). No preliminary associations between treatment and first test milk yield (CON = 38.1 ± 0.2 vs. BOL = 38.1 ± 0.2 kg/d, P = 0.88) or culling within 30 d in milk [CON = 4.1% (80/1,973) vs. BOL = 4.9% (96/1,976), P = 0.22] were observed. Administration of a single dose of an oral Ca bolus after parturition did not increase blood Ca concentration, and preliminary results indicate no association between treatment and early lactation milk production or culling.

**Key Words:** hypocalcemia, oral calcium, transition cow

**M94 Serum mineral concentrations and their association with calcemic status at calving on multiparous Jersey cows.** A. Valldecabres⁴, J. A. A. Pires⁵, and N. Silva-del-Rio⁵, ⁴Veterinary Medicine Teaching and Research Center, University of California-Davis, ⁵J. Dairy Sci. Vol. 100, Suppl. 2
The objective was to evaluate the association of serum Ca concentration and calcemic status with P, Mg, K, Na, Fe, Zn and Cu concentrations at calving on 768 multiparous Jersey cows from 2 commercial dairies. Blood samples for serum Ca, P, Mg, K, Na, Fe, Zn and Cu analyses were collected from the coccygeal vessels 3 h 14 min (±2 h 04 min) after calving. Serum samples were analyzed by Inductively Coupled Plasma – Optical Emission Spectrometry method (ICP-OES). Based on serum Ca concentration cows were classified as subclinical hypocalcemic (SCH; Ca ≤8.5 mg/dL; n = 250). Associations among variables and with calcemic status (SCH; Ca ≤8.5 mg/dL; n = 518) and normocalcemic (NC; Ca >8.5 mg/dL; n = 250). Associations among variables and with calcemic status with P, Mg, K, Na, Fe, Zn and Cu concentrations at calving are shown in the Table 1. Serum mineral concentrations at calving were classified as subclinical hypocalcemic (SCH; Ca ≤8.5 mg/dL; n = 518) and normocalcemic (NC; Ca >8.5 mg/dL; n = 250). Associations among variables and with calcemic status were analyzed using spearman rank correlations and mixed models, respectively (CORR and MIXED procedures of SAS). Descriptive statistics, including first (Q1), second (Q2) and third (Q3) quartiles, of serum mineral concentrations at calving are shown in the Table 1. Serum Ca concentration was correlated negatively with Mg and positively with P, Na and Zn (P < 0.001; | r | > 0.15). Cows with SCH had higher serum Mg concentration than NC cows (2.53 vs. 2.42 mg/dL; P < 0.001) but lower P (3.84 vs. 4.77 mg/dL; P < 0.001), K (4.58 vs. 4.73 mEq/L; P = 0.01), Na (137.4 vs. 142.3 mEq/L; P < 0.001), Fe (1.00 vs. 1.11 ppm; P = 0.001), Zn (0.59 vs.0.69; P < 0.001) and Cu (0.80 vs. 0.86 ppm; P < 0.001). Our results suggest that the negative effects of postpartum subclinical hypocalcemia on dairy performance may be in some measure mediated by variations in other serum minerals.

**Key Words:** serum calcium, serum minerals, hypocalcemia

### Table 1 (abstract M94). Quartile distribution (Q1 = 25th percentile, Q2 = 50th percentile, Q3 = 75th percentile) of serum mineral concentrations at calving

<table>
<thead>
<tr>
<th></th>
<th>Ca (mg/dL)</th>
<th>P (mg/dL)</th>
<th>Mg (mg/dL)</th>
<th>K (mEq/L)</th>
<th>Na (mEq/L)</th>
<th>Fe (ppm)</th>
<th>Zn (ppm)</th>
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<td>SCH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>7.4</td>
<td>3.1</td>
<td>2.2</td>
<td>4.2</td>
<td>133</td>
<td>0.72</td>
<td>0.49</td>
<td>0.66</td>
</tr>
<tr>
<td>Q2</td>
<td>7.9</td>
<td>3.8</td>
<td>2.4</td>
<td>4.5</td>
<td>137</td>
<td>0.94</td>
<td>0.60</td>
<td>0.76</td>
</tr>
<tr>
<td>Q3</td>
<td>8.2</td>
<td>4.6</td>
<td>2.7</td>
<td>4.8</td>
<td>141</td>
<td>1.20</td>
<td>0.72</td>
<td>0.88</td>
</tr>
<tr>
<td>NC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>4.0</td>
<td>2.2</td>
<td>4.4</td>
<td>139</td>
<td>0.79</td>
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<tr>
<td>Q2</td>
<td>8.9</td>
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<td>4.6</td>
<td>142</td>
<td>1.00</td>
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<td>5.6</td>
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<td>4.9</td>
<td>145</td>
<td>1.30</td>
<td>0.80</td>
<td>0.95</td>
</tr>
</tbody>
</table>
toxic effects of punicalagin even with 10 mg/kg daily intake in male New Zealand rabbits.

**Key Words:** antioxidant, polyphenol, toxicity

**M98 Subacute bisphenol A toxicity in male New Zealand White rabbits.** H. Karabulut and M. S. Gulay*, Mehmet Akif Ersoy University, Burdur, Turkey.

Because of the possible effects of bisphenol A (BPA) on human and other animals, there are several studies about the possible effects of BPA toxicity. Even so, the current literature lacks studies about the potential effects of BPA on rabbits. Therefore, the objectives of the present study were to document the effects of different doses of bisphenol A (BPA) on hematological and biochemical parameters, liver enzymes, weight gain and feed intake of male New Zealand White rabbits. Prior to the experiment male rabbits (n = 24) were acclimatized to laboratory conditions for 14 d. After the adaptation, rabbits were divided into 4 groups of 6; positive controls (Group 1, corn oil), and 3 different doses of BPA (10, 20 and 100 mg/kg BPA in corn oil) for 9 weeks. Body weights and feed intakes of the rabbits were evaluated weekly. At the end of the experiment blood samples from the ear artery were taken for the analyses of hematological and biochemical parameters. PROC GLM procedure was used for statistical evaluations. To compare the individual means of the groups, Dunnett post hoc analysis was performed. The results of the current study indicated no changes in weight gain and feed intake among the treatments. Similarly, the mean values of total white blood cells, lymphocytes, monocytes, granulocytes, platelets, mean platelet volume, mean corpuscular volume and mean corpuscular hemoglobin were within the physiological ranges for rabbits and not affected by BPA treatment at the end of the study. However, hemoglobin, red blood cells and mean corpuscular hemoglobin concentrations were reduced significantly due to 20 and 100 mg/kg BPA exposure ($P < 0.05$). Among the biochemical parameters, serum glucose, cholesterol, and triglyceride concentrations were not affected by BPA treatments. On the other hand, serum alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, urea, and creatinine levels were significantly elevated in the 20 and 100 mg/kg BPA dose groups ($P < 0.05$). In conclusion, the current subacute study suggested a no-observed adverse effect level (NOAEL) of 10 mg BPA/kg in male New Zealand White rabbits.

**Key Words:** bisphenol A (BPA), rabbit, subacute toxicity

**M99 Omnigen supplementation during the first 150 days of life decreases the incidence of tick fever in dairy calves.** B. B. Leme*1, L. F. Barbosa2,1, I. C. Marabíza4, A. C. Mariano4, S. H. Casonato4, and J. L. M. Vasconcelos3,1, 1Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, São Paulo, Brazil, 2Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil, 3Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, 4Fazenda Agrindus S/A, Descalvado, São Paulo, Brazil.

The aim of this study was to evaluate the rate of infection of tick fever (disease caused by agents *Babesia bigemina, Babesia bovis*, and *Anaplasma marginale*, which are transmitted by the tick) in dairy calves supplemented or not with Omnigen-AF (OMN, Phibro Animal Health, Teaneck, NJ) before and after weaning. One hundred twenty calves ranked by serum protein (refractometer), evaluated 24–48 h after calving, were distributed to receive OMN (n = 60; 10 g/calf/d up to 60 d, added to milk, and later 20gr/calf/d, added to the concentrate, from 61 to 150 d) or not (CON; n = 60). The calves were kept in individual cages, for 75 d, where they received 6L of milk per day plus ad libitum concentrate. After 75 d, they were introduced into group housing (n = 4), where they received a total mixed ration (TMR). During the experimental period, weekly evaluations were made for blood hematocrit, serum protein, stained smear to detect the presence of *Anaplasma spp.*, or *Babesia spp.*, mucosal color and rectal temperature. The treatment for tick fever (TF) and others disease were recorded daily. Blood samples were collected from the TF treated animals for determination of hematocrit, serum protein, and presence of TF. There was no effect ($P > 0.10$) of treatment for serum protein parameters, rectal temperatures, and detection of hemoparasites by stained smear. Animals that received OMN in relation to the control group required fewer treatment days for diarrhea and pneumonia (mean of 12.67 vs 15.12 ± 1.1 d, $P = 0.1$) and for TF (mean of 0.88 versus 1.35 ± 0.13, $P = 0.012$). OMN group had lower number of animals affected by TF (mean 60% vs 77% ± 6%, $P = 0.05$), lower number of weeks with hematocrit below normal range (mean of 2.17 vs 3.12 ± 0.32, $P = 0.035$), lower number of animals that needed to repeat treatment by TF (mean of 0.23 vs 0.37 ± 0.059, $P = 0.1$), and fewer days of treatment (clinical symptoms) (mean of 0.22 vs 0.4 ± 0.06, $P = 0.03$). For descriptive statistical analysis it was conducted by the Minitab program (Minitab Inc., State College, PA), generalized linear model, assuming statistical significance for $P < 0.05$. Omnigen-AF supplementation for dairy calves may minimize infections and clinical symptoms of tick fever.

**Key Words:** calf, Omnigen-AF, health
Predicted transmitting abilities (PTA) for gestation length (GL) were developed for all dairy breeds and crossbreds. Initial GL PTA were computed for all 6.8 million Holstein and 5 million Brown Swiss and Guernsey dairy cows born since 1990. They included effects of conception month, age-parity of dam, breed of dam, offspring sex, herd-month, service sire, permanent environment of dam, and error. PTA were computed for all 73 million animals from their additive relationships to the sires. An animal model for GL as a trait of the calf might provide higher reliability by using maternal genetic relationships for dams, but the focus here was on sire sire direct genetic effects, which were nearly 4 d shorter for Holsteins than for Brown Swiss and Guernseys. Heritability estimates computed as 4 times the service sire variance were 0.48 from heifers and 0.44 from all lactations (heifers and cows) based on 5 million Holstein GL records. The GL PTA for recent Holstein bulls (born 1995 or later with 90% or higher reliability) have a minimum of −5.6 and maximum of +6.4 and a SD of about 1.4 d. Jersey and Brown Swiss bulls both had the same SD of about 1.4 after adjustment to within-breed bases but had smaller ranges of values than Holstein due to fewer bulls: −4.2 to +5.0 for Jersey; −3.6 to +5.6 for Brown Swiss. Genomic predictions for Holsteins averaged 65% reliability. Short GL is favorably correlated by about 0.38 with daughter calving ease and by about 0.24 to 0.29 with yield and productive life. Thus, current strong selection for these correlated traits has already decreased GL in recent years. Gestation length (GL) can be useful in mating programs to group all birth dates together in seasonal calving, managing maternity pens, or improving calving ease as a correlated trait. Official GL evaluations are expected in 2017.

Key Words: gestation length, genomic prediction, calving management
M103  Genome-wide copy number variant analysis in Holstein cattle reveals variants associated with 10 production traits including residual feed intake and dry matter intake. E. E. Connor*1, Y. Zhou1,3, G. R. Wiggans1, Y. Lu4, R. J. Tempelman5, S. G. Schroeder1, H. Chen1, and G. Liu6,  1 USDA-ARS, Animal Genomics and Improvement Laboratory, Beltsville, MD, 3 University of Guelph, Guelph, ON, Canada, 1 USDA-ARS, Animal Genomics and Improvement Laboratory, Beltsville, MD, 5 Michigan State University, East Lansing, MI, 6 Northwest A&F University, Yangling, Shaanxi, China.

Copy number variation (CNV) is an important type of genetic variation contributing to phenotypic differences among mammals and may serve as an alternative molecular marker to single nucleotide polymorphism (SNP) for genome-wide association study (GWAS). Recently, GWAS analysis using CNV has been applied in livestock, although few studies have focused on Holstein cattle. Here, we describe 191 CNV of high confidence that were detected using SNP genotypes generated with the BovineHD Genotyping BeadChip (Illumina, San Diego, CA) among 528 Holstein cows. The CNV were used for GWAS analysis of 10 important production traits of cattle related to feed intake, milk quality, and female fertility, as well as 2 composite traits of net merit and productive life. In total, we detected 57 CNV associated ($P < 0.05$ after false discovery rate correction) with at least one of the 10 phenotypes. Focusing on feed efficiency and intake-related phenotypes of residual feed intake and dry matter intake, we detected a single CNV (CNV1) associated with both traits which overlaps predicted olfactory receptor gene OR2A4 (LOC787786). Additionally, 2 CNV (CNV32 and CNV66) within the RXFP4 and 2 additional olfactory receptor gene regions, respectively, were associated with residual feed intake. The RXFP4 gene encodes a receptor for an orexigenic peptide, insulin-like peptide 5 produced in the hypothalamus that is involved in the regulation of appetite, and has been implicated in participating in appetite regulation. Our results identify CNV for genomic evaluation in Holstein cattle, and provide candidate genes contributing to variation in feed efficiency and feed intake-related traits.

Key Words: dairy cow, genome-wide association study, copy number variation

M104  Association of residual feed intake with disease indicator traits in Holsteins. D. Hailemariam*1, 1, G. Manafazar1, J. Basarab1, F. Miglior1,2, G. Plastow1, and Z. Wang1, 1 Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, 2 Alberta Agriculture and Forestry, Lacombe Research Centre, Lacombe, AB, Canada, 3 Canadian Dairy Network, University of Guelph, Guelph, ON, Canada, 4 CGIL Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.

The objective of this study was to investigate the association of residual feed intake (RFI) with routinely measured milk components that are indicators of subclinical mastitis and ketosis. Milk somatic cell count (SCC, $10^3$ cells/mL) is commonly used to diagnose subclinical mastitis while β-hydroxybutyrate (BHB, mmol/L) and acetone (ACT, mmol/L) are indicators of ketosis. RFI was phenotyped in 71 lactating Holstein dairy cows at the Dairy Research and Technology Center–University of Alberta with components of metabolic body weight, empty body weight change, and milk production energy requirements over 255 d in milk using random regression and multiple linear regression models. Correspondingly, test-day milk samples were collected twice a week and analyzed at DHI lab by a MIR spectrometer (MilkoScan FT+, Foss, Hillerød, Denmark) during the same period as for RFI prediction. A total of 3,810 test day records for each of the traits; SCC, BHB and ACT were obtained from April to August 2016. The data were analyzed using a MIXED model procedure of SAS with fixed effects of RFI (-RFI and +RFI), lactation number (1, 2 and 3+), milking time (AM and PM), interactions of RFI x lactation, RFI x milking time and random effects of cow. Days in milk was included in the model as a covariate. The result indicated that -RFI and +RFI groups did not differ in SCC ($381.01 ± 55.77$ vs. $359.47 ± 47.14; P = 0.76$), BHB ($0.53 ± 0.07$ vs. $0.64 ± 0.05; P = 0.25$) and ACT ($0.30 ± 0.06$ vs. $0.32 ± 0.04; P = 0.75$). The correlation analysis showed no evidence of RFI association with SCC ($r = 0.01; P = 0.91$), BHB ($r = 0.17; P = 0.17$) and ACT ($r = -0.042; P = 0.72$). The result suggests that selection for RFI may not be negatively correlated with incidence of subclinical mastitis or ketosis in dairy cattle.

Evaluation of the bovine transcriptome using RNA-Sequencing (RNA-Seq) has made substantial impact in assessing functional and structural genomes in cattle. A preliminary study evaluated the metatranscriptome of bovine milk to determine the composition and structure of bacterial populations influencing subclinical mastitis. Differences in bacterial presence in milk between healthy and mastitic quarters were found in Holstein cows using RNA-Seq technology. The objective of this study is to further evaluate the use of RNA-Seq technology to assess the non-mapped milk bacteria genome in dairy cattle. Transcriptomic and metagenomic analysis were performed using RNA-Seq technology and 16S ribosome sequencing on milk collected from 4 quarters of healthy (n = 4) and mastitic (n = 4) dairy cows. Milk samples were collected 3 h after morning milking to obtain a high percentage of epithelial cells. Cow teats were cleaned with gauze (70% isopropanol) and milk was collected by hand milking directly into sterile 50 mL Falcon tubes or using a 3 cm plastic cannula to collect milk within the teat canal to avoid external contamination. Total RNA was extracted from somatic cells (SC) and milk fat globule (MFG) membrane from both hand milking and cannula milk samples. Using a RNA-seq analysis pipeline, preliminary results revealed that 60 to 75% of reads were categorized as mapped to the bovine reference sequence. All reads not mapping to the bovine genome were annotated for MFG (32% hand milking, 20% cannula) and SC (25% hand milking, 12% cannula). Analysis of SC non-mapped reads identified differences in microbial species present in healthy and mastitic milk. Further analysis will lead to more precise mapping of sequence data and improved understanding of bacterial gene expression, integrating data generated from RNA-Seq and 16S sequencing. Future assessment of the non-mapped reads using RNA-Seq will be performed to study the ability of RNA-Seq technology to capture invasive pathogens in milk and their association to genes differentially expressed in healthy and mastitic quarters. This assessment may lead to a comparative approach to examine the immune response to infection in dairy cattle.

Key Words: genome/host, transcriptomics/metatranscriptomics, RNA-sequencing technology

M105  Use of RNA-Sequencing technology for detection of microbial species. S. Lam*1, F. Miglior1,2, L. L. Guan3, A. Islas-Trejo4, D. Seymour1, V. Asselstine1, L. F. Brito1, J. F. Medrano4, and A. Canovas1, 1 Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada, 2 Canadian Dairy Network, University of Alberta, Edmonton, AB, Canada, 3 Department of Animal Science, University of California-Davis, Davis, CA.

Evaluation of the bovine transcriptome using RNA-Sequencing (RNA-Seq) has made substantial impact in assessing functional and structural genomes in cattle. A preliminary study evaluated the metatranscriptome of bovine milk to determine the composition and structure of bacterial populations influencing subclinical mastitis. Differences in bacterial presence in milk between healthy and mastitic quarters were found in Holstein cows using RNA-Seq technology. The objective of this study is to further evaluate the use of RNA-Seq technology to assess the non-mapped milk bacteria genome in dairy cattle. Transcriptomic and metagenomic analysis were performed using RNA-Seq technology and 16S ribosome sequencing on milk collected from 4 quarters of healthy (n = 4) and mastitic (n = 4) dairy cows. Milk samples were collected 3 h after morning milking to obtain a high percentage of epithelial cells. Cow teats were cleaned with gauze (70% isopropanol) and milk was collected by hand milking directly into sterile 50 mL Falcon tubes or using a 3 cm plastic cannula to collect milk within the teat canal to avoid external contamination. Total RNA was extracted from somatic cells (SC) and milk fat globule (MFG) membrane from both hand milking and cannula milk samples. Using a RNA-seq analysis pipeline, preliminary results revealed that 60 to 75% of reads were categorized as mapped to the bovine reference sequence. All reads not mapping to the bovine genome were annotated for MFG (32% hand milking, 20% cannula) and SC (25% hand milking, 12% cannula). Analysis of SC non-mapped reads identified differences in microbial species present in healthy and mastitic milk. Further analysis will lead to more precise mapping of sequence data and improved understanding of bacterial gene expression, integrating data generated from RNA-Seq and 16S sequencing. Future assessment of the non-mapped reads using RNA-Seq will be performed to study the ability of RNA-Seq technology to capture invasive pathogens in milk and their association to genes differentially expressed in healthy and mastitic quarters. This assessment may lead to a comparative approach to examine the immune response to infection in dairy cattle.
M106  Genetic trends of linear type traits for validation of genomic evaluation in US Holsteins, S. Tsuruta\textsuperscript{1,}, T. J. Lawlor\textsuperscript{2,}, D. A. L. Lourenco\textsuperscript{1,}, Y. Masuda\textsuperscript{1,}, and I. Misztal\textsuperscript{1,}  
\textsuperscript{1}University of Georgia, Athens, GA, \textsuperscript{2}Holstein Association USA, Brattleboro, VT.

Proper modeling of genetic evaluations is necessary to obtain accurate forward predictions. Differences in genetic trends for genomic (G)PTA, traditional PTA, parent averages (PA), and daughter yield deviations (DYD) can illustrate a model’s ability to control bias due to genomic preselection and improper parameter choice. Phenotypes for 18 linear type traits and genotypes were provided by Holstein Association USA and USDA-ARS, respectively. The full data consisted of 10,067,745 records up to 2014 calving, 9,730,943 animals in pedigree, and 569,404 genotyped animals with 60K SNP. For validation of young genotyped animals who did not have phenotypes or daughters in 2010, 9,235,355 records and 105,116 genotyped animals were used to estimate genetic trends, comparing with those estimated from the full data set. The BLUP-P90IOD2 program was used to predict GPTA in 2010 and in 2014 with single-step genomic BLUP using the algorithm of proven and young animals. The trends were calculated separately for bulls with at least 50 daughters in 2014 and for cows with records. Assuming that GPTA in 2014 were the most accurate, GPTA in 2010 for more than half of the traits, when no parameter adjustments are made, showed some bias. Traits with directional selection, i.e., body size and udder traits, were overpredicted. Parent averages in 2014 were similar to PTA and DYD in 2014 and lower than GPTA in 2014. Traits with an intermediated optimum, such as rump angle and foot angle, showed little or no bias. Lowering the heritability slightly improved both the accuracy and predictability. Including an adjustment (weight <1.0) to the inverse of the relationship matrix of the genomic tested animals dramatically improved the predictability of the model with a slight decrease in accuracy. Future research is ongoing to fully understand how this adjustment is altering our assumptions in the basic model; e.g., how this adjustment is related to genetic parameters that could be different by generation and why some traits are not overpredicted without any adjustment.

**Key Words:** genomic evaluation, genetic trend, linear type trait

M107  Sources of variation in minor milk components and their potential prediction using mid-infrared spectroscopy, A. Fleming\textsuperscript{1,}, F. S. Schenkel\textsuperscript{1,}, S. Nayeri\textsuperscript{1,}, C. Baes\textsuperscript{1,}, R. A. Ali\textsuperscript{2,}, M. Corredig\textsuperscript{1,3}, and F. Miglior\textsuperscript{1,5,}  
\textsuperscript{1}Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, \textsuperscript{2}Department of Mathematics and Statistics, University of Guelph, Guelph, ON, Canada, \textsuperscript{3}Department of Food Science, University of Guelph, Guelph, ON, Canada, \textsuperscript{4}Gay Lea Foods Co-operative, Mississauga, ON, Canada, \textsuperscript{5}Canadian Dairy Network, Guelph, ON, Canada.

Variation in minor milk components is of interest to consumers and milk processors but additional knowledge is needed to ascertain its sources. A phenotyping tool such as mid-infrared (MIR) spectroscopy would help future inquiry into these traits on a large-scale routine basis. The objectives of this work were to examine the variation of characteristics and contents of minor milk components and assess the ability to predict them from MIR spectra of the milk. A total of 4,116 milk samples were collected from 421 cows representing 4 dairy breeds coming from 47 Canadian herds. The MIR spectra of the milk samples were obtained through DHI labs. Not all samples were analyzed for all traits, but some overlap occurred. Samples were analyzed for casein micelle size ($n = 3,117$), total ($n = 986$) and soluble ($n = 937$) calcium, lactoferrin ($n = 2,054$), and $\alpha_-, \beta_-, \kappa$-casein proportions ($n = 2,067$). Milk component traits were examined separately using a linear model including the effects of herd nested within breed, days in milk class, parity, season, and animal. Herd nested within breed, days in milk class, and season had significant effects on all examined traits. Significant differences were noted between breeds for casein micelle size, total calcium, lactoferrin, and casein proportions. Lactoferrin and casein proportions also differed between parities. Moderate and negative Pearson correlation coefficients were found for lactoferrin content with test-day milk, fat, and protein yields ($P < 0.001$). Moderate and positive Pearson correlation coefficients were found between lactoferrin and somatic cell score as well as total calcium and protein percentage ($P < 0.001$). Partial least squares regression using a 10-fold random subset cross-validation was used to predict the milk components from the MIR spectra. The average cross-validation $R^2$ ($R^2_{cv}$) values were very low for casein micelle size, total and soluble calcium, and $\alpha_-\beta_-\kappa$-casein proportions at 0.31, 0.24, 0.16, 0.03, 0.10, and 0.08, respectively. For lactoferrin a $R^2_{cv}$ of 0.55 was found, which may allow for MIR predicted lactoferrin to serve as a limited indicator of the true lactoferrin content in milk.
M13  Dry heat treatment affects solubility, whey protein denaturation, and soluble aggregates formation in nonfat dry milk. K. S. Alán and K. Schmidt*, Kansas State University, Manhattan, KS.

Heat treatments are used in the dairy industry to ensure microbiologically safe products, extend shelf life, and improve functional properties. Dependent upon temperature and time, these treatments can cause protein denaturation, whey protein aggregation as well as initiate the Maillard reaction in liquid milk systems. However, insufficient evidence exists about the impact of a dry heat treatment in a dry milk system, such as nonfat dry milk (NDM). The aim of this research was to apply a dry heat treatment to low heat (LH) NDM to elucidate the effect of the dry heat on proteins. Radiofrequency dielectric heat was the mechanism bring LH-NDM to 1 of 3 temperatures: 80, 85, and 90°C and all samples were held at that temperature for 90 min. LH-NDM and high heat (HH) NDM that were not dry-heated were included in the assessments. All samples were evaluated for whey protein nitrogen index (WPNI), solubility (NSI), XTT reduction (Maillard reaction) and soluble aggregates content. One-way ANOVA was used to determine differences among the 5 samples, with significant means differentiated with the Tukey’s test ($P \leq 0.05$). Results showed that LH-NDM treated at 85°C had similar content of soluble whey protein aggregates as HH-NDM, further these samples contained significantly more soluble than did the LH-NDM at 80°C, and 90°C. The WPNI for HH-NDM was significantly less than the 4 LH-NDM samples, regardless of dry heat treatment. The WPNI decreased in all dry-heated LH-NDM samples when compared with non-treated LH-NDM (2% at 80°C, 7% at 85°C and 15% at 90°C). LH-NDM treated at 80°C maintained good solubility; however, LH-NDM treated at 85°C and 90°C had losses in solubility (0.64% and 3.34%, respectively) when compared with LH-NDM. Results from the XTT test indicated that the Maillard reaction products were greater in the HH-NDM, LH-NDM treated at 85°C and 90°C. These results suggest that dry heat treatment can affect the proteins and their interactions in LH-NDM. It is important to understand the effects of dry heat in dairy powders, as these treatments could provide ideal conditions to improve the functional properties of NDM.

Key Words: nonfat dry milk, milk proteins, dry heat treatment

M108  Rapid determination of lactulose in heat-treated milk using ultraperformance convergence chromatography coupled with mass spectrometry. F. Wen1,2, Y. Tian1,4, Y. W. Xu3, N. Zheng1,2, Q. L. Sun2, S. L. Li1,3, and J. Q. Wang*1,2. 1Ministry of Agriculture-Key Laboratory of Quality & Safety Control for Milk and Dairy Products, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2Ministry of Agriculture-Laboratory of Quality and Safety Risk Assessment for Dairy Products, Beijing, China, 3Ministry of Agriculture-Milk and Dairy Product Inspection Center, Beijing, China, 4State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 5Waters Corporation, Shanghai, China.

Lactulose represents the most widely studied index for distinction of heat-treated milk and for evaluating the heat load to which milk was subjected. Several methods have been reported and applied to detect lactulose in milk, including enzymatic, GC and HPLC methods. The major disadvantage of these methods is that they are laborious, time consuming and less sensitive. One of the main problems in measuring lactulose in milk is the coexistence of lactose and lactulose, which have a similar retention time using HPLC. In particular, the amount of lactose is 2 orders of magnitude larger than that of lactulose. Here we present a rapid and sensitive quantification of lactulose in milk based on the utilization of supercritical fluid chromatography coupled with mass detector. Quantification of lactulose was performed by employing selected ion recording (SIR) mode. Lactulose and lactose were well resolved from the peaks with a resolution value ($R_s$) of 1.8. It is worth noting that lactulose peak has a shorter retention time than lactose peak, and the tail effect is lowered. Under the optimal conditions, a linear relationship between the concentrations of lactulose and peak areas was obtained in the range of 0.1 to 10.0 mg/L ($r^2 = 0.999$), with a LOD and LOQ of 0.03 mg/L and 0.1 mg/L in matrix solution, respectively. Considering the dilution factor of sample pretreatment, the LOD and LOQ could be lower than 0.75 and 2.5 mg/L in milk sample. The relative standard deviation was 3.3% for UHT milk, indicating good precision. Compared with the methods developed using HPLC, the proposed method shows some superiorities, such as shorter retention time, less solvent consumption, better peak resolution and higher sensitivity.

Key Words: lactulose, milk, ultraperformance convergence chromatography

M109  Characterization of microdomains in bovine milk phospholipid monolayers that contain GM3 and GD3 gangliosides. L. Real Hernandez* and R. Jimenez Flores, The Ohio State University, Columbus, OH.

GM3 and GD3 are the 2 main gangliosides present in the outer layer of bovine milk fat globule membranes (MFGM). Lipids in the outer layer of the MFGM can condense into liquid-ordered phases known as microdomains, but the role GM3 and GD3 have on the formation of MFGM microdomains is unknown. Furthermore, studies have not described the microdomains formed in lipid layers where both GM3 and GD3 are present, as they are in the MFGM. In this study, Langmuir-Blodgett films were made from lipid monolayers containing cholesterol, buttermilk phosphatidylcholine, buttermilk sphingomyelin, and milk gangliosides GM3 and GD3. Lipids were spread at the air-water interface on a 740 mm x 75 mm high-compression Langmuir trough, and Langmuir-Blodgett films were analyzed using a 3-dimensional laser scanning confocal microscope. Observed microdomains were analyzed for shape and size among lipid monolayers of varying ratios of the lipids studied. Understanding how the major gangliosides in milk affect microdomains in the MFGM allows us to understand the structural role of GM3 and GD3 in milk.

Key Words: gangliosides, microdomains, milk fat globule membrane

M110  Effect of caprine casein in combination with arabinogalactan on the chemical stability of lutein in corn oil-in-water emulsions. A. Mora-Gutierrez*, R. Attaie, M. Gonzalez, Y. Jung, and S. Woldesenbet, Prairie View A&M University, Prairie View, TX.

The objective of this study was to determine the effects of caprine casein in combination with arabinogalactan on the chemical stability of lutein in corn oil-in-water emulation at 37°C for 96 h. Previous studies have reported an increase in the chemical stability of lutein-loaded oil-in-water emulsions prepared using bovine casein. However, the chemical degradation of lutein in corn oil-in-water emulsions stabilized by bovine...
casein increased by raising the temperature from 5 to 70°C. The concentration of lutein in oil-in-water emulsion stabilized by bovine casein was reduced by 12% at 37°C during the 96-h incubation as compared with the control. On the other hand, the ability of arabinogalactan, a condensation product of galactose and arabinose at 6:1 ratio, to form an inclusion complex with a guest molecule, can improve lutein application, bioavailability, and stability in foods. Corn oil-in-water emulsion (5% wt/wt) stabilized with bovine casein (0.5% wt/wt) or caprine casein (0.05% wt/wt), and arabinogalactan at a concentration of 0.25% (wt/wt) was prepared using a high-pressure homogenizer. The pH of the emulsions was adjusted to 7.0. The chemical stability of lutein was assessed by measuring the changes in lutein concentration in the emulsions during incubation at 37°C for 96 h using a spectrophotometric method. The chemical stability of lutein increased significantly (P < 0.05) in the caprine casein types I and II as compared with bovine casein. Microencapsulation of lutein with either caprine casein-arabinogalactan or bovine casein-arabinogalactan protected lutein in corn oil-in-water emulsions and significantly reduced (P < 0.05) the chemical degradation of lutein at 72 and 96 h of incubation at 37°C, compared with the treatments without arabinogalactan. This information will be useful for understanding the behavior of lutein emulsions in food products.

Key Words: casein, lutein, arabinogalactan

M111 Lipidomics approach reveals integrated triglycerides profiles associated with different thermal treatments on dairy cow milk. Y. Zhang*1,4, N. Zheng1,2, S. Li3,4, S. Zhao1,4, F. Wen1,4, M. Li1,4, L. Meng2,3, and J. Wang1,2,4, 1Ministry of Agriculture-Key Laboratory of Quality & Safety Control for Milk and Dairy Products, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2Ministry of Agriculture-Laboratory of Quality and Safety Risk Assessment for Dairy Products, Beijing, China, 3Laboratory of Quality & Safety Control for Milk and Dairy Products, Ministry of Agriculture-Milk and Dairy Product Inspection Center, Beijing, China, 4State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

Different triglycerides (TG) profiles in raw milk (RM), pasteurized (BM, 85°C for 15 s) and indirect ultra-high temperature (UHT) treated cow milk (UM, 135°C for 15 s) was investigated using UPLC-MS/MS. Raw milk was treated similarly for pasteurization process and UHT treatment, 3 replicated trials were undertaken using different batches of raw milk. Three replicates of each milk samples and 1 pool from each milk sample were analyzed. The results showed that 94 TGs were found in all milk samples, and the contents of the 94 TGs decreased significantly in UM milk compared with that in either RM milk (P < 0.05) or BM milk (P < 0.05), but there was no significant difference between RM milk and BM milk (P > 0.05). However, the contents of the free fatty acids increased significantly in UM milk compared with that of RM milk (P < 0.05) or BM milk (P < 0.05). Moreover, there was no significant difference between BM milk and UM milk (P > 0.05) for the free fatty acid. The results indicated that lipolysis significantly occurred in UM treated compared with that in pasteurized milk. The difference in TGs of milk under different thermal treatment may have influence on milk nutritional content.

Key Words: lipidomics, triglycerides, thermal treatment

M112 Evaluation of electrical bioimpedance spectroscopy for detection of milk adulteration—Preliminary results. E. A. Veiga*2, C. M. M. R. Martins3, R. Frizon2, and M. V. Santos1, 1Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, São Paulo, Brazil, 2Bionexus Tecnology, Chapecó, Brazil.

Electrical bioimpedance spectroscopy (EBS) is a fast, easy and low-cost methodology used for measurement the electrical properties of biological materials. Thus, the present study aimed to evaluate an automatic equipment based on EBS (MilkSpec) for identification of milk adulterations. Bulk milk samples from 2 dairy farms were collected and stored for 24 h at 5°C. The milk from each farm were split into 24 subsamples of 20 mL, which were experimentally adulterated: (a) sodium bicarbonate (NaHCO3) or caustic soda (NaOH50%): 0, 0.1, 0.2, 0.5, 1.0, 2.0, and 4.0%; (b) Formaldehyde37%: 0, 0.5, 1.0, 2.0, 4.0 and 8.0%; (c) Mix: (a) Water (10%) + urea (0.5%), 2) Milk slightly acid (pH = 6.55) + NaOH50% (0.1%) (final pH = 6.9). 3) Milk slightly acid (pH = 6.55) + NaOH37% (0.1%) (final pH = 6.9) + Formaldehyde37% (0.5%). 4) Water (10%) + Formaldehyde37% (0.5%) + Urea (0.5%) + NaHCO3 (0.5%). The EBS analysis were made by Milkspec FS317 (Bionexus, Brazil) considering as experimental unit, which required only 20 mL of raw milk and less than a minute to obtain the bioimpedance spectra. The EBS results were calculated using Bionexus InterCurve EBS software for curve fitting and inverse Fourier transform. Also, a Δ from maximal and minimal of impedance spectra was obtained in each sample. NaHCO3 and NaOH50% were detected in milk from concentration of 0.1% by changes in curve fitting parameters and on Δ of impedance spectra in comparison with non-adulterated milk. Formaldehyde was detected from 0.5% by changes in curve fitting and by inverse Fourier transform. Adulteration by adding water and urea were also detected by curve fitting and inverse Fourier transform. Milk slightly acid and NaOH50% was detected by curve fitting, and Milk slightly acid and NaOH37% + Formaldehyde37% (0.5%) by changes in curve fitting and Δ of impedance spectra. The addition of water, formaldehyde, urea and sodium bicarbonate was detected by changes in curve fitting and Δ of impedance spectra. This preliminary results suggest that an automatic equipment based on EBS may be used in the dairy industry to detect milk adulterations. Additionally, EBS would have easier operating procedures and lower cost than traditional methods.

Key Words: electrical bioimpedance spectroscopy, milk quality, MilkSpec
of adulterants monitoring in milk, since they affect the analytical results of milk quality, obtained by electronic methods.

**Key Words:** FTIR, adulterant in raw milk, compositional analysis

**M114 Effect of extraction conditions on the extraction efficiency for the HS-SPME-GC/MS analysis of volatile compounds in Turkish white cheese using central composite rotatable design.**

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Aroma of cheese is one of the most important features due to the product quality and consumer’s acceptance. Although most of the studies related to the analysis of volatile compounds of cheeses were carried out with headspace solid-phase microextraction (HS-SPME) method, there is lack of information about the effects of SPME parameters on extraction efficiency. In this study, the effects of principal extraction conditions (extraction temperature, time, and agitation speed) on the extraction efficiency were evaluated by using response surface methodology. Experimental data were processed using multiple linear regression analysis and second order polynomial models were created to predict the FID areas for volatile compounds. As a result, the variations of FID areas for 26 volatile compounds were successfully modeled. While 10 of these volatile compounds were acids, there were 7 alcohols, 3 ketones, 3 esters, 1 aldehyde, 1 lactone, and 1 phenol. The extraction temperature, time, and interaction effects were found to be significant on all volatile compounds (P < 0.01). On the other hand, the effect of agitation speed on FID areas for 8 volatile compounds (especially for acids) was not statistically important. Consequently, the most important factor was determined to be extraction temperature, followed by extraction time. This work was supported by The Scientific and Technological Research Council of Turkey (TUBİTAK) [project no: 115O229].

**Key Words:** SPME, volatile, response surface methodology

**M115 Sodium reduction and flavor enhancers addition in probiotic Prato cheese: Effect on the probiotic survival and functionality, proteolysis, antioxidant and angiotensin I-converting enzyme inhibitory activity.**

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The present study aimed to evaluate the microbiological (lactic acid bacteria count and probiotic bacteria count) and survival to gastrointestinal condition), physicochemical (pH, proteolysis) and bioactivity (antioxidant and angiotensin I-converting enzyme inhibitory activity) parameters of low sodium probiotic Prato cheese added with different flavor enhancers at 3 different moments: immediately after the processing, ripening time and refrigerated storage (1, 30 and 60 d, respectively). Five formulations of probiotic Prato cheese (L. lactis and L. casei 01, 6 and 8 log cfu/g, respectively), were manufactured: CI (control - 100% NaCl), CII (1 NaCl:1 KCl (wt/wt)), CIII (1 NaCl:1 KCl (wt/wt) plus 1% wt/wt arginine), CIV (1 NaCl:1 KCl (wt/wt) plus 1% wt/wt yeast extract) and CV (1 NaCl:1 KCl (wt/w) plus 1% wt/wt oregano extract). Sodium reduction and addition of flavor enhancers did not presented negative effect for lactic bacteria count and probiotic survival as well as probiotic survival to gastrointestinal condition once L. lactis counts ranged from 8.4 to 6.2 and for L. casei 01 these values were above 8 log cfu/g in all cheeses during all period (P > 0.05). Cheeses CII to CV showed increased pH values when compared with the cheese CI (values ranged from 5.5 to 7.6 against 5.3 to 6.1, P < 0.05). Low-sodium probiotic Prato cheeses showed higher proteolysis when compared with cheese CI (0.386 to 0.804 to 0.331 to 0.478, P > 0.05, respectively). The same tendency was observed with the antioxidant activity (65.68 to 89.1%, CII-CV against 52.04 to 63.30%, CI, respectively, P > 0.05) and angiotensin-I-converting enzyme inhibitory activity (13.0 to 69.9, CII-CV, 9.7 to 45.3% CI, respectively, P > 0.05) during all period. In conclusion, the partial replacement of sodium chloride by potassium chloride and the flavor enhancer addition in probiotic Prato Cheese formulation contributed to increased values of proteolysis, antioxidant activity and ACE-inhibitory activity, showing the benefits of the reformulation in Prato cheese without negative effect for probiotic and lactic bacteria survival.

**Key Words:** fatty acid profile, probiotic Prato cheese, sodium reduction
Prato cheese, a traditional semi-hard Brazilian cheese is a dairy food largely manufactured for dairy companies. However, the high sodium level Prato cheese formulation constitutes a negative aspect for consumers with present cardiovascular disease. This study aimed to evaluate the effect of sodium reduction and flavor enhancers on the availability of calcium, magnesium, zinc, phosphorus, and potassium of probiotic Prato cheese formulations. The cheese was manufactured considering 5 different formulations: 100% NaCl, NaCl/KCl 50/50%, and NaCl/KCl 50/50% wt/wt using the following flavor enhancers (oregano extract, arginine, and yeast extract, respectively). Mineral content and availability were evaluated at 3 different periods: 1, 30 and 60 d (immediately after the processing, ripening and refrigerated storage). Sodium reduction and addition of flavor enhancers influenced the mineral content and availability (P > 0.05), being the performance dependent of the ingredients added. Oregano extract addition increased the calcium, magnesium, zinc, phosphorus and potassium content (195.63, 146.62, 7.61, 528.36, 367.11 mg/100g, respectively) and their availability (19.78, 65.85, 19.25, 9.22, 8.55%, respectively) after the ripening (60 d), being followed by yeast extract and arginine addition. On the converse, conventional Prato cheese (normal sodium level and without flavor enhancer had lower calcium, magnesium, zinc, phosphorus and potassium content (99.59, 112.20, 3.18, 214.38, 84.72 mg/100g, respectively) and lower availability values (10.63, 52.65, 7.4, 4.32, 8.01%, respectively). Overall, the reformulation of Prato cheese by decreasing sodium content and addition of flavor enhancers increased the mineral content and availability and should take in account for the Brazilian cheese industry.

Key Words: availability, mineral, probiotic Prato cheese

M118 Micro-vesicles in milk: Identification and characterization of exosomes, ectosomes and small MFGM particles. J. Ortega-Anaya* and R. Jimenez-Flores, The Ohio State University, Columbus, OH.

Exosomes and ectosomes in general were considered fragments derived from death cells; however, it is now known their participation in short and long communication between cells. Even though they have different origin since exosomes have intracellular biogenesis and ectosomes are assembled from the cell plasma membrane, these milk micro-vesicles share molecular features between each other and with the MFGM such as protein composition; however, not much has been addressed regarding lipid composition, vesicle size and distribution, or surface characteristics (Z-potential). The aim of this study was to isolate the micro-vesicles from raw milk fractions by ultracentrifugation (110,000 × g) on a sucrose cushion (30%) and perform the characterization of the different vesicles based on their colloidal behavior in solution (reconstituted in PBS buffer). The components in each fraction were isolated by SEC and subjected to dynamic light scattering (NanoBrook 90 PlusPALS, Brookheaven Instruments) collecting the dispersed light at angles of 90° and 15° to resolve isolated particles from large aggregates. We found significant differences between the size of MFGM particles, exosomes (ranging from 50 to 100 nm) and ectosomes (ranging from 100 to 350 nm). We also determined lipid composition of each fraction by extracting lipids (Bligh and Dyer methodology) and analyzing them by liquid chromatography on an HPLC system coupled to a charged aerosol detector (Corona Veo RS, Thermo Scientific). Even though every fraction is composed of typical phospholipids (phosphatidylcholine, phosphatidylethanolamine), cholesterol and sphingomyelin found in milk, their distribution in each group of vesicles is different, thus, correlating with the different values of surface Z-potential determined in solution. The characterization and differentiation of milk extracellular micro-vesicles derived from this work, along with the protein and nucleotide composition already reported elsewhere, will help understand the function of these structures during digestion, and as transporters of biological active agents with promising application in human therapy diseases.

Key Words: milk micro-vesicle, Z-potential, lipid

M119 Hydrogen and methane in biogas from anaerobic digestion of manure and whey mixtures. D. J. McMahon*, D. S. Fallon, and C. L. Hansen, Utah State University, Logan, UT.

Whey (pH 4, 6 and 8) was substituted for manure at 25, 50, 75 and 100% to study the effect of whey on anaerobic biogas production. The sample weights were adjusted to provide the same total chemical oxygen demand since on a weight basis whey has double the chemical oxygen demand of manure. The mixtures, along with an activated sludge and mineral and vitamin supplement, were placed in 140-mL glass bottles fitted with a syringe inserted through a rubber septum. The samples were incubated at 35°C, and when gas had filled the syringe (~60 mL) the syringe was withdrawn and gas composition measured using gas chromatography and volume of methane and hydrogen produced was calculated. Then the syringe was replaced and digestion and gas collection continued. Statistical analysis was performed with effects of percent whey (n = 4), whey pH (n = 3) and sampling time (n = 3) and their 2-way and 3-way interactions. Percent whey significantly (P ≤ 0.01) affected volume of hydrogen and methane produced as well as pH at the end of digestion. Biogas from mixtures containing 25% or 50% whey contained primarily methane and no or little hydrogen and were not significantly different from each other. Biogas from mixtures containing 75% or 100% whey contained primarily hydrogen and no or little hydrogen and were not significantly different from each other. The pH of the whey had less effect on gas production than whey percent. The difference in biogas composition was explained by the higher level of fermentable carbohydrate (lactose) in whey. After digestion, pH was significantly decreased when whey percent was increased to 75%. Mean pH values were 6.51, 6.50, 3.63, and 6.19 for mixtures containing 25, 50, 75, and 100% whey, respectively. Adding more than 25% whey caused more inconsistent gas production (i.e., greater variation in time to produce the first 60 mL of gas, and more failures occurring during digestion (i.e., gas production ceased before the end of the experiment). Having a highly fermentable sugar such as lactose as part of a waste stream, causes a drop in pH during anaerobic digestion and this needs to be considered when designing digester systems for use by artisan cheese makers.

Key Words: manure, whey, anaerobic digestion

M120 Economic feasibility of anaerobic digestion for treating manure and whey from small-scale dairy farm combined with artisan cheese making. S. C. Lund*, D. J. McMahon*, A. J. Young, C. L. Hansen, and D. V. Bailey, Department of Nutrition, Dietetics and Food Sciences, Utah State University, Logan, UT; Department of Applied Economics, Utah State University, Logan, UT; Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT.
This study analyzed economic feasibility of implementing anaerobic digestion for waste treatment of manure and whey from an artisan cheese making operation combined with a small-scale dairy farm. Enterprise budgets were used to calculate net present value (NPV) and internal rate of return (IRR) from equipment price quotes, estimations from literature and using estimated annual receipts and costs for a 210-cow dairy farm in Utah, an artisan cheese plant producing bottled milk and cheese, and an inverted bed reactor anaerobic digester to handle manure and whey. Each enterprise was analyzed separately and integrated together to provide hypothetical models of annual costs and returns that can be viewed as a tool to help farmers make decisions about investments. Total costs for the dairy farm was $758,538 and based on 2015 milk prices this provided a net income (NI) of -$371 per head of cow. Initial investment cost for the artisan milk processing, cheese making and retail facility was $1,658,984 ($7,900 per head). Total operating costs were $898,835 with NI of $198,020 ($943 per head). How the cheese is marketed impacts NI as cheese can be sold directly by the artisan cheese maker (either through a retail store or online) at $29/kg compared with $17/kg or $9/kg if sold wholesale or through a distributor, respectively. Total cost for the anaerobic digester was $320,621 (after a 10% investment tax credit) that equates to $1,527 per head. Total operating cost was $66,238 with NI of $2,105 ($10/head) based on electricity generated and sale of digester biomass, carbon offsets and services for managing digestion of whey, manure and other organic wastes. For adding an artisanal cheese making facility producing 47,000 kg of cheese per year, NPV was estimated at $580,739 with 39% IRR. In comparison, NPV for the digester system was -$65,378 with IRR of −5.2%. For investment in a digester to be acceptable, a 12% discount rate is needed, meaning that 35% of the investment cost must be subsidized. Currently, small-scale dairy farmers facing urban encroachment cannot economically adopt anaerobic digestion to manage waste without an appropriate investment subsidy reflecting its social value.

**Key Words:** artisan cheese, whey, anaerobic digester
Dairy Foods II: Chemistry II

M121  Rheological properties, size distribution and optical microscopy of vanilla dairy desserts added with arrowroot flour. R. Oliveira¹, M. V. Ferreira*¹, J. L. Barbosa Junior¹, M. I. Barbosa¹, R. Bisagio², M. Cristina³, and A. Cruz³,¹Universidade Federal Rural of Rio de Janeiro (UFRJR), Seropédica, RJ, Brazil, ²Instituto Federal de Ciência e Tecnologia do Rio de Janeiro (IFRJ), Rio de Janeiro, RJ, Brazil.

Arrowroot (Maranta arundinacea L.) is a non-conventional food plant (NCFP) from Marantaceae species. Although this crop presents high nutritional, functional, and technological potential, its use has been neglected. To overcome this, studies on the NCFP exploitation by food producers have been recently intensified, mainly those applied in dairy products, since these products presented a great increase on their demand in the last decades. The aim of this work was to evaluate the effect of arrowroot flour contents (0, 1.5, 3.0, 4.0%, wt/wt) on the rheological properties, size distribution and optical microscopy in vanilla dairy dessert. The addition of flour showed a non-Newtonian shear-thinning flow behavior, by observing shear stress variation with shear rate, suggesting pseudoplastic behavior (n < 1). However, control samples had a complex modulus (G*) and loss angle tangent (tan δ) lower than the flour-added samples, suggesting that the flour addition increases the dessert’s hardness. Regarding size distribution, increased D[4,3] values were seen in the samples with flour added compared in the control dessert (ranging from 12 to 50 μm and 5 μm, respectively, P > 0.05). Optical microscopy confirmed the impact of the addition of the diameter increase in all the samples as the addition of increased flour content samples (4% wt/wt) resulted in great agglomeration of starch granules. Overall, the arrowroot flour addition improved technological properties of vanilla dairy desserts.

Key Words: non-conventional food plant, dairy dessert

M122  Supercritical carbon dioxide technology for processing of whey grape juice beverage: Assessing rheological parameters and particle size distribution. G. Amaral¹, M. V. Ferreira*¹, E. Silva², M. A. Meireles², E. Esmerino³, and A. Cruz³,¹Universidade Federal Rural of Rio de Janeiro (UFRJR), Seropédica, RJ, Brazil, ²Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil, ³Universidade Federal Fluminense (UFF), Niterói, RJ, Brazil, 4Instituto Federal de Ciência e Tecnologia do Rio de Janeiro (IFRJ), Rio de Janeiro, RJ, Brazil.

Supercritical carbon dioxide (SCCD) technology has been developed as a non-thermal food preservation methodology for dairy processing. In this sense, the objective of this study was to investigate the effects of treatment of SCCD (140, 160 and 180 bar at 35°C ± 2°C for 10 min) compared with conventional heat treatment (HTST) (72°C for 15 s) on rheological parameters (flow curves) and particle size distribution (D[3,2] values) of whey grape juice drink. SCCD decreased the D[3,2] values in a significant way when compared with the pasteurized beverage (62-64% reduction against 17.7%, P > 0.05). Regarding the rheological parameters it was observed all samples presented non-Newtonian behavior, being classified as pseudoplastic fluids (n < 1) while for the consistency index, the SCCD proportioned a higher reduction when compared with pasteurized sample (P < 0.05). In this sense, the findings of this study suggest the potential of SCCD technology is an adequate method to be adopted during the whey drinks processing, in particular whey grape juice drink.

Key Words: supercritical carbon dioxide technology, whey grape juice drink, rheology

M123  Lactobacillus casei 01 in probiotic and symbiotic sheep milk ice cream: Viability, survival under simulated gastrointestinal conditions and Caco-2 cells adhesion. C. Balthazar¹, H. Silva*¹, E. Esmerino¹, M. Carmo², L. Azevedo², I. Camps², and A. Cruz²,¹Universidade Federal Fluminense (UFF), Niterói, RJ, Brazil, ²Universidade Federal de Alfenas, Alfenas, MG, Brasil, ³Instituto Federal de Ciência e Tecnologia do Rio de Janeiro (IFRJ), Rio de Janeiro, RJ, Brazil.

The addition of probiotic bacteria and prebiotic ingredient in sheep milk dairy foods increases their status as a functional food. This study evaluated the Lactobacillus casei-01 viability, survival under simulated gastrointestinal conditions and Caco-2 cell adhesion in probiotic (L. casei 01, 7 log cfu/mL) and symbiotic (L. casei 01, 7 log cfu/mL + 10% inulin wt/wt) sheep milk ice cream along the frozen storage (105 d, −18°C). After overrun (about 60%), L. casei count in probiotic ice cream decreased 0.74 log cycle, while the symbiotic ice cream also did present similar values (7.90 to 7.86 log cfu/g, P > 0.05) along the study. The survival under simulated gastrointestinal conditions was improved in symbiotic when compared with probiotic ice cream (7.06 vs. 5.72 log cfu/g, respectively, P < 0.05) at 1st frozen storage day. However, a decrease of this value was observed in both formulations (6.44 and 5.26 log cfu/g in symbiotic and probiotic ice cream, respectively, P > 0.05) during the frozen storage. Regarding the Caco-2 cells adhesion, probiotic and symbiotic ice cream presented 5.69 and 5.70 log cfu/g l. casei 01, respectively and also a decrease was noted (5.24 and 5.12 log cfu/g, respectively, P < 0.05) during the frozen storage. In this sense, sheep milk ice cream may be considered an adequate food carrier to deliver probiotic bacteria with potential benefits for consumers. Additionally, the addition of inulin might exert a positive effect on the L. casei 01 viability and survival under gastrointestinal condition but without influence on the CaCO-2 cells adhesion values.

Key Words: sheep milk, ice cream, probiotics

M124  Physical-chemical and functional characteristics and volatile compounds of vanilla dairy desserts: Effect of arrowroot flour addition. R. Oliveira¹, M. V. Ferreira*¹, L. Cappato¹, K. Nascimento¹, J. Moraes², J. L. Barbosa Junior¹, M. I. Barbosa¹, M. Cristina³, and A. Cruz³,¹Universidade Federal Rural of Rio de Janeiro (UFRJR), Seropédica, RJ, Brazil, ²Instituto Federal de Ciência e Tecnologia do Rio de Janeiro (IFRJ), Rio de Janeiro, RJ, Brazil.

Non-conventional food plants (NCFP) have shown a great potential as new sources of prebiotics (oligossaccharides, resistant starch) and should be considered in dairy food formulations improving the technological and functional parameters. In this study, the addition of arrowroot flour (Maranta arundinacea L., 0; 1.5; 3.0 and 4%, wt/wt) in vanilla dairy dessert formulation was evaluated taking in account some physico-chemical and functional parameters. Glycemic index (GI), color parameters, pH,
syneresis index and volatile compounds profile refrigerated storage (4°C) were performed. Overall, the arrowroot flour addition presented low GI (41.8%) without however, present effect on whiteness (P > 0.05), although chroma value was negatively affected (P < 0.05) by the addition of 3 and 4% wt/wt of flour (15.31 ± 0.09 and 13.80 ± 0.29, respectively). On the reverse, pH values was not influenced (P > 0.05) by flour addition and ranged from 6.14 ± 0.03 to 6.27 ± 0.17, respectively, while the syneresis index decreased along the refrigerated storage (28 d/4°C), which suggests that arrowroot flour addition improves an important technological for this dairy product. Regarding the volatile profile, Di-hydro-4-hydroxi-2 (3H)-furanone and benzoic acid were the major compounds and alcohols were the most abundant substance type found in the dairy desserts samples added with arrowroot flour. Arrowroot flour presented a great potential as non-conventional food plant ingredient for vanilla dairy dessert formulation as it proportionate a decrease of glycemic index and syneresis index, improving the technological and functional properties of vanilla dairy desserts

**Key Words:** syneresis, glycemic index, arrowroot flour

### M125 Effect of ultrasound processing on physical properties of prebiotic soursop-flavored whey beverage

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This study aimed to evaluate the effect of different ultrasound (US) powers on physical properties of a prebiotic soursop-flavored whey beverage and compare to conventional method of rapid pasteurization (RP). The whey beverage was manufactured with whole pasteurized and homogenized milk (30 g 100 g−1), whey powder (6 g 100 g−1), soursop pulp (15 g 100 g−1), sugar (8 g 100 g−1) and inulin HP (6 g 100 g−1) of DP (degree of polymerization) ≥23. The US was applied in 25-cm3 samples, for 3 min at different powers (200, 400 and 600 W), one sample was submitted to RP (72–75°C for 15–20 s), and one was unprocessed (US 0 W). US effects on whey beverage properties was evaluated, immediately after its manufacturing, using particle size distribution (PSD), color (L* a* b* measurements) and rheological properties (flow curves). US technology presented color difference from US 0 (ΔΕ*) similar to RP (2.1) at 200 W (1.9) (P > 0.34), but significantly higher at 400 W (2.6) and 600 W (2.4) (P < 0.01). All samples presented a pseudo plastic behavior, however, the US increased the flow behavior index (n) and reduced the consistency index (k) (P < 0.01), unlike RP sample which remained unaltered for n (P = 0.73) and for k (P = 0.88). Furthermore, no influence of US power were observed for rheological parameters, including apparent viscosity, which was not influenced by US or RP (P > 0.05). The greater D32 value reduction, compared to US 0 W, was 26.6% at US 200 W (P < 0.02) and for D43 values were 58.1% and 44.2% at US 600 W and RP (P < 0.01), respectively. The PSD value suggest a considerable change in large particles for US 600 W and RP and size reduction of smaller particles for US 200 W. Based at the conditions used in this study, US technology presented effects on the color parameters of soursop-flavored whey beverage; however no effect was observed the rheology behavior.

**Key Words:** soursop-flavored whey beverage, ultrasound, prebiotic

### M126 Physical stability study of a prebiotic soursop-flavored whey beverage formulation

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The objective of this study was to evaluate how the degree of polymerization (DP) of the inulin affects the physical properties of the whey beverage stability. Nine formulations were manufactured using full-fat pasteurized and homogenized milk (30 g 100 g−1), whey powder (6 g 100 g−1), soursop pulp (15 g 100 g−1), sugar (8 g 100 g−1), inulin (6 g 100 g−1) of 2 different DP, GR (DP ≥10) or HP (DP ≥23), and 2 different stabilizers, gellan gum (GG) (0.05 g 100 g−1) or gum acacia (GA) (0.5 g 100 g−1). The ingredients were homogenized with a multiple phase disperser (10 min). The phase separation was evaluated during storage of 24, 48 and 72 h at 6 ± 2°C, using separation index (SI) and light backscatter scan analyzer. Rheological properties were evaluated immediately after its processing. Mean pH values was 5.4 ± 0.1 with no difference among the samples (P > 0.05). It was observed a slight increase of SI through storage time (P > 0.05). The addition of HP inulin was effective to decrease the SI beverage value when compared with control at 72 h (14%, P < 0.05), but GR was not effective (43%, P > 0.05). However, the GG stabilizer was effective to reduce the SI from GR samples (15%). Using the light backscatter scan analyzer it was possible to make backscattering profiles until 3 d of storage, thus, the visual examination of graphics showed longer stability of the HP-GG formulation with increasing of backscattering during the storage time. All samples presented a pseudo plastic flow behavior (P > 0.05), being the consistency index (k) influenced by different formulations, but the addition of only inulin was not significant (P > 0.05). However, the association of inulin and GG greatly influenced the consistency index (P < 0.05). Regards the viscosity values, it was noted an effect of inulin and stabilizer content (P < 0.05), but different DP provided similar results. Overall, the inulin had a great effect on physical stability, but without a stabilizer, a higher DP was more effective and the influence of DP reduced when GG was used.

**Key Words:** prebiotic, soursop-flavored whey beverage, functional food

### M127 Impact of ultrasound processing in bioactive compounds content of a prebiotic soursop-flavored whey beverage

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The study aimed to evaluate the influence of different ultrasound (US) powers in nutritional parameters of a prebiotic soursop-flavored whey beverage, comparing with the traditional dairy method of rapid pasteurization (RP). The whey beverage was manufactured with whole pasteurized and homogenized milk, whey powder, soursop pulp, sugar, inulin and gellan gum. The US processing was realized using 25-cm3 samples, for 3 min and 3 different powers (200, 400 and 600 W), one sample was submitted to RP (72–75°C for 15–20 s) and one was unprocessed (US 0 W). The samples were frozen immediately after manufacturing and stored at −18°C until the analysis. The nutritional parameters evaluated were total phenolic compounds (TPC), ascorbic acid (AA), antioxidant activity (DPPH reduction) and antihypertensive activity (ACE inhibitory values). US 200, 400 and 600 W did not significantly degraded the AA, compared with US 0 W (19, 15 and 14 mg 100 cm3−1, and 18 mg 100 cm3−1, respectively, P > 0.05). However, it was observed a slightly decrease in AA content of US 400 W and 600 W compared with RP (19 mg 100 cm3−1, P = 0.049 and P = 0.027, respectively). The TPC values...
increased with both technologies ($P < 0.05$), and although RP, US 400 and 600 W presented similar values (750, 736 and 777 EAG mg 100 cm$^{-3}$, respectively, $P > 0.05$), it was observed an increased extraction of TPC with US powers. The US processing (200, 400 and 600 W) improved the ACE inhibition (63, 78 and 80%, respectively) and DPPH reduction (21, 21 and 23%, respectively) compared with US 0 W (57 and 18%, respectively, $P < 0.05$), unlike RP (46 and 19%, respectively), that was worse than US 0 W for ACE inhibition ($P < 0.05$) and unaltered for DPPH reduction ($P > 0.05$). Furthermore, antioxidant and antihypertensive activity increased as US power increased as well. Overall, our results suggest a potential use of US technology for extracting and releasing bioactive peptides with antioxidant and antihypertensive activity, thus, improving the functional value of the product.

**Key Words:** ultrasound, bioactive compounds, prebiotic soursop-flavored whey beverage

**M128 Effect of ultrasound processing on microbial inactivation of prebiotic soursop-flavored whey beverage.** J. Guimarães*1, E. Silva2, M. A. Meireles2, E. Esmerino1, and A. Cruz3, 1Universidade Federal Fluminense (UFF), Niterói, RJ, Brazil, 2Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil, 3Instituto Federal de Ciência e Tecnologia do Rio de Janeiro (IFRJ), Rio de Janeiro, RJ, Brazil.

The present study aimed to evaluate the effect of the ultrasound technology (US) on microbial inactivation (MI) of a prebiotic whey soursop-flavored beverage compared with conventional method of rapid pasteurization (RP). The whey beverage was manufactured with whole pasteurized and homogenized milk (30 g 100 g$^{-1}$), whey powder (6 g 100 g$^{-1}$), soursop pulp (15 g 100 g$^{-1}$), sugar (8 g 100 g$^{-1}$) and inulin (6 g 100 g$^{-1}$). The US processing was realized in 25-cm$^3$ samples, for 3 min at 3 different powers (200, 400 and 600 W), being one treatment submitted to RP (72–75°C for 15–20 s) and one was unprocessed (US 0 W), which was used as a control to calculate the log reductions. The whey beverages were submitted to microbial group counts (total and thermotolerant coliforms, aerobic mesophilic bacteria and yeasts counts) and some physico-chemical parameters (pH and zeta potential). Total and thermotolerant coliforms values were <3 MPN/mL in all samples, suggesting good hygienic quality during all processing while for aerobic mesophilic bacteria counts, the samples submitted to RP, US 600 W and US 400 W presented log reductions of 2, 2 and 1 respectively ($P > 0.05$), suggesting that higher US powers influence directly in MI. Finally, the yeasts counts presented absence of significant difference between US and RP ($P > 0.05$), despite the slightly higher reduction in US 600 W and 400 W treatments. pH values remained at 5.4 ± 0.01 regardless of the treatments ($P > 0.05$), while zeta-potential values of US samples ranged from −18 to −19 mV (US 200 and 600 W, respectively, $P < 0.05$), suggesting that the intensity of US powers changed the negative charges of the whey beverage particle surfaces; however no difference was observed when compared with US 0 W and RP (−19 mV for both processing, $P > 0.05$). Overall, US technology presented effective impact on microbial inactivation in a prebiotic soursop-flavored whey beverage.

**Key Words:** prebiotic, soursop-flavored whey beverage, ultrasound

**M130 Whey acerola-flavored drink processed by ohmic heating: Effect on ascorbic acid degradation and color parameters.** L. Cappato1, M. V. Ferreira*1, G. Mercali2, L. Marzak2, and A. Cruz3, 1Universidade Federal Rural of Rio de Janeiro (UFRRJ), Seropédica, RJ, Brazil, 2Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil, 3Instituto Federal de Ciência e Tecnologia do Rio de Janeiro (IFRJ), Rio de Janeiro, RJ, Brazil.

Ohmic heating (OH) consists of the passage of electric current in the food itself, which promotes a fast and homogeneous heating, due to the conversion of the electric energy into thermal by the joule effect, thus resulting in a greater retention of bioactive compounds. The aim of the present work is to evaluate the OH effect and determine the best processing parameters (10, 100, 1000 Hz at 25 V, 45, 60, 80 V at 60 Hz) compared with conventional processing (pasteurization – 64°C/30 min) of acerola-flavored whey beverage (35% pasteurized milk + 65% whey, 30% acerola pulp w/wt) regards the impact on color parameters and ascorbic acid degradation. Samples were collected at the following pre-determined interval times (0, 15, 30, 45, 60, 80, 100 min) to determine the AA degradation kinetics to obtain the D value (time to degrade 90% of the initial concentration). The data were adjusted by
the first-order model \( [C = C_0 \times \exp(-Kt)] \). For colorimetric analysis the parameters hue angle (\( \theta \)), the chromaticity (\( C^* \)) and the color variation (\( \Delta E^* \)) were calculated. The OH performed at 1000 Hz - 25 V (\( D = 675 \) min) and 60 V - 60 Hz (\( D = 759 \) min) presented a lower D value (\( P < 0.05 \)), whereas for the other treatments there were no significant difference in relation to the conventional (\( D = 864.8 \) min). In relation to the color degradation after the pasteurization, a significant difference (\( P < 0.05 \)) in hue and color values was observed. Color parameters changes were observed just for the OH processing parameters (80V – 60 Hz) in relation to the conventional processing (\( \theta = 2.15 \) and 1.13 and \( \Delta E^* = 4.58 \) and 1.31, respectively, \( P < 0.05 \)). Regarding the Chroma values, no OH effect was observed (\( P > 0.05 \)). The findings suggests that OH is presented as a promising technology for the production of whey dairy drinks, which in this context, the knowledge of the best process parameters is a crucial point to guarantee the best retention capacity of AA, without influencing the color aspects.

**Key Words:** ohmic heating, acerola-flavored whey drink,ascorbic acid degradation

**M131 Whey acerola-flavored drink processed by ohmic heating: Rheological behavior, particle size distribution, and microstructure.** L. Cappato1, M. V. Ferreira*1, G. Mercali2, L. Marczak2, and A. Cruz3, 1Universidade Federal Rural of Rio de Janeiro (UFRRJ), Seropédica, RJ, Brazil, 2Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil, 3Instituto Federal de Ciência e Tecnologia do Rio de Janeiro (IFRJ), Rio de Janeiro, RJ, Brazil.

The increase in demand for dairy products has driven industries and research centers to develop new technologies, including ohmic heating (OH), to minimize the deleterious effects of conventional processing. However, the study of the physical properties presents great importance for the knowledge of the effect that the OH processing will result in the quality parameters of dairy food. The present study aims to evaluate the effect of the OH parameters (10, 100, 1000 Hz at 25 V, 45, 60, 80 V at 60 Hz) on the acerola-flavored whey beverage. Rheological behavior (flow curves), physical properties (particle size distribution) and microstructure (optical microscopy) were performed. All the results were compared with the conventional processing (64°C/30 min), which all the samples were performed under the same time \( \times \) temperature profile. OH processing resulted in a pseudoplastic behavior (\( n < 1 \)) and consistency index values (\( k \)) were lower than the conventional (12.66 \( \pm \) 1.31 mPa\( \cdot \)s\(^n\)), which indicates a loss in the viscosity, except for the treatments: 100 Hz-25 V and 80 V - 60 Hz (44.72 \( \pm \) 2.31 and 14.82 \( \pm \) 0.35 mPa\( \cdot \)s\(^n\), respectively). Regarding the particle size distribution, \( D [4.3] \) and \( D [3.2] \) values presented a significant reduction (\( P < 0.05 \)) for treatments: 100 Hz; (\( D [4.3] = 26.1, 21.8, 25.5, 21.9 \)) respectively, compared with the conventional (\( D [4.3] = 30.4 \) \( \pm \) 0.6) while for \( D [3.2] \) values, no significant difference was observed in comparison to the conventional (\( D [3.2] = 3.00 \) \( \pm \) 0.09, \( P > 0.05 \)). Our findings suggest an effect of the OH in the cell disruption and the leaking of the cell material, which may have influenced the increase in the viscosity. The optical microscopy showed changes in the cellular structure regardless of OH parameters used. Overall, the findings can contribute for a better understanding of OH processing in physical and microstructural parameters of acerola-flavored whey beverage.

**Key Words:** ohmic heating, acerola-flavored whey beverage, rheology

**M132 Effect of the ohmic heating in the bioactive compounds (antioxidant capacity and ACE inhibitory peptides) in acerola-flavored whey beverage.** L. Cappato1, M. V. Ferreira*1, G. Mercali2, L. Marczak2, and A. Cruz3, 1Universidade Federal Rural de Rio de Janeiro (UFRRJ), Seropédica, RJ, Brazil, 2Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil, 3Instituto Federal de Ciência e Tecnologia do Rio de Janeiro (IFRJ), Rio de Janeiro, RJ, Brazil.

The fast and homogeneous heating provided by ohmic heating (OH) results in a less thermic intensity, decreasing the degradation of bioactive compounds in dairy foods. This study aimed to evaluate the OH effect (10, 100, 1000 Hz 25 V; 45, 60, 80 V and 60 Hz) on the degradation of bioactive compounds in raspberry-flavored whey beverage. All treatments were performed under the same temperature profiles (64°C/30 min). To evaluate the bioactive compounds the DPPH and FRAP (expressed in Trolox Equivalent/g) and Total Phenolic compounds (expressed in Acid Gallic/g), besides the bioactive peptides using the Angiotensin Enzyme inhibitor (ACE). Regarding the ACE analysis, the results showed that OH increased the enzyme inhibition when compared with the conventional (\( \approx 66\% \)), except for the 45 V–60Hz (\( \approx 58\% \)). An increase in frequency resulted in a higher inhibition. The greater inhibition was observed in the 100 and 1000 Hz (\( \approx 93\% \) and 97%, respectively, \( P > 0.05 \)) compared with the conventional. In the treatments where the voltage were tested, only the 80V (\( \approx 85\% \)) had a significant difference compared with the conventional. Regarding the antioxidant capacity corresponding to the phenolic compounds, it was observed that the conventional treatment resulted in a lower degradation (\( \approx 61\% \)) followed by the 1000 Hz (\( \approx 63\% \)), while for the other samples the degradations were higher when compared with the conventional process. The FRAP assay showed same phenolic compounds as the conventional process (\( \approx 248 \mu M \)) had a less degradation in the bioactive compounds, where the 80V–60 Hz had the worst scenario (\( \approx 196 \mu M \)). For the DPPH analysis, OH resulted in a better stability compared with the conventional (\( \approx 8.5 \mu M \)), where the treatment 80V–60 Hz had the best condition (\( \approx 8.88 \mu M \)), followed by 1000 Hz–25 V (\( \approx 8.86 \mu M \)) and 60V–60 Hz (\( \approx 8.86 \mu M \)). According to the results, OH processing may presents an alternative to conventional processing due to lower degradation in the bioactive compounds in the whey beverages.

**Key Words:** ohmic heating, acerola-flavored whey beverage, bioactive compounds
M133 Comparison of the adhesion characteristics of common dairy spore formers and their spores. S. Jindal and S. Anand*, South Dakota State University, Brookings, SD.

The initial attachment of aerobic spore forming bacteria to the surfaces of dairy processing equipment leads to biofilm formation and biofouling. Although spore formers may vary in attachment, various surface modifications are being studied to develop a surface that is least vulnerable to attachment. The aim of this study was to compare the extent of adhesion of spores and vegetative cells of high-heat-resistant spore formers (HHRS) such as *B. sporothermodurans*, and *G. stearothermophilus*, and thermo-tolerant species *B. licheniformis* and, on both native and modified stainless steel surfaces. Influence of various contact surface and cell surface properties including surface energy, surface hydrophobicity, cell surface hydrophobicity, and zeta-potential on the adhesion tendency of bacteria were compared. The ability of the vegetative cells and spores of different aerobic spore former to attach to native and modified (Ni-P-PTFE) stainless steel surfaces was determined by allowing the interaction between the contact surface, and spores or vegetative cells for an hour at ambient temperature. Hexadecane assay was employed to determine the hydrophobicity of vegetative cells and spores of aerobic spore-forming bacteria, while the surface charge (expressed as zeta potential) was determined using Zeta sizer Nano series instrument. The results indicated higher adhesion tendency of spores over vegetative cells of aerobic spore forming bacteria. On comparing the sporeformers, *B. sporothermodurans* demonstrated greatest adhesion tendency followed by *G. stearothermophilus* and *B. licheniformis*, respectively. As the vegetative cells and spores of *B. sporothermodurans* and *G. stearothermophilus* demonstrated significantly greater attachment as compared with *B. licheniformis* thus it can be interpreted that HHRS show great attachment tendency as compared with thermo-tolerant spore formers. The tendency to adhere varied with the variations in cell surface properties as it decreased with lower cell surface hydrophobicity and higher cell surface charge. On the other hand, modifying the contact surface properties caused the attachment tendency to decrease with the lowering surface energy and increasing surface hydrophobicity.

Key Words: aerobic sporeformer, hydrophobicity, zeta-potential

M134 Evaluating enzyme formulations for biofilm removal from dairy separation membranes. N. Garcia-Fernandez1,2 and S. Anand*, 1Midwest Dairy Foods Research Center; Brookings, SD, 2Department of Dairy and Food Science, South Dakota State University, Brookings, SD.

Enzymatic cleaners are generally used during cleaning in place (CIP) processes to improve the cleanability of dairy separation membranes. Many of the commercial enzymatic cleaners, however, contain general action enzymes, not specifically designed to degrade recalcitrant biofilm matrices. In our previous screening, some enzymes showed a greater biofilm removal on reverse osmosis (RO) membranes, as compared with commercial enzyme-based cleaners. This project aims to evaluate the efficacy of a protease (EC 3.4.24.31, named S1), an alkaline phosphatase (EC 3.1.3.1, S2), and a lactase (EC 3.2.1.23, S3) in removing biofilms on diverse dairy separation membranes, for RO (KMS HRX: TFC polyamide), and Ultrafiltration (UF) processes (HFK-131: polyethersulfone, and HFM-180: polyvinylidene difluoride) (Koch membrane systems, Wilmington, MA). Forty-eight-hour-old mixed species biofilms, constituting common dairy sporeformers *Bacillus licheniformis*, *B. coagulans*, *B. sporothermodurans*, and *Geobacillus stearothermophilus*, were developed on the respective membranes (4cm²) under lab conditions. Tryptic soy broth at 37°C served as the immersion medium. All enzymes and buffer solutions used were prepared following manufacturer recommendations (Sigma-Aldrich, Saint Louis, MO). Membranes in triplicates were rinsed with sterile distilled water, followed by separately cleaning for 45 min at 55°C with individual enzyme solutions at 0.2 U/mL (S1), 0.1 DEA/mL (S2), and 0.01 U/mL (S3). All assays were repeated 3 times, and data were statistically analyzed. The residual viable cell numbers were estimated by swabbing, and plating on plate count agar. Percentage reductions in viable counts for S1, S2 and S3, respectively, were 99.93, 99.69 and 90.74% cfu/cm² for biofilms formed on RO, 99.99, 99.91 and 99.40% for UF HFK-131, and 98.23, 0.0 and 48.0% for UF FM-180. In conclusion, S1 was the most effective enzyme for reducing multispecies biofilms on all membrane types. Additionally, the most resistant biofilms were observed on HFM-180. These findings suggest that for better cleaning of any membrane material, it will be critical to design a specific enzyme-based formulation, depending on a particular biofilm matrix.

Key Words: membrane cleaning, biofilm, enzyme


In dairy manufacturing plants, biofouling of separation membrane represents a serious quality issue. Besides hydrodynamic conditions, the adhesion of pioneer bacteria and the formation of biofilms during filtration of dairy fluids could be influenced by membrane material properties. Consequently, the objective of this study was to characterize the impact of 3 different membrane materials (polyethersulfone [PES], polyvinylidene fluoride [PVDF], polyacrylonitrile [PAN]) on the diversity of early bacterial communities formed on membranes after ultrafiltration (UF) of dairy fluids. A laboratory-scale crossflow filtration system equipped with parallel modules, each containing with a different membrane of 42 cm², was used for UF of pasteurized skim milk and cheese whey. The UF system was operated at 50°C during 5h- and 20h-periods in the concentration mode with the feed maintained at 10°C between passages in the UF system. Membranes were cleaned with an alkaline solution prior and following each UF experiments. The bacterial diversity was assessed on cleaned membrane coupons and in filtered fluids after UF of 5 h and 20 h through a metabarcoding approach targeting the 16S rRNA gene. The bacteria numeration in samples was also estimated using qPCR targeting the same gene target. Bacterial genus ratios within the biofilms were found dependent of the composition of the membrane material used during UF of milk and whey. Interestingly, the qPCR quantification revealed a similar number of bacteria for each condition (P > 0.05). According to a PERMANOVA analysis, the diversity observed on membranes was dependent of the nature of the filtered fluids and the filtration duration, explaining respectively 53.24% and 21.75% (P = 0.002) of the variances among bacterial communities. Consequently, this study suggests that the membrane material may affect the biofilm formation on UF membranes, but other operational parameters such as...
the duration of filtration between cleaning cycles should be prioritized to control the biofouling issue.

Key Words: ultrafiltration, membrane material, biofilm

M136 Investigation of Escherichia coli survival in powdered whole goat milk during four months of storage. B. I. Davis, A. Siddique, A. K. Mahapatra, and Y. W. Park*, Fort Valley State University, Fort Valley, GA.

Low water activity (aw) is essential for extending shelf life as well as attaining microbiologically safe foods, such as in dehydrated milk products. Certain harmful microbes can enter the food chain opportunistically during processing and survive in dehydrated foods, causing serious concerns over food safety. The objective of this study was to investigate the survivability of Escherichia coli in powdered goat milk (PGM) at 4°C and 22°C during 0, 2 and 4 mo storage. Three different lots of commercial whole goat milk powder products were purchased from a local retail outlet, and the total amount of each lot was divided into 2 equal quantities to assign them to 2 treatment groups as control and E. coli inoculated groups. Ten grams of the experimental PGM samples were inoculated with 50 µL of E. coli K12. Both of the treated and control samples without inoculation of the pathogens were subjected to the 2 temperature and 3 storage treatments. All experimental PGM samples were microbiologically analyzed according to the manufacturer’s procedure (3M Center, St. Paul, MN). The PGM samples in duplicates were serially diluted, plated on the 3M Petrifilm EC plates, and colonies were counted after 48 h incubation at 37°C. The initial inoculation rate was at least 8 log cfu/g for each sample. Results showed that the inoculated experimental PGM contained average 5.01 log cfu/g E. coli in the initial samples. Mean E. coli counts of 4 and 22°C at 0, 2 and 4 mo storages were: 5.01, 4.16, 3.43, 1.85; and 3.77, 1.48 cfu/g, respectively, indicating that E. coli counts significantly (< 0.01) decreased during 4 mo storage period. There were significant (P < 0.01) differences in E. coli counts between temperatures and between storage periods for both of main factors. E. coli counts of the powder milk samples were not affected by batch effect up to 2 mo, but did affect at 4 mo storage. It was concluded that the survivability of E. coli in the powdered whole goat milk significantly decreased as the storage time advanced.

Key Words: Escherichia coli, powdered goat milk, storage

M137 Evaluation of relationship between water activity, pH and Escherichia coli survival of powdered whole caprine milk during 4 months of storage. B. I. Davis*, A. Siddique, and Y. W. Park, Fort Valley State University, Fort Valley, GA.

Water activity (aw) is important indicator for food quality, safety and storage stability, where aw is directly related to bacterial growth, especially when aw is above 0.90. Water activity in relation to bacterial counts of dehydrated bovine milk may have been studied extensively, while no report has been available for the correlation between aw, pH and Escherichia coli survival in powdered caprine milk (PCM). The objectives of this study were to determine aw, pH and Escherichia coli counts of PCM, and evaluate correlations among these parameters at 4°C and 22°C for 0, 2, and 4 mo storage. Three different lots of commercial whole PCM were purchased at a local retail outlet and divided the total amount of each lot into 2 equal portions to assign them to 2 treatment groups: control and E. coli inoculated groups. Ten grams of the experimental PGM samples were inoculated with 50 µL of E. coli K12, and control samples without inoculation of the pathogens were subjected to the temperature and storage treatments. Water activity was measured by AquaLab aw meter (cx-2; Decagon Devices, Pullman, WA). All experimental PGM samples were also analyzed for E. coli counts according to the manufacturer’s procedure (3M Center, St. Paul, MN). Results showed that aw values were significantly (P < 0.05) reduced, where mean aw for 0, 2 and 4 mo storage were 0.266, 0.251, 0.243; 0.291, 0.266, 0.219, respectively. No differences in pHs were found between 2 temperature groups, while pHs were slightly higher at 2 mo storage. Mean E. coli counts of 4 and 22°C at 0, 2 and 4 mo storages were: 5.01, 4.16; 3.43, 1.85; and 3.77, 1.48 cfu/g, respectively, indicating that E. coli counts significantly (< 0.01) decreased during 4 mo storage. E. coli counts were significantly correlated with aw, having r = −0.857 at 4°C and −0.771 at 22°C, respectively. Correlations between levels of pH and aw at both temperature treatments were also negatively correlated. It was concluded that E. coli counts of the powdered goat milk were negatively correlated with levels of water activity as the storage time advanced.

Key Words: water activity, Escherichia coli, powdered goat milk


The objective of this research was to determine the concentration of lactose oxidase (LO) needed to activate the lactoperoxidase system (LPS) in skim milk and assess its ability to inhibit the growth of Pseudomonas fragi, a milk spoilage strain. LO oxidizes the lactose in milk and produces hydrogen peroxide needed for the activation of the antimicrobial system. Seven treatments were evaluated at 4 and 21°C: the control, and three levels of LO or LPS+LO (0.012, 0.12, and 1.2 g/L). The base LPS was obtained adding 30 mg/L of bovine lactoperoxidase and 14 mg/L of NaSCN to ultra-pasteurized skim milk. Three independent trials of the experiment were performed and the microbial reduction was calculated for 1, 4, and 7 d. The effect of treatment, temperature, time, and their interactions was determined through a multi-factorial analysis. Also, for both temperatures, a one-way ANOVA was conducted separately for each day to determine the significance of the treatments followed by a Tukey’s test. The results showed that treatments were more effective at refrigeration temperature (P < 0.001). At 4°C, LO at 0.12 and 1.2 g/L showed a significantly higher reduction than the control (P < 0.001) when added alone and combined with the system for every time point. An increase in the concentration of LO caused higher reductions of P. fragi at d 7, achieving a >2.93 log cfu/mL reduction for the 1.2 g/L treatments. At 21°C, treatments with a concentration of 1.2 g/L of LO achieved a reduction of >2.93 log cfu/mL, while under the other conditions reductions were not significantly different from the reduction observed for the control (P > 0.05). Results confirm that lactose oxidase can be used to inhibit the growth of P. fragi and represents a new way to extend the shelf-life of dairy products. The application of LO serves as an opportunity to reduce food waste and for the dairy industry to benefit from a longer shelf-life while meeting the consumers’ demand for clean label products. Further research will assess the inhibition of other spoilage microorganisms in different dairy products, as well as the effect of the inoculation level and thiocyanate concentrations.*

Key Words: lactoperoxidase, lactose oxidase, spoilage

*Corrected abstract

M139 Selective primer development for rapid detection of the gas-producing non-starter bacterium Lactobacillus wasatchensis. M. Culumber1, T. Oberg2, T. Allen2, F. Ortakci2, C. Oberg2*, and D.
Lactobacillus wasatchensis is a slow-growing non-starter lactic acid bacterium (NSLAB) recently implicated in gassy defects in aged Cheddar cheese. This organism has been detected in cheeses from 7 cheese processing facilities in different regions of the United States and is of significant concern to cheese producers. Rapid detection of Lb. wasatchensis would allow for better control of the organism, and help determine where it is entering the manufacturing process. A set of 16S rRNA primers were developed using NCBI Primer-Blast against the Lb. wasatchensis genome and selected based on product length, melting temperature, and primer self-complementarity. In silico analysis against the NCBI database indicated the primers should have high specificity for Lb. wasatchensis. PCR optimum conditions were determined experimentally with Lactobacillus casei and Lactobacillus curvatus DNA as non-target template. To determine specificity, the primers were tested against DNA extracted from 22 different common NSLAB, including strains of Lb. wasatchensis isolated from cheese and the original Lb. wasatchensis WDC04. Only strains identified previously as Lb. wasatchensis amplified with the primers. Even the mostly closely related NSLAB species (such as Lb. curvatus) to Lb. wasatchensis could be differentiated with these primers. DNA from all isolates amplified using standard bacterial 16S rRNA primers. The new primers, LW86Fa and LW258Ra, will be used in traditional and real-time PCR for rapid detection of Lb. wasatchensis in gassy cheeses and the cheese processing environment. Rapid molecular detection will help diagnose and track Lb. wasatchensis contamination, and help control the occurrence of gassy-cheese defects.

Key Words: Lactobacillus, gassy defect, cheese

M140 Effect of bio-protective lactic acid bacteria cultures on Lactobacillus wasatchensis. A. Lavigne1, S. Smith1, C. Oberg*1, I. Bowen2, and D. McMahon2, 1Weber State University, Ogden, UT, 2Utah State University, Logan, UT.

The nonstarter lactic acid bacteria (NSLAB) Lactobacillus wasatchensis can cause late gassy defect when it grows to high numbers during Cheddar cheese storage. A potential strategy for preventing such growth is incorporation of specific lactic acid bacteria strains (termed bio-protective LAB) into the cheese during manufacture, which may specifically inhibit growth of Lb. wasatchensis. Determination of inhibition by common NSLAB lactobacilli and potential bio-protective LAB (BPLAB) strains against Lb. wasatchensis was done using the spot test along with the agar flip method. MRS agar supplemented with 1.5% ribose (MRS-R) was inoculated with each NSLAB or BPLAB using the spread plate method and incubated anaerobically at 25°C for 48 or 72 h. Inoculated agar was then flipped over and either Lb. wasatchensis WDC04 or CGL04 swabbed on the newly exposed agar plates. Examining the antagonism between bio-protective cultures and NSLABs for Lb. wasatchensis strains allows for selection of lactic acid bacteria strains that could inhibit this problematic bacterium during cheese ripening.

Key Words: lactic acid bacteria, Lactobacillus, gassy defect

M141 The antibacterial effect of addition of citrulline in fermented milk against foodborne pathogens. S. W. Ho* and Nangendra P. Shah, The University of Hong Kong, Hong Kong, China.

LAB contribute to antibacterial effect against pathogens by generating antimicrobial agents, however, a sufficient cell concentration is required. Citrulline, a non-protein amino acid, provides extra energy to LAB by arginine deiminase pathway to improve cell growth. Citrulline is also a precursor of nitric oxide (NO), which plays an important role in protecting from enteric pathogens. The aims of this study were (1) to investigate the effect of adding citrulline on NO production by LAB and its antibacterial activity, (2) to investigate the antibacterial mechanisms of LAB against foodborne pathogens, and (3) to examine the stimulating effect of NO production in the intestinal epithelial cells and its anti-adhesive effect. The selected LAB were incubated with 0, 0.1, or 0.2% of citrulline in de Man, Rogosa, Sharpe (MRS) broth and in milk at 37°C for 18–20h. The antimicrobial activities against the pathogens were determined by measuring the diameter of the zones of inhibition. The NO production ability of LAB was determined by using metmyoglobin supplemented MRS plates and the bacteriocin-like inhibitory substances (BLIS) production ability of LAB was determined by eliminating the effect of acids and hydrogen peroxide. The stimulating effect of NO production by citrulline and LAB and the anti-adhesive effect were evaluated using IPEC-J2 cell line. The selected LAB when fermented with citrulline addition in MRS and fermented milk with Lactobacillus helveticus ATCC 511 significantly inhibited the tested pathogens (all P < 0.001). Milk with added citrulline when fermented with L. helveticus ATCC 511 and L. bulgaricus ATCC 756 showed the dose-dependent effect on the inhibitory activity of Shigella sonnei ATCC 25931. None of the selected LAB were capable of producing NO for converting metmyoglobin to nitrosomyoglobin in MRS-Mb agar. Addition of citrulline to milk fermented with LAB might enhance the antibacterial effect of LAB against selected pathogens in vitro. The cell culture work has shown some interesting data with regard to stimulation effect of NO production in the intestinal epithelial cells and its anti-adhesive effect.

Key Words: lactic acid bacteria, citrulline, antibacterial activity

M142 Influence of the antimicrobial myrrh on yogurt culture bacteria over yogurt shelf life. M. Alhejaili*, D. Olson, M. Janes, C. Boeneke, and K. Aryana, Louisiana State University Agricultural Center, Baton Rouge, LA.

Myrrh is a natural flavoring substance approved by FDA as a food flavor and essential oil. Also, myrrh has antibacterial and antiinflugal activity against pathogens. The objective was to determine the effect of myrrh on Streptococcus thermophilus and Lactobacillus bulgaricus counts, pH and titratable acidity of yogurt during 5 wk of storage. Myrrh dispersion was prepared and incorporated at 1% vol/vol yogurt mix. A control with no myrrh was also prepared. Three replications were conducted. Streptococcus thermophilus was enumerated using Streptococcus ther- mophilus agar with aerobic incubation at 37°C for 24 h, and Lactobacillus bulgaricus was enumerated using MRS agar adjusted to pH 5.2 with

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Lac-fermented, antimicrobial, yogurt bacteria can survive in the presence of myrrh within yogurt. Little to no change in yogurt pH and titratable acidity, yogurt culture around 1.2% expressed as lactic acid for both yogurt types throughout other at any given week. The titratable acidity values remained steady were significantly lower for the myrrh yogurts than for the control, the yogurt (5.3 log cfu/mL). Although the log counts for L. bulgaricus not significantly different than S. thermophilus counts in yogurt containing myrrh (5.4 log cfu/mL) were significantly lower for the myrrh yogurts than for the control, the yogurts containing myrrh was significantly higher than the control yogurt, but their pH values were within 0.1 pH units of each other at any given week. The titratable acidity values remained steady around 1.2% expressed as lactic acid for both yogurt types throughout the storage period with no significant differences between them. With little to no change in yogurt pH and titratable acidity, yogurt culture bacteria can survive in the presence of myrrh within yogurt.

Key Words: fermented, antimicrobial, yogurt

M143 Influence of the food matrix on the viability of Lactobacillus casei and Lactobacillus fermentum strains. B. M. Salotti-Souza, T. F. Borgonovi, and A. L. B. Penna*, São Paulo State University, São José do Rio Preto, SP, Brazil.

The consumption of probiotic products, which is proven to provide health benefits, has increased significantly in the last few years. A proper selection of strains and food matrix should be conducted for the processing of probiotic food products, because certain components in the food matrix may interact with these probiotics, altering their functional performance. Therefore, the application of probiotic strains in different food matrices could represent a great challenge to maximize their effectiveness. This research aimed to evaluate the viability of potentially probiotic strains in fermented milk prepared using different matrices. L. casei SJRP38 and L. fermentum SJRP43, previously selected by their good technological features, safety and high probiotic potential, were evaluated and used in coculture with the commercial strain of S. thermophilus TA040 for fermentation. The influence of matrices: M1 - reconstituted skim milk powder (RSMP) + 7% sucrose and M2 - RSMP + 7% sucrose + 5% flaxseed (Linum usitatissimum L.) were evaluated on the acidifying kinetic parameters and viability of the strains under simulated gastrointestinal (GI) conditions during refrigerated storage. Times to reach the maximum acidification rate, pH 5.0, and pH 4.6 (end of fermentation) were influenced by the food matrix. M2 had a negative effect on the fermentation time, causing an increase of up to 3 h for finishing the process. All strains in the M1 matrix survived well (>7 log cfu/mL) during the simulated GI, which is equivalent to the passage of bacteria through the human GI tract. M2 affected the counts of L. casei SJRP38, L. fermentum SJRP43, and S. thermophilus TA040, compared with the M1 matrix, and they had a reduction of up to 3 log cfu/mL after intestinal passage. Additionally, the population reduction after the assay was influenced by the storage period for both matrices. Considering the overall results, Lactobacillus casei SJRP38 in coculture with S. thermophilus TA040 in the M1 matrix presented a high probiotic potential for further application in functional fermented products.

Key Words: probiotic viability, acidifying kinetic parameters, functional food

M144 Properties of Enterococcus faecium strains isolated from traditional Carpathian ewe’s cheese. O. Tisaryk*, I. Slyvka1, L. Musiy1, I. Kushnir1, and T. Bocer2, 1Lviv National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine, 2Rzeszow University, Rzeszow, Poland. The purpose of our work was to study the technological properties and sensitivity to antibiotics of the 4 strains of Enterococcus faecium isolated from traditional Carpathian cheese. These strains are classified as Enterococcus faecium based on the microbial and genotypic properties (RAPD-PCR, RFLP-PCR, sequence 16S RNA), but are not registered in Gene Bank as a nucleotide sequence. The strains were labeled as SB20, SB18, SB6, SB12. The studies included morphological characteristics, optimal growth temperature, the ability to produce CO2 from glucose, hydrolysis of arginine, catalase activity and fermentation of spectrum carbohydrates. Technological properties were evaluated as the ability to form lactic acid and the ability to grow in the presence of 2, 4, and 6.5% NaCl. The sensitivity to antibiotics (11 group) was determined by disc diffusion method. It was established that the strains SB20, SB18, SB6, SB12 were gram-positive cocci, grew well on MRS at temperatures of 15–45°C, did not ferment fructose, raffinose, xylose and sorbitol, were catalase negative, did not form CO2 from glucose, hydrolyzed arginine, grew in the environment of 6.5% NaCl. The acidity of skim milk increased to 80–82 °T and the pH decreased to 5.1 upon 24 h fermentation. It was established that all strains of Enterococcus faecium were sensitive to a wide range of antibiotic (penicillin, makrolides, tetracyclines, fluoroquinolones, cephalosporins, nitrofurans, chloramphenicol, glycopeptides, polimikany, rifampicin) except for aminoglycosides (gentamicin, streptomycin, kanamycin). The natural resistance to aminoglycosides is explained by the absence of a system transfer of antibiotics through the cell membrane by anaerobic Enterococcus faecium. It was concluded that enterococci strains SB20, SB18, SB6, SB12 are showing good technological properties and high sensitivity to antibiotics of all groups and may be considered as potentially promising for the industry, but further research on their virulence and pathogenicity is required.

Key Words: Carpathian cheese, Enterococcus faecium, antibiotic resistance


During milk fermentation with lactic acid bacteria (LAB) can be produced biologically active peptide sequences known as bioactive peptides. One of the most important biological activities is angiotensin-converting enzyme (ACE) inhibitory activity. The ACE-inhibitory activity depends on the fermentations conditions (temperature, pH, and inoculum). To evaluate the effect of some fermentation conditions on ACE-inhibitory activity a central composite design was used. Previously, 3 probiotic strains Lactobacillus plantarum, L. pentosus and L. acidipiscis were isolated from double cream cheese produced in the state of Chiapas (Mexico). These probiotic bacteria were shown to generate an ACE-inhibitory activity (more than 50%) in vitro tests. Lactobacillus plantarum was chosen for evaluating the effect of temperature (X1), initial pH (X2) and inoculum concentration (X3) on the generation of ACE-inhibitory activity (Y). This study aims to optimize the ACE-inhibitory activity during milk fermentation by Lactobacillus plantarum using response surface methodology (RSM). For the optimization of fermentation process, a central composite design was used. ACE-inhibitory activity (response variable) was measured by the Cushman and Cheung method at initial and final fermentation time (16 h). The equation for the proposed model and model parameters where calculated with NCSS 11 Data Analysis Software. The mathematical model for the generation of
ACE-inhibitory activity of fermented milk with *Lactobacillus plantarum* was the following: 

\[ Y = 524.99 - 122.70X_1 + 344.85X_2 + 186.79X_3 + 1.66X_1^2 - 32.33X_2^2 - 0.86X_3^2 + 7.47X_1X_2 + 3.81X_1X_3 - 89.00X_2X_3 - 0.100X_1^2X_2 - 0.10X_1^2X_3 + 6.03X_2^2X_3. \]

The results of regression analysis showed that initial pH was the most important factor positively affecting the ACE-inhibitory activity. Other factors significantly affecting the activity were inoculum and temperature (negative correlation). This mathematical model predicted the ACE-inhibitory activity in 86.99% of the cases.

**Key Words:** *Lactobacillus plantarum*, ACE-inhibitory activity, optimization
The University of Missouri Extension Dairy Team has a rich history of providing educational programming to the dairy industry. However, surveys suggest the majority of participants involved are male. Additionally, women dairy operators in Missouri have recently eroded. The 2012 census of Missouri Agriculture reflects a sharp decline of 39% of women dairy operators when compared with 2007. Women who have no off-farm income or women who work <99 d off farm have decreased at a slower rate, 9% and 2%, respectively. The establishment of the “Women in Dairy” program targets women as primary operators and/or active women participants on the farm to address this educational gap. The objectives of the program were to provide a non-confrontational learning environment for women operators to obtain best management practices (BMP). Program content delivered by University of Missouri extension specialists included topics selected by a producer panel: calf care, farm succession-planning, reproduction and stress management. Many women participants reported plans for implementing BMPs on the farm. Post-program surveys were collected at monthly events throughout the year. Sixty-one surveys were returned (48.4% response rate) and reflected a 57% increase in understanding of farm succession and a 94% increase in knowledge and skill for dealing with stress and stressful situations. Altered from traditional extension format of the program delivery, meetings deliberately engage interaction. Women participants reported to enjoy: “the ability to chat freely” and to “learn so much (while) meeting other dairy wives.” The comradeship of the group is tangible, yet difficult to quantify. By educating a previously underserved audience, we anticipate increased productivity of Missouri dairy farms. Through education, women operators will become more effective contributors to their operations and therefore the families’ livelihood.

Key Words: women, underserved, extension

We developed a nonlinear programming model that selects the optimal cropping plan and diet ration to maximize farm income over feed cost (IOFC) or minimize greenhouse gas emission (GHG) in a representative 200-lactating cow, 100-ha south-central Wisconsin farm. Nutrition requirements for 6 cow-groups were formulated according to the 2001 Dairy NRC equations. In each of 25 weather scenarios, farm-produced feed, forage quality, and feed production costs were simulated with the Integrated Farm System Model using 25-year daily weather data (1986 to 2010). Feed prices, collected as the monthly market prices from the Understanding Dairy Market website during 2015 and 2016, were randomly assigned to each scenario. The model contained 3 sections: (1) Maximizing IOFC under a fixed or flexible cropping plan; (2) Minimizing farm GHG emissions under the fixed cropping plan; (3) Maximizing IOFC by constraining the GHG emissions. Farm IOFC included milk and surplus feed sale, feed production cost, and feed purchasing costs. Hedging decisions were included in the first section to compare the difference from contracting feed or milk prices with different cropping plans. The optimal solution maximized the total IOFC across 25 scenarios through the expected utility theory. Aggregated IOFC across 25 scenarios was $8.31/cow per d with the original cropping plan of 54.6 ha of corn and 45.4 ha of alfalfa while the GHG emission was 1.33 kg CO2eq. per kg of FPCM. The model chose to produce at the greatest production for all scenarios with the original cropping plan. Diet formulation and purchasing strategies changed for each weather scenario to maximize IOFC according to farm-grown feed condition. Flexible optimal cropping plans for each scenario improved IOFC slightly (0.2–0.4% depending on risk attitude and elasticity); however, incorporating both flexible cropping plans and commodity hedging improved farm IOFC by 16%. The minimum GHG emission was 1.20 kg CO2eq. per kg of FPCM with IOFC at $7.04/cow per d. The farm reduced milk production and changed the rations in some groups and scenarios to minimize GHG emission. By increasing the upper limit of GHG emission from the minimum emission in the third section, farm IOFC increased with a declining rate.

Key Words: whole-farm optimization, feed allocation, income over feed cost

In 2014, the National Animal Health Monitoring System reported that roughly 24.1% of all cows in the top 17 dairy producing states suffered from clinical or subclinical mastitis. Many milk processors award producers with bonus incentives for reaching higher milk quality. Most dairymen are aware of their bulk tank somatic cell count (SCC), however, what they lack is a way of determining how much monetary loss is incurred by not receiving a milk quality bonus. The Dairy Focus SCC Calculator (DFSCCC), which is an Excel-based program, allows producers to analyze their test day milk numbers and take appropriate action regarding SCC. The main goal of the DFSCCC is to assist dairy producers in making management decisions on an individual herd level, which will improve overall health and decrease economic losses due to mastitis. The DFSCCC allows producers to identify cows in the herd that are contributing the highest percentage to the bulk tank SCC. Also, the calculator identifies cows that have chronic or new cases of mastitis by sorting cows by highest current and previous test day SCC. The program also includes an ‘Economic Gains’ table which allows the user to view the differences between bulk tank values with and without high SCC cows. These values are influenced by the bulk tank milk amount, bulk tank SCC, current milk price, and milk quality bonuses per CWT if a SCC parameter is achieved once certain cows are removed. One case showed that if a producer removed one high SCC contributing cow from the bulk tank, that their pounds of milk shipped per month would be decreased by 1,905 kg, while the monetary value of the milk shipped increased by $10,370 per month. It is important to note that the high SCC milk that is no longer used in the bulk tank could be used for alternate purposes, such as calf feeding. The DFSCCC is very easy to operate and is free to download under the ‘Tools’ page at www.dairyfocus.illinois.edu. There are currently versions available for Dairy Comp 305 and PCDart, as well as a version for dairymen who prefer to enter data manually.

Key Words: SCC, mastitis, milk quality
The Penn State Extension dairy team has worked with 143 dairy operations consistently in the past 5 years to develop cash flow plans, monitor income over feed costs and cost of production. From this group, 50 farms were selected to test corn silage quality, fecal starch and milk urea nitrogen and evaluate its impact on farm profitability. Forty-four farms completed their actual cash flow plan for 2013 and sampled their corn silage in the fall of 2013 and spring of 2014. Producers responded to questions related to corn hybrids planted and feeding management practices. Additional farms were added for 2014–2015. The total number of farms completing the project was 56. Farms utilized between 1 to 13 different corn hybrids and the process for selection ranged from the cheapest seed to crop yields. Quality parameters such as neutral detergent fiber and starch digestibility did not routinely factor into the decision process. Farms incorporating best feeding and cropping management practices showed a 5.8-lb milk increase versus their counterparts. Over the 2-year period milk urea nitrogen and fecal starch levels consistently fell within recommended parameters and there was no association to forage quality or feeding management practices. Average changes in 7-h starch digestibility fall to spring tended to increase for farms that had the same corn hybrid and same structure during the seasonal sampling period (M = 6.5 SD = 5.01), which is expected and illustrated in controlled research studies. However, this trend was not observed for farms that had either hybrid blends or that changed hybrid and or structure during the sampling period (M = 1.8 SD = 4.85). They had more varied changes in starch digestibility than the same farms F(1,50) = 7.135, P = 0.01. Forage quality and quantity are the foundation for developing successful and profitable rations. Producers benefit from advisors that understand cropping, feeding and economics to help them make smarter decisions. There are opportunities for producers to examine more closely hybrid selection decisions and evaluate how quality parameters affect animal production and cash surplus.

**Key Words:** forage quality, income over feed cost, feed practices

### Table 1 (abstract M150). Average and range of gross milk price ($/cwt) and total milk cow feed cost ($/cwt) for 44 Pennsylvania dairies, 2013-2015

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**M149**  Education and decision support strategy for farm-level economic and environmental assessment of dairy feed-focused best management practices. T. J. Beck2, R. C. Goodling1, M. M. Haan3, V. A. Ishler1, R. D. Weaver1, and H. A. Weeks2,4, 1The Pennsylvania State University, University Park, PA, 2Penn State Extension, Carlisle, PA, 3Penn State Extension, Leesport, PA, 4AgChoice Farm Credit, Mechanicsburg, PA.

**M150**  Feed management practices and corn silage quality effects on income over feed cost. T. J. Beck2, R. C. Goodling1, M. M. Haan3, V. A. Ishler1, R. D. Weaver1, and H. A. Weeks2,4, 1The Pennsylvania State University, University Park, PA, 2Penn State Extension, Carlisle, PA, 3Penn State Extension, Leesport, PA, 4AgChoice Farm Credit, Mechanicsburg, PA.

Feeding and cropping management practices are critical to the profitability of a dairy business. In a 2015 cash flow plans summary, dairy farm breakeven milk price ranged from $12.23/cwt to $38.72/cwt (n = 107). Between 2013 and 2015, 60 farms were sampled 4 times over 2 years for corn silage, fecal starch, and milk urea nitrogen (MUN), and surveyed about best management practices. 44 of those farms provided actual income and expenses for their dairy enterprise for all 3 years. The objective of the project was to determine the affect corn silage quality and feed management practices on income over feed cost. Table 1 depicts the average, standard deviation, and range of gross milk price and total milk cow feed cost (purchased and home raised) per cwt for each year and the 3 year average. Gross milk price and total milk cow feed cost varied both within years and across years. There was greater variation in total milk cow feed cost among the 44 farms than in gross milk price. The next step was to evaluate the top and bottom 25% herds for income over feed cost and their feed management practices and corn silage quality. The top 25% had the highest percentage of implementation of feeding management practices as compared with the bottom 25%. The top 25% tended to have slightly better corn silage in terms of higher average dry matter percent, 7 h starch digestibility, and 30 h NDF digestibility both in the fall and spring sampling periods. During the next phase of this project, a more complete analysis of corn silage within rations will be analyzed with the addition of actual dry matter intakes and an analysis of the total mixed ration.

**Key Words:** feed cost, milk price, profitability
Near-infrared reflectance spectroscopy (NIRS) analysis is based on the development of calibration equations that relate constituents and the infrared information spectrum. This study compares 2 chemometric tools for developing NIRS prediction models: the GLOBAL modified partial least squares and the recently calibration strategy known as LOCAL. The LOCAL procedure is designed to select, from a large database, samples with spectra resembling the sample being analyzed. A multispecies data sets of 2,448 forage samples (1,872 grass forages, 315 legume forages, and 261 forage trees) were used for the prediction of crude protein (CP), crude ash (CA), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). The samples were randomly divided into a calibration set (n = 2204) and a validation set (n = 244). Spectra were collected using a Foss NIRSystems model 6500 scanning VIS/NIR spectrometer, each spectrum was collected in the range of 1108–2492 nm every 2 nm, the spectra were normalized by standard normal variate and detrend, transformation followed by a first order derivation (1,4,4,1; first derivative, 4nm gap, 4 points of first smoothing, 1 point of second smoothing). Calibration performance for each model was assessed by standard error of calibration, coefficient of determination of calibration, standard error of cross-validation, coefficient of determination of cross-validation, and residual predictive deviation. LOCAL calibration reduced the bias and produced a significant decrease in the standard prediction error compared with the GLOBAL calibration; CP (0.86 vs 0.99), CA (0.68 vs 0.79), NDF (1.29 vs 2.71), ADF (1.78 vs 2.02), and ADL (0.87 vs 1.23). The coefficient of determination values were improved using the LOCAL strategy exceeding 0.90 for all chemical constituents. The use of LOCAL algorithm accurately predict the composition of forages and could offer a practical way to develop a robust equation taking into account the biodiversity of Colombian forage resources.

Key Words: tropical forage, nutritional value, fiber content

M152 Characterization of forage resources of Colombian highlands grazing systems using LOCAL algorithm with near-infrared spectroscopy. C. Ariza-Nieto1, B. Mojica2, O. L. Mayorga1, A. Sierra1, E. Mancipe1, J. Vargas1, and G. Afanador2, CORPOICA, Bogota, Colombia, Universidad Nacional de Colombia, Bogota, Colombia.

Fiber fractions and protein content are important chemical characteristics of forage resources because of their close relationship with factors affecting the performance of dairy cows. Advances in methodologies for evaluating these characteristics and the use of indirect methods such as the near-infrared spectroscopy (NIRS) promote the chemical characterization of forage as a fundamental tool to assess the efficiency of milk production under specific conditions in production. In this study, crude protein (CP), crude ash (CA), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were estimated using the NIRS LOCAL algorithm in the spectrum of 1100–2500 nm every 2 nm using the FOSS NIRSystems 6500. Two hundred seventy forage resources were contextualized in terms of their topographical location (wavy, slope and flat), pasture harvest season or cut grass (rainfall, drought, rainfall-drought transition, drought–rain transition), the fertilization (yes or no) and altitude. The topography affected the CP level of the forage resources, being this lower in the slope (12.8%, P < 0.05). Harvest season affected NDF and ADF (P < 0.05). The NDF level was higher in the rainfall-drought transition compared with the drought-rain transition (57.5 vs 59.6%, P < 0.05). The ADF level was higher in the drought-rain transition compared with drought (29 vs 27.7%, P < 0.05). Fertilization affected levels of CP, CA, NDF, and ADL (P < 0.05); CP was higher when fertilization was applied to the grasslands (14.5 vs 11.4%, P < 0.05), while NDF was lower when fertilization was applied (59.2 vs 57.7%, P < 0.05). The CA level was also higher when fertilization was applied (10.6 vs 9.5%, P < 0.05), while ADL level was lower when fertilizer was applied (30.7 vs 29.4%, P < 0.05). Concentration of CP was affected by the altitude where the value was higher for samples above 2500 m above sea level. (14.2%, P < 0.001). At this level, the concentrations of NDF (55.9%) and ADL (5.2%) were lower (P < 0.001). It was concluded that the most critical variables in the determination of forages quality in dairy feeding systems are overcome taking advantage of the new technologies as NIRS.

Key Words: forage resources, global calibration, local calibration

M153 Effects of a chemical additive on aerobic stability and fungal microbiome of corn silage. E. Benjamim da Silva*1,2, R. M. Savage1, S. A. Polukis1, M. L. Smith1, R. N. Mester1, A. M. Gray1, and L. Kung Jr.1, University of Delaware, Newark, DE, 2CAPES Foundation, Brasilia, DF, Brazil.

The objective of this experiment was to determine the effectiveness of Safesil (SF; 20% sodium benzoate, 10% potassium sorbate, 5% sodium nitrite) from Salinity, Sweden on improving aerobic stability of corn silage, and to evaluate its effects on fungi population by analysis of the internal transcribed space 1 (ITS1). Whole-plant corn was harvested at 39% DM, chopped, and untreated (CTRL) or treated with SF (2 L/t). Four replicated silos (7.5 L) were packed (224 kg of DM/m3) and ensiled for 85 d. Portions of CTRL and SF silages were analyzed for fungal composition. DNA extraction, amplification of ITS1, library preparation, and sequencing, by Illumina MiSeq (San Diego, CA) platform, were performed by Research and Testing Laboratory (Lubbock, TX). Data were analyzed using JMP 12.0 and QIIME 1.9.1. SF had fewer yeasts (P < 0.01), lower pH (P < 0.01), and lower concentration of ethanol (P < 0.01) compared with CTRL. SF (241 h), TMR with SF silage (71 h), and TMR containing silage treated with SF after ensiling (82 h) had improved (P < 0.01) aerobic stability compared with CTRL silage (37 h) and CTRL TMR (41 h). Chao1 index indicated that fungal diversity was higher (P < 0.05) in fresh forage and decreased after ensiling. The relative abundance of genus Candida was lower (P < 0.01) in SF (6.3%) than CTRL (46.2%). The chemical additive greatly improved the aerobic stability of silage. Additionally, it has the potential to be used as a TMR stabilizer, if applied after ensiling. The improvement on aerobic stability by the chemical additive might be due to its capacity to reduce total numbers of yeasts,
and to decrease specific populations of yeasts associated with lactate assimilation, such as *Candida*.

**Key Words:** chemical additive, silage, microbiome

**M154**  A sensory additive increased milk response to concentrate supplementation in dairy cows grazing kikuyu pastures. L. M. Gómez1, P. Aguirre1, P. Fargo2,3, G. Tedo4, and I. Iparraguirre5,6,7,8,9, Solla, Medellín, Colombia, 2Lucta SA, Barcelona, Spain, 3Universidad Buenos Aires, Buenos Aires, Argentina, 4University of Kiel, Kiel, Germany.

Forty-five Holstein dairy cows (147 d-in-milk and 544 kg BW on average) were assigned to a 3 x 3 Latin square design replicated 15 times to evaluate the effect of a sensory additive (ProEfficient, PE; Lucta SA, Barcelona, Spain) on milk response to concentrate supplementation on pasture. Cows were blocked by parity in 15 blocks and within blocks randomly assigned to one of 3 treatments: 0 kg/d concentrate (OC); 5 kg/d control concentrate (CC); and 5 kg/d CC with 30 g/d of PE (PEC). Cows grazed a kikuyu (*Pennisetum clandestinum*) pasture for 84 d in 3 28-d periods. Targeted pasture allowance was 35 kg DM/cow. Pasture averaged 24.2% CP, 55.5% NDF, and 60.3% in vitro DM digestibility (IVDMD). Concentrates were fed twice daily at milking. Concentrate (44.3% corn, 11.2% wheat bran, 10.0% corn gluten feed, 8.6% rice meal, 8.4% soybean meal, 8.0% of sunflower meal, 9.5% mineral premix) averaged 18.4% CP, 17.8% NDF, and 85.5% IVDMD. Data were analyzed using a mixed model that included the fixed effects of treatment, block, period, their 2-way interactions, and the random effect of cow within block. On average, supplementation with the starch-based concentrate increased \( P < 0.05 \) milk yield at 6.2 kg/d compared with OC (15.9 vs. 22.1 kg/d, SEM 0.51). However, cows supplemented with PEC produced more \( P < 0.05 \) milk than CC cows (22.7 vs. 21.4 kg/d, respectively; SEM 0.51). Milk production response to concentrate supplementation was improved at 15% \( P < 0.05 \) by the addition of PE into the concentrate (1.32 kg vs. 1.15 kg milk/kg concentrate for PEC and CC, respectively). Concentrate supplementation reduced \( P < 0.05 \) milk fat percentage (3.69 vs. 3.93%, SEM 0.065) and milk urea N (17.0 vs. 19.3 mg/dl, SEM 0.45). Milk fat and protein yield were increased \( P < 0.05 \) by concentrate supplementation (0.794 vs. 0.620 kg/d, SEM 0.022 and 0.686 vs. 0.497 kg/d, SEM 0.016; respectively) compared with CC. The milk response triggered by PE might be associated with a reduction in the substitution rate of CC for pasture.

**Key Words:** sensory additive, milk response to supplementation, grazing dairy cows

**M155**  Feed laboratory demographics and utilization in the United States. J. Severe* and A. J. Young, Utah State University, Logan, UT.

Feed analysis is the basis for buying and selling many feeds of interest. The point of contact between these 2 entities is the laboratory that analyzed the sample. Little research has been conducted specifically to describe feed laboratories in the United States; therefore, the objective of these surveys was to determine US feed laboratory demographics and utilization. In the first survey, laboratory demographic data were compiled from internet and laboratory sources. Based on this information, 114 feed laboratories were identified and selected. About 76% of the laboratories were commercial entities; the rest were state departments of agriculture (17%), universities (5%), and USDA (2%). Interestingly, universities in the Gulf and southeast US sponsored 63% of feed laboratories in that region. Approximately 80% of labs used wet chemistry and 52% used NIR analysis. Mean participation in NFTA certification from 2010 to 2014 was 67%. Commercial laboratories credited in refereed journals increased by 450% from 2004 to 2013; 3 laboratories accounted for 85% of credits. The second survey collected data from members of a trade organization with a response rate of 52% (161 out of 308); 72% indicated that they used a laboratory. Laboratory use by trade sector was beef (47%), equine (46%), retail feeds (60%), dairy (100%), and export (100%). Respondents reported using 45 different feed laboratories; one was used by 22% of respondents. Qualities that determined lab selection from most to least important were certification, reputation, sample turnaround time, and cost. Preference was for NIR (47%) compared with chemical analysis (21%), or no preference (32%). Preference for NIR was primarily due to turnaround time, while preference for wet chemistry was chiefly due to accuracy. Fifty percent of respondents were dissatisfied with feed laboratory performance, 49% reported financial loss due to feed analysis concerns, and 35% indicated harm to business relationships from feed analysis issues. These surveys show that out of 114 labs, 2 or 3 analyze most of the samples. All individuals associated with the dairy and export market use laboratories and NIR is evolving toward becoming the preferred method for analysis.

**Key Words:** feed laboratories, NIR, survey

**M156**  Effects of nitrogen fertilization on the nutritive value of oat forages. W. K. Coble�,*1, M. S. Akins1, and J. S. Cavadin1.1US Dairy Forage Research Center, Marshfield, WI, 2Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, 3University of Wisconsin Marshfield Agricultural Research Station, Marshfield, WI.

Nitrogen fertilization is a routine part of forage management strategies for grasses, but the effects on forage nutritive value have been inconsistent. Our objectives were to evaluate the effects of N fertilization on the nutritive value of fall-grown oat fertilized at planting with 20, 40, 60, 80, or 100 kg N/ha of urea or 2 rates of dairy slurry (42,300 or 84,600 L/ha). Oat forages fertilized with urea or dairy slurry had greater \( P < 0.001 \) concentrations of fiber components compared with those harvested from unfertilized plots (0 kg N/ha), and fiber concentrations increased linearly \( P < 0.001 \) with urea fertilization rate. In contrast, concentrations of water-soluble carbohydrates were greatest for unfertilized forages (21.2%), but declined linearly \( P < 0.001 \) with urea fertilization. Approximately, non-fiber carbohydrate also declined linearly \( P < 0.001 \) from 34.8% for unfertilized plots to a minimum of 24.6% at the 80 kg N/ha application rate. Similarly, non-fiber carbohydrate also declined linearly \( P < 0.001 \) from 34.8% for unfertilized plots to a minimum of 24.6% at the 80 kg N/ha urea application rate. Expressed on a percentage of DM basis, fertilization with urea resulted in linear \( P < 0.001 \) increases in crude protein (CP), neutral-detergent soluble CP, neutral-detergent insoluble CP, and acid-detergent insoluble CP, but effects on subcomponents of the total CP pool were not observed when concentrations were expressed on a percentage of CP basis \( P > 0.117 \). The summative calculation of energy (TDN) was closely related to N fertilization rate during both the 2013 \( (Y = −0.038 x + 72.2; r^2 = 0.961) \) and 2014 \( (Y = −0.040 x + 69.2; r^2 = 0.771) \) production years. Following 30- or 48-h incubations in buffered rumen fluid, in vitro DM disappearance was greater \( P ≤ 0.024 \) for unfertilized forages compared with those fertilized with either urea or dairy slurry, and DM disappearance declined linearly \( P ≤ 0.001 \) with urea fertilization rate; however, these responses were not detected \( P ≥ 0.109 \) for neutral-detergent fiber disappearance. Overall, the forage nutritive value of fall-grown oat declined mildly in response to N fertilization, resulting in losses of approximately 0.4 percentage units of TDN for every 10 kg N/ha applied as urea.

**Key Words:** N fertilization, nutritive value, oat
M157  Winter supplementation of ground whole flaxseed impacts milk fatty acid composition on organic dairy farms in the northeastern United States. A. N. Hafla1, K. J. Soder*1, A. F. Brito2, R. Kersbergen3, A. F. Benson4, H. Darby5, M. D. Rubano1, S. L. Dillard1, J. Kraft1, and S. F. Reis5, 1USDA-ARS, University Park, PA, 2University of Maine, Orono, ME, 3Cornell University, Cortland, NY, 4University of Vermont, Albans, VT.

Fourteen organic dairy farms were used to (1) evaluate seasonal variation of bioactive fatty acids in milk; and (2) evaluate supplementation of ground whole flaxseed to maintain levels of bioactive fatty acid concentrations during the non-grazing season. During year round farm visits (twice a month during the grazing season and once monthly during the non-grazing season) from April 2012 until April 2015, milk, feed, and pasture samples were collected, and diet and milk production and composition recorded. During the winters of 2013–14 and 2014–15, 9 farms supplemented ground whole flaxseed at 6% of diet dry matter to half of the cows within each herd (n = 238 cows/treatment). Milk samples were collected and pooled by treatment (flaxseed or control). Data were analyzed using the MIXED procedure of SAS. A month x year interaction (P < 0.01) for omega-3 fatty acid concentrations indicated an increase beginning in April of 2014 through the end of the study. Total milk conjugated linoleic acid (CLA) concentrations were seasonal with greatest (P < 0.01) concentrations (1.32% of total fatty acids) during the grazing season. Winter flaxseed supplementation did not impact concentrations of milk fat and milk protein, or body condition score. Compared with the control diet, flaxseed decreased total milk saturated fatty acid concentrations (P < 0.01) by 3.1 percentage units, increased omega-3 fatty acid concentrations (P < 0.01) by 88%, and tended (P = 0.13) to increase total CLA concentrations (P = 0.13) by 9.0%. While flaxseed supplementation increased milk omega-3 fatty acid concentrations, minimal impacts on saturated fatty acid and total CLA concentrations indicated a greater level of winter supplementation is required to maintain concentration of all beneficial fatty acids comparable to the grazing season.

Key Words: flaxseed, milk fatty acids, grazing

M158  Nutrient composition and management characteristics of California sorghum silage. J. Heguy*,1, J. Dahlberg2, P. Price4, J. Martins3, N. Clark1, N. Silva-del-Rio3, and D. Meyer3, 1University of California, Ag & Natural Resources, Parlier, CA, 2University of California, Ag & Natural Resources, Modesto, CA, 3University of California, Ag & Natural Resources, Tulare, CA, 4University of California, Davis, Davis, CA, 5University of California, Veterinary Medicine Teaching & Research Center, Tulare, CA.

The aim of this study was to obtain information on current sorghum management practices and sorghum silage quality from dairy farms (n = 16) located in California’s San Joaquin Valley. Herd size ranged from 320 to 5,500 lactating dairy cows (median = 2,013). Dairy producers answered short agronomic and harvest management surveys. At harvest, during summer and fall of 2016, 10 consecutive truckloads of chopped sorghum were sampled and composited for wet chemistry nutrient analysis (Table 1). Hectares of farmed sorghum ranged from 16.9 to 232.3 ha (median = 76.1); sorghum types were grain (n = 5), brown midrib (n = 10) and unknown (n = 1). Sorghum was stored in piles (n = 12) and bags (n = 4). Dairies with piles built one (n = 7), 2 (n = 3) or 3 (n = 2) sorghum silage piles, while bagged silage was stored in 5 or more bags on all dairies. Half of the dairies stored their sorghum silage on dirt surfaces. Delivery rate of the 10 truckloads of sorghum ranged from 12 to 78 min (median = 40). All dairies utilized custom harvesting services. Quality of sorghum harvested for silage was variable, with lower starch and NFC content and higher ash content than the traditional summer corn crop grown in California.

Table 1 (abstract M158). Nutrient composition of chopped sorghum sampled at harvest from dairy farms (n = 16) in California’s San Joaquin Valley

<table>
<thead>
<tr>
<th></th>
<th>DM</th>
<th>CP</th>
<th>ADF</th>
<th>NDF</th>
<th>Starch</th>
<th>NFC</th>
<th>Ash</th>
<th>% of DM</th>
<th>NDFD 30, % of NDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>28.7</td>
<td>9.5</td>
<td>34.6</td>
<td>49.7</td>
<td>10.9</td>
<td>26.3</td>
<td>12.2</td>
<td>48.6</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>28.4</td>
<td>9.7</td>
<td>34.9</td>
<td>50.4</td>
<td>9.6</td>
<td>27.4</td>
<td>11.8</td>
<td>50.5</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>23.2</td>
<td>5.7</td>
<td>30.4</td>
<td>44.9</td>
<td>1.9</td>
<td>14.4</td>
<td>9.2</td>
<td>35.1</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>34.6</td>
<td>11.7</td>
<td>40.2</td>
<td>55.3</td>
<td>22.5</td>
<td>35.6</td>
<td>21.5</td>
<td>60.3</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>3.3</td>
<td>1.8</td>
<td>3.1</td>
<td>3.8</td>
<td>6.7</td>
<td>6.0</td>
<td>2.9</td>
<td>7.8</td>
<td></td>
</tr>
</tbody>
</table>

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and 0.57%, respectively. Loss of DM was minor but lower \( (P = 0.05; 2.42\% \text{ vs. } 2.73\%) \) for SHRD. A 4.6%-units greater CSPS was observed \( (P = 0.001; 68.1\% \text{ vs. } 63.53\% \) starch passing through 4.75 mm sieve \) for SHRD than for CONV samples. In contrast, peNDF and ivNDFD were \( (P = 0.001) \) 1.8%- and 1.6%-units greater for CONV. A gradual increase in CSPS from Sep to Dec was observed \( (P = 0.001) \), followed by a decreased in Jan/Feb and a subsequent increase from Mar to Aug. Our results suggest that harvesting WPCS as SHRD improve kernal breakage while maintaining adequate fermentation patterns.

**Key Words:** corn silage processing score, shredlage, fermentation

### M160 Evaluation of yield and quality of photoperiod-sensitive sorghums in central Wisconsin.

E. Remick\(^1\), M. Akins\(^1\), A. Grisham\(^1\), H. Su\(^2\), W. Cobleitz\(^2\), and R. Ogden\(^1\), \(^1\)Department of Dairy Science, University of Wisconsin, Madison, WI, \(^2\)College of Animal Science and Technology, China Agricultural University, Beijing, China. \(^3\)US Dairy Forage Research Center, Marshfield, WI.

A 2-year study (2015, 2016) was conducted at 2 sites (Marshfield, Hancock) in central Wisconsin to assess yield and quality of photoperiod sensitive (PS) and non-PS sorghums in relation to corn planted on 2 dates and harvested once or twice. At each site, treatments were arranged in a split-split plot in a randomized complete block with 4 replications. Main plots of planting date (early or mid-June) were randomized within block. Subplots of harvest strategy (harvested once or twice) were randomized within planting date. Within harvest strategy, 8 forages were assigned (corn, PS sorghum, PS sorghum-sudangrass, sorghum, brown midrib (BMR) sorghum, sorghum-sudangrass, BMR sorghum-sudangrass, or PS-BMR sudangrass). Multi-harvests occurred in mid-summer and fall, and single harvest was based on maturity or after a frost. Data were analyzed using the Mixed model of SAS. Single harvest plots had greater yields than multi-harvest \( (18,961 \text{ vs. } 9,970 \text{ kg DM/ha}; P < 0.01) \), a site by harvest interaction \( (P < 0.01) \) suggested 2 harvests were more similar to 1 harvest at Marshfield than Hancock. Yields were greater at Hancock than at Marshfield \( (16,562 \text{ vs. } 12,370 \text{ kg DM/ha}; P < 0.01) \) and were greater in 2015 than 2015 \( (18,262 \text{ vs. } 10,669 \text{ kg DM/ha}; P < 0.01) \). The early June planting had greater yields than mid-June \( (15,320 \text{ vs. } 13,612 \text{ kg DM/ha}; P = 0.02) \). There was a harvest x variety (Table 1, \( P < 0.01 \) ) interaction; single harvest PS varieties and non-PS sorghum-sudangrass yielded more than BMR varieties, corn and forage sorghum were intermediate. Sorghum-sudangrass and sudangrass had more similar yields using either 1 or 2 harvests than other varieties. Overall, sorghum can provide high yields of moderate quality forage.

**Table 1 (abstract M160).** DM yields (kg/ha) for sorghums and corn using single or multiple harvests at Hancock and Marshfield in 2015 and 2016

<table>
<thead>
<tr>
<th>Forage(^1)</th>
<th>Harvest</th>
<th>Single</th>
<th>Multi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td></td>
<td>17,551</td>
<td>5,159</td>
</tr>
<tr>
<td>PS forage sorghum</td>
<td></td>
<td>23,606</td>
<td>9,024</td>
</tr>
<tr>
<td>PS sorghum-sudangrass</td>
<td></td>
<td>25,218</td>
<td>13,850</td>
</tr>
<tr>
<td>Forage sorghum</td>
<td></td>
<td>18,054</td>
<td>10,677</td>
</tr>
<tr>
<td>Sorghum-sudan</td>
<td></td>
<td>21,067</td>
<td>14,412</td>
</tr>
<tr>
<td>BMR forage sorghum</td>
<td></td>
<td>16,372</td>
<td>8,128</td>
</tr>
<tr>
<td>BMR sorghum-sudan</td>
<td></td>
<td>19,964</td>
<td>9,206</td>
</tr>
<tr>
<td>PS BMR sudangrass</td>
<td></td>
<td>14,857</td>
<td>9,307</td>
</tr>
<tr>
<td>SEM</td>
<td>1,038</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Variety x harvest \( (P-value) \) <0.01

\(^1\)PS = photoperiod sensitive; BMR = brown midrib.

**Key Words:** sorghum forage, harvest strategy

### M161 Comparison of two in situ reference methods to estimate indigestible NDF by near infrared reflectance spectroscopy.

G. J. Zhang\(^*1\), Y. H. Yan\(^2\), M.H. Hall\(^1\), D. J. undersander\(^1\), and D. K. Combs\(^1\), \(^1\)Ningxia University, Yinchuan, Ningxia Hui, China, \(^2\)Sichuan Agriculture University, Chengdu, Sichuan, China, \(^3\)Pennsylvania State University, State College, PA, \(^4\)University of Wisconsin, Madison, WI.

Undigested forage NDF residues (uNDF) from long-term ruminal in situ incubations are used to estimate indigestible fiber (iNDF). Estimation of iNDF is important in forage evaluation because it defines the potentially digestible pool of NDF. Near-infrared reflectance spectroscopy (NIRS) can be calibrated to in situ reference sets to rapidly predict uNDF. Our objective was to compare uNDF estimates after 240 h of incubation when 2 types of bags were used in the in situ reference method. The bags compared were 4 cm × 5 cm Ankum F57 bags (25-µm pore size), and 5 cm × 10 cm Ankum in situ bags (50-µm pore size). Alfalfa samples taken from Pennsylvania and Wisconsin \( (n = 144) \) of different varieties and harvest intervals were used. Forages were dried 48h at 60°C and ground through a 2 mm screen in Wiley mill. One-half or 2 g samples, respectively, were weighed into the small and large bags in triplicate. Mass to surface area was 0.05 and 0.02 g/cm² for the small and large bags, respectively. The uNDF content after 240 h incubation were evaluated by 2 bag types in 3 rumen-cannulated Holstein cows. Each dried and ground forage was also scanned to determine the visible–near-infrared-reflectance spectra using with a FOSS NIRSystems 6500 spectrophotometer. Prediction equations were developed for each bag type using partial least square regressions. The estimated uNDF fraction from small and large bags were 13.75% and 9.97%, respectively \( (SED = 0.39, P < 0.001) \). The coefficient of determination for calibration (RSQ), cross-validation \( (1 \text{ – } VR) \), calibration standard deviation \( (SEC) \), and interactive authentication standard deviation \( (SECV) \) was 0.94, 0.92, 0.85 and 0.98 for uNDF values determined with the small bag and 0.88, 0.85, 1.12 and 1.27 for uNDF values determined with the large bag calibration sets, respectively. Results indicate that uNDF vary and NIRS can be used to quickly and quantitatively estimate iNDF content in alfalfa. Bag type influences 240-h NDF residues. NIRS predictions of uNDF from the small bag calibration had higher RSQ and lower SEC and SECV than the large bag calibrations.

**Key Words:** NIRS, undigested NDF, alfalfa digestibility

### M162 Simulating the effects of forage harvesting strategies on dairy farm profitability and agro-environmental performance in Canada.

V. Ouellet\(^*1\), G. Belanger\(^2\), S. Binggeli\(^1\), D. Pellerin\(^1\), J. Dairy Sci. Vol. 100, Suppl. 2

Maintaining cow productivity, while decreasing the proportion of concentrates may require cutting forages at an early stage of development with reduction in yield and persistence. Our objective was to use the whole-farm model N-CyCLES to assess the effect of 2 forage harvesting strategies on dairy farm profits, N and P balance, and greenhouse gas emissions, while optimizing the management practices required to achieve maximum profits. Three model Canadian dairy farms under contrasting climate (Maritimes, Central Canada, and Prairies) were built in the model. Adaptations made to the model included modification to crop rotations, adjustment in the optimization constraints, evaluation of crop production cost, evaluation of forage nutritive value and yield, and update in fertilization requirements. Two harvesting strategies of divergent intensity of alfalfa-based forages were compared within each
region: HS1: frequent harvests at an early development stage producing highly digestible forages and HS2: less frequent harvests at the recommended development stage producing higher forage dry matter yields of medium digestibility. Highest dairy farm profits were achieved under HS2 for the 3 model farms. This is explained by the greater income arising from higher forage yields produced under HS2. Moreover, HS2 achieved better performance for most agro-environmental parameters when compared with HS1. These results suggested that harvesting forages at the usual maturity stage is more profitable and, allows for better agro-environmental performance than harvesting more frequently for higher nutritive value.

Key Words: harvesting strategy, whole-farm model, profitability

M164 Simulating the effect of corn silage substitution by sweet pearl millet or sweet sorghum silages on dairy farm profitability and agro-environmental performance in Canada. J. Velarde-Guilherme1, D. Pellerin1, L. Guerra-Aranda1, A. Vanasse2, M. Chantigny2, V. Baron3, and É Charbonneau1, 1Agriculture and Agri-Food Canada, Lacombe, AB, Canada, 2Agriculture and Agri-Food Canada, Québec, QC, Canada, 3Agriculture and Agri-Food Canada, Lacombe, AB, Canada.

Corn silage (CS) is widely used on dairy farms because of its high yields and energy content. Sweet pearl millet (SPM) and sweet sorghum (SS) are forages that can produce yields similar to CS, but with lower N needs. The aim of this study was to evaluate the economic and environmental differences of CS substitution by SPM or SS through simulations with the whole-farm model N-CyCLES. Three virtual farms were built in the model for contrasted climate and agricultural regions (Maritimes: 63 cows and 513 584 kg/yr, Québec/Ontario: 71 cows and 613 841 kg/yr, Prairies: 144 cows and 1 212 875 kg/yr). The model was adjusted for crop growth and rotations, fertilization needs, and constraints specific to each virtual farm type. The SPM was evaluated as a replacement for CS in the 3 climate regions, whereas SS was tested for Québec/Ontario, the only region with the appropriate climate for this plant. Results show that replacing CS for SPM and SS caused a slight decrease in net income per kg of FPCM (−7% in average). Environmentally, replacing CS for SPS and SS reduced greenhouse gas emissions per kg of FPCM (−4% on average), increased P balances (+93% on average), but lowered N balances for 2 out of 3 regions (−9% on average). These results suggest that, although CS is a good option, its substitution by SPM or SS is a viable alternative for Canadian dairy production.

Key Words: corn silage, sweet pearl millet silage, sweet sorghum silage

| Table 1 (abstract M162). Effects of two different harvesting strategies on dairy farm net income, greenhouse gas emission, P and N balance |
|---------------------------------|----------------|----------------|----------------|
|                                 | Maritimes1 | Central Canada2 | Prairies3 |
| Profit ($/100 kg FPCM4)                                   | HS1 | HS2 | HS1 | HS2 | HS1 | HS2 |
| Net income                                                 | 10.3 | 12.2 | 20.4 | 22.2 | 22.1 | 24.0 |
| Crop income                                                | 3.5  | 4.7  | 10.4 | 12.8 | 5.0  | 6.5  |
| Greenhouse gas emission                                    | Total CO2-eq. FPCM (kg/yr) | 1.4 | 1.4 | 1.3 | 1.4 | 1.3 | 1.3 |
| Mineral balance (g/kg FPCM)                                 | N    | 13.6 | 12.2 | 14.6 | 14.3 | 14.5 | 14.3 |
| P                                                           | 1.7  | 1.6  | 0.5  | −0.1 | 1.0  | 1.3  |

163 cows; 513 584 kg/yr.
71 cows; 613 841 kg/yr.
144 cows; 1 212 875 kg/yr.
Fat- and protein-corrected milk.
The objective of this study was to evaluate how canopy heights (1.00, 1.40, 1.80, 2.20 and 2.60 m) affect fiber digestibility of elephantgrass (*Pennisetum purpureum* cv. Cameroon). The experiment was conducted in 25 plots with 6 rows each in Lavras, MG, Brazil. Harvests were performed from Oct/2014 to Oct/2016. The experimental period was divided in 4 periods, 2 rainy seasons (RS) and 2 dry seasons (DS). The plants used for the evaluations were harvested at soil level. Forages were analyzed for aNDF with the Ankom procedure with NDF solution containing amylase and sulfite. Fiber digestibility was measured by the total-tract NDF digestibility (TTNDFD) in vitro method. Indigestible NDF (iNDF) of the samples was obtained via 240 h in situ incubation in 2 cannulated dairy cows. A randomized complete block design with 5 replications was used. Data were analyzed with PROC MIXED in SAS, and canopy height (CH), season and its interaction were considered fixed effects. There were differences between all variables (*P* < 0.01) reduction in TTNDFD values occurred with the increase of the CH. As CH increased, the fiber digestibility of elephantgrass decreased.

**Key Words:** TTNDFD, elephantgrass, fiber digestibility

**Table 1 (abstract M164).** Farm economic and environmental impacts of forage type

<table>
<thead>
<tr>
<th></th>
<th>Maritimes</th>
<th>Quebec/Ontario</th>
<th>Prairies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CS</td>
<td>SPM</td>
<td>SS</td>
</tr>
<tr>
<td>Dairy farm profit ($/kg FPCM&lt;sup&gt;1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net income</td>
<td>0.10</td>
<td>0.08</td>
<td>0.21</td>
</tr>
<tr>
<td>Crop income</td>
<td>0.03</td>
<td>0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Greenhouse gas emission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(kg CO₂ eq./kg FPCM)</td>
<td>Total</td>
<td>1.39</td>
<td>1.37</td>
</tr>
<tr>
<td>Milk allocation</td>
<td>1.10</td>
<td>1.09</td>
<td>0.99</td>
</tr>
<tr>
<td>Cash crop allocation</td>
<td>0.06</td>
<td>0.06</td>
<td>0.13</td>
</tr>
<tr>
<td>Mineral balance (g/kg FPCM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>12.5</td>
<td>10.92</td>
<td>13.36</td>
</tr>
<tr>
<td>P</td>
<td>1.86</td>
<td>2.70</td>
<td>0.36</td>
</tr>
</tbody>
</table>

<sup>1</sup>Fat- and protein-corrected milk.
Table 1 (abstract M166). Dry matter yields (kg/ha) for sorghums and corn at various irrigation rates

<table>
<thead>
<tr>
<th>Variety</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>15,508</td>
<td>15,053</td>
<td>17,338</td>
<td>19,301</td>
<td>22,452</td>
</tr>
<tr>
<td>PS forage sorghum</td>
<td>20,384</td>
<td>23,498</td>
<td>35,407</td>
<td>39,782</td>
<td>37,438</td>
</tr>
<tr>
<td>PS sorghum-sudan</td>
<td>21,108</td>
<td>26,917</td>
<td>31,666</td>
<td>38,752</td>
<td>44,457</td>
</tr>
<tr>
<td>Forage sorghum</td>
<td>26,089</td>
<td>24,707</td>
<td>25,163</td>
<td>26,805</td>
<td>31,465</td>
</tr>
<tr>
<td>Sorghum-sudan</td>
<td>19,787</td>
<td>19,249</td>
<td>25,095</td>
<td>25,977</td>
<td>21,691</td>
</tr>
<tr>
<td>BMR forage sorghum</td>
<td>15,915</td>
<td>22,624</td>
<td>21,273</td>
<td>29,837</td>
<td>34,787</td>
</tr>
<tr>
<td>BMR sorghum-sudan</td>
<td>18,510</td>
<td>18,928</td>
<td>19,428</td>
<td>18,674</td>
<td>24,633</td>
</tr>
<tr>
<td>PS BMR sudangrass</td>
<td>21,280</td>
<td>23,378</td>
<td>19,749</td>
<td>28,426</td>
<td>24,603</td>
</tr>
<tr>
<td>SEM</td>
<td>3,536</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variety × Irrigation (P-value)</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1PS = photoperiod sensitive.

Key Words: irrigation, sorghum forage

M167  Packing density of corn and winter forage silage structures on California dairies. M. Cuffia1, J. Lawrence2, J. Heguy3, and N. Silva-del-Rio1, 1University of California, Veterinary Medicine Teaching & Research Center, Tulare, CA, 2Alltech, Fresno, CA, 3University of California, Agriculture & Natural Resources, Modesto, CA,

The aim of this study was to describe packing density of corn (n = 177) and winter forage (n = 73) silage structures on 99 California dairies. Dairies were visited at least once from 2010 to 2016. To determine density, silage structures were probed at 3 different face locations (right, middle and left) at 1.8 m from the bottom surface. Cut length was evaluated with a measuring tape. Silage samples were composited and a sample was sent to a commercial lab for dry matter (DM) determination. The overall density for each of the silage structures was calculated as an average of the 3 samples collected. Descriptive statistics, including first (Q1), second (Q2) and third (Q3) quartiles as well as means, range and coefficient of variation (CV) were computed with the UNIVARIATE procedure of SAS. Means comparisons were performed with TTEST procedure of SAS. Corn silage structures were conventional piles (n = 118), bunkers (n = 6), bags (n = 10), roller piles (n = 36), or hybrid piles (n = 7). Winter forage silage structures were conventional piles (n = 45), roller piles (n = 18), bags (n = 8) or hybrid piles (n = 2). Average cut length was 1.7 cm (range: 1.3 to 2.2 cm) for corn silage and 1.6 cm (range: 1.3 to 1.9 cm) for winter forage. Corn silage DM was 34% (range: 28 to 45% of DM), while winter forage was 32% (range: 27 to 45% of DM). Corn silage structures had a higher (P < 0.01) packing density at the bottom-middle (269 kg of DM/m3) than at the bottom-right (247 kg of DM/m3) or bottom-left (239 kg of DM/m3). Average packing density was 223 (Q1), 251 (Q2) and 280 (Q3) kg of DM/m3 for corn silage. In winter silage structures, higher (P < 0.01) packing density was observed at the bottom-middle (184 kg of DM/m3) than at the bottom-right (160 kg of DM/m3) or bottom-left (160 kg of DM/m3). Average packing density for winter forage was 135 (Q1), 168 (Q2) and 205 (Q3) kg of DM/m3. In the bottom-middle sample location, 75% of corn silage structures were above 240 kg of DM/m3, whereas 50% of winter forage structures were below 183 kg of DM/m3. Bagged silage was below recommended minimum packing densities for both corn and winter forage. California silage structure density can be improved, especially in winter forage structures.

Key Words: corn silage, winter forage, packing density

M168  Nitrogen fertilization effects on sorghum forage yield and quality. A. Grisham1, M. Akins1, E. Remick1, H. Su2, R. Ogden2, and W. Coblenz1, 1Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, 2College of Animal Science and Technology, China Agricultural University, Beijing, China, 3US Dairy Forage Research Center, Marshfield, WI.

The study objective was to determine the effect of N fertilization on yield and quality of photoperiod sensitive (PS) and non-PS sorghums compared with corn. This study was done in a randomized complete block design with treatments arranged in a 4 x 8 factorial with 3 replicate blocks. Within each block, N rates (0, 56, 112, and 168 kg N/ha) were randomized with 7 kg N applied as urea by hand at planting and the remaining N applied at the 4–6 leaf stage. The 0 kg N/ha application rate did not receive pre-plant N. Within each N rate, varieties (corn, PS forage sorghum, PS sorghum-sudangrass, forage sorghum, brown midrib (BMR) forage sorghum, sorghum-sudangrass, BMR sorghum sudangrass, PS BMR sudangrass) were randomly assigned. Plots were planted June 3, 2016 and harvested at 1/3 to 1/2 kernel milk-line for corn, soft to hard-dough for sorghum, or after a killing frost. Data were analyzed using MIXED procedure of SAS. The interaction of variety and N rate was not significant (P = 0.67). Nitrogen rate affected yield (Table 1; P = 0.02) with reduced yield for 0 kg N/ha plots compared with 56 kg N/ha, 112 kg N/ha, and 168 kg N/ha plots (11375 vs 17199, 16627, 18981 kg DM/ha respectively). However, there were no significant differences in yield across N rates of 56, 112, and 168 kg N/ha (P = 0.21). Yield was affected by variety (Table 1; P < 0.01) with PS varieties, forage sorghum, and BMR forage sorghum having greater yields than BMR sorghum-sudangrass, sudangrass, and corn. Overall, some sorghum varieties can provide excellent yields of forage with modest N fertilization in central Wisconsin.

Table 1 (abstract M168). Dry matter yields (kg/ha) for sorghums and corn using nitrogen rates in WI in 2016

<table>
<thead>
<tr>
<th>Variety</th>
<th>0</th>
<th>56</th>
<th>112</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>12,391</td>
<td>11,499</td>
<td>14,127</td>
<td>15,717</td>
</tr>
<tr>
<td>PS forage sorghum</td>
<td>9,983</td>
<td>21,399</td>
<td>21,885</td>
<td>25,118</td>
</tr>
<tr>
<td>PS sorghum-sudan</td>
<td>11,715</td>
<td>18,726</td>
<td>21,302</td>
<td>23,647</td>
</tr>
<tr>
<td>Forage sorghum</td>
<td>13,410</td>
<td>20,660</td>
<td>19,130</td>
<td>20,250</td>
</tr>
<tr>
<td>Sorghum-sudan</td>
<td>13,836</td>
<td>17,278</td>
<td>15,269</td>
<td>20,085</td>
</tr>
<tr>
<td>BMR forage sorghum</td>
<td>14,205</td>
<td>24,289</td>
<td>17,226</td>
<td>21,332</td>
</tr>
<tr>
<td>BMR sorghum-sudan</td>
<td>8,609</td>
<td>14,859</td>
<td>12,566</td>
<td>13,306</td>
</tr>
<tr>
<td>PS BMR sudangrass</td>
<td>6,854</td>
<td>8,885</td>
<td>11,514</td>
<td>12,395</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
<td>3,181</td>
<td></td>
</tr>
<tr>
<td>Variety × Irrigation (P-value)</td>
<td></td>
<td>0.67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1PS = photoperiod sensitive; BMR = brown midrib.

Key Words: sorghum forage, corn, nitrogen fertilization

M169  Pearl millet morphological composition at three sowing densities and two cutting heights. J. S. Trindade1, V. L. Banys1, M. Dias1, F. J. S. Dias1, and E. A. Collao-Saenz2, 1Universidade Federal de Goiás-UGF, Jataí, Goiás, Brazil, 2UNIVAR, Barra do Garças, Mato Grosso, Brazil.

West Central region of Brazil presents a well-defined dry season during the winter and a rainy summer season, therefore, forage alternatives such as pearl millet are evaluated to reduce the effects of the dry period. The objective of this study was to evaluate the morphological composition of millet at the south-east region of Goias State of Brazil.
Plots were disposed in a completely randomized block design using a 3 × 2 factorial arrangement (3 sowing densities: 10, 15 and 20 kg ha⁻¹ and 2 cutting heights: 50 and 70 cm) with 20 cm residual height and 6 replicates. The samples were collected at 3 points in the plots and separated and weighed in stem, leaves, dead/senescent material and panicles. Statistical analyses were performed at 5% probability and means comparison evaluated by Tukey test. There was no significant effect of seeding density on the plant structural fractions nor interaction between sowing density and cutting height. Average values for leaf, stem, panicle and dead matter fractions were 27.24; 46.26; 19.63 and 6.42, respectively, showing a compensatory effect of the sowing rate on the plant components distribution, which allowed that the canopy structure was preserved even when sown changed the final population (53, 68 and 78 plants/m², respectively for 10, 15 and 20 kg seeds/ha densities). The effect of cutting height was significant for all fractions (Table 1).

The higher cutting height decreased the leaves percentage and increased the stem percentage resulting in a lower leaf/stem ratio, probably due to the longer period necessary to reach 70 cm height, with maturity, the plants also presented higher percentage of panicle and dead material. Considering that the grazing aims harvesting green leaves and a longer grazing period, it is recommended to use millet management at 50 cm.

**Table 1 (abstract M169).** Morphological composition of millet off-season on two cutting heights

<table>
<thead>
<tr>
<th>Fraction (%)</th>
<th>Cutting Height (cm)</th>
<th>CV (%)¹</th>
<th>P-value²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>50</td>
<td>18.74</td>
<td>15.80</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem</td>
<td>44.63</td>
<td>47.89</td>
<td>4.24</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panicle</td>
<td>14.54</td>
<td>24.72</td>
<td>7.09</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead material</td>
<td>4.67</td>
<td>8.39</td>
<td>27.12</td>
</tr>
</tbody>
</table>

¹Coefficient of variation.
²F test.

**Key Words:** flowering, grass, leaf/stem ratio

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**M170 Effect of plant population and hybrids varying in relative maturity on yield, nutrient composition and ruminal in vitro NDF digestibility in whole-plant corn forage.** L. F. Ferraretto¹, B. G. Wasdin¹, C. R. Staples¹ and D. Grabow², 1University of Florida, Gainesville, FL, 2Grabow Seed Services Inc., Atlanta, GA.

The objective of this study was to evaluate the effect of plant population and hybrid relative maturity on yield, nutrient composition and ruminal in vitro NDF digestibility at 30 h (ivNDFD) in whole-plant corn forage. Five hybrids varying in relative maturity (115 [M115], 118 [M118a, M118b], 124 [M124] and 130 [M130] days) were each planted at 2 different plant populations (60,000 [60K] or 70,000 [70K] plants/ hectare) during the summer, in quadruplicate. Samples were analyzed via NIRS at Dairyland Labs (Arcadia, WI). Data were analyzed as a completely randomized design using Proc Glimmix of SAS with the Fixed effects of hybrid, plant population, and their interaction. A plant population × hybrid interaction was observed (P = 0.001) for DM content, with greater values observed for 70K than 60K on M118a (33.9% vs. 32.1%) but the opposite on M118b (28.6% vs. 31.1%). Despite the greater (P = 0.02; 8.9% vs. 8.3%) CP content for 60K than 70K, plant population did not affect (P > 0.10) ADF, aNDFom, and starch concentrations. Greater starch (40.0% vs. 26.3% on average) but reduced CP (7.5% vs. 8.0% on average), ADF (22.7% vs. 28.8% on average) and aNDFom (36.0% vs. 44.7% on average) concentrations were observed (P < 0.01) for M118a than other hybrids. Increasing plant population from 60K to 70K tended (P = 0.08) to increase ivNDFD by 1.5%-units. This is related to reduced lignin (P = 0.02; 4.5% vs. 4.0%) and a trend for decreased aNDFom (P = 0.09; 14.3% vs. 13.0%) concentrations for 70K. Yield of DM, milk/t and milk/ha were unaffected by plant population and averaged 13.2 t of DM/ha, 1263 kg of milk/t of DM, and 13952 kg of milk/ha, respectively. In addition, M118a tended to have greater ivNDFD (P = 0.06; 51.1 vs. 47.4% of NDF on average). This is related to the 1.1 % - and 4.0% -unit lower (P < 0.01) lignin and aNDFom concentrations, and led to enhanced (P = 0.01) milk/t and milk/ha estimates. Yield of DM was 1.2 t/ha greater (P = 0.01) for M130 than other hybrids, on average. Plant population slightly improved NDF digestibility whereas hybrids varying in relative maturity affected yield and quality of whole-plant corn forage.

**Key Words:** corn silage, plant population, NDF digestibility

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**M171 Productivity of lactating dairy cows fed diets with teff hay as the sole forage.** B. Saylor*, D. Min, and B. Bradford, Kansas State University, Manhattan, KS.

Groundwater depletion is one of the most pressing issues facing the dairy industry today. One strategy to improve the industry’s drought resilience involves feeding drought tolerant forage crops in place of traditional forage crops like alfalfa and corn silage. The objective of this study was to assess the productivity of lactating dairy cows fed diets with teff hay (Eragrostis tef) as the sole forage. Teff is a warm-season annual grass native to Ethiopia that is well adapted to drought conditions. Nine multiparous Holstein cows (185 ± 31 d in milk; mean ± SD) were randomly assigned to 1 of 3 diets in a 3 × 3 Latin square design with 18-d periods (14 d acclimation and 4 d sampling). Diets were either control (CON), where dietary forage consisted of a combination of corn silage, alfalfa hay, and prairie hay, or 1 of 2 teff diets (TEFF-A and TEFF-B), where teff hay (13.97 ± 0.32% CP, DM basis) was the sole forage. All 3 diets were formulated for similar dry matter (DM), crude protein (CP) and starch concentrations. CON and TEFF-A were matched for concentrations of neutral detergent fiber (NDF) from forage (18.23 ± 0.15% of DM), and TEFF-B included slightly less, providing 16.63% NDF from forage. Dry matter intake (DMI), milk and component production, body weight (BW), body condition score (BCS), as well as DM and NDF digestibility (DM and NDFD) were monitored and assessed using mixed model analysis. Treatment had no effect (P = 0.76) on DMI (28.14 ± 0.75 kg/d). Similarly, treatment had no effect (P = 0.65) on milk production (40.68 ± 1.79 kg/d). Concentrations of milk fat (3.90 ± 0.23%) and protein (3.07 vs. 3.16 ± 0.09%) were the same for all treatments. Treatment had no effect (P > 0.10) on energy-corrected milk (ECM) yield (43.37 ± 1.26 kg/d), BW, or BCS change (all P > 0.10). Additionally, treatment had no effect (P = 0.47) on total-tract DM or NDF (P = 0.58) digestibility. Results from this study indicate that teff hay has potential to replace alfalfa and corn silage in the diets of lactating dairy cattle without loss of productivity.

**Key Words:** drought, teff hay, dairy cattle
**Lactation Biology I**


Isolation of good quality ribonucleic acid (RNA) from mammary glands of elite animals is often hindered by invasive method of mammary tissue sampling, ethical permission, time consuming, and repeated biopsies from the same animal at different time points. The aim of this study was to optimize a protocol for RNA isolation from milk fat globules (MFG) that is suitable for transcriptome analysis. We isolated good quality RNA from milk fat of goats and buffalo milk by combining 2 methods namely Trizol and GenElute mammalian RNA isolation kit. The concentration of RNA ranges from 385 to 3000 ng/µl (1267.5 ± 186.5 ng/µL) in 20 µl of elution volume. Our improved protocol resulted in optical density (OD) ratios of RNA close to 2.0 (OD260/280 = 2.05 ± 0.01 and OD260/230 = 1.99 ± 0.03) indicating its purity. RNA integrity number (RIN) value of representative sample was 8.1 indicating suitability of RNA samples for next generation sequencing like RNA-Seq. Functional validation of total RNA isolated from MFG, were tested for the expression of milk protein genes like α-lactalbumin (LALBA), β-lactoglobulin (BLG4), β-casein (CNS2) and ribosomal protein genes like RPS23 for quantitative PCR analysis. We concluded that our results could be used to obtain high quality and abundant quantity of RNA for transcriptome analysis of mammary glands and could be serve as non-invasive method of expression analysis in other species.

**Key Words**: milk fat globule, RNA isolation, transcriptome analysis


Lactation activity is extremely important for dairy cows, but the underlying metabolic mechanisms is not well understood. This study was conducted to investigate the lactation-related maintenance and initiation metabolic mechanisms in dairy cow using overall biofluid and partial tissue metabolomics. Six mid-lactation Holstein cows were used to analyze the relationships between 4 biofluids (rumen fluid, serum, milk and urine) and to compare mammary gland metabolism with 6 nonlactating cows using gas chromatography-time of flight/mass spectrometry and multivariate analysis. Totally, 33 mutual metabolites and 274 metabolites were identified in 4 biofluids and mammary gland tissues, respectively. The sub-clusters of heatmap analysis for rumen fluid and serum were grouped together and highly correlated with each other, but separated from milk. Creatine was identified as key metabolite to explain the biological variation among 4 biofluids. Pathways of gluconeogenesis, pyruvate metabolism, tricarboxylic acid (TCA) cycle, glycerolipid metabolism and aspartate metabolism, demonstrated most functional enrichment among 4 biofluids (false discovery rate <0.05, fold enrichment >2). Clear discriminations were observed between lactating and nonlactating cows, with 54 significantly higher (\(P < 0.05, \text{VIP}>1\)) metabolites in lactation group. Lactobionic acid, citric acid, orotic acid and oxamide were extracted by S-plot as putative biomarkers. The TCA cycle, glyoxylate and dicarboxylylate metabolism, glutamate metabolism and glyoxylate metabolism were determined as functional impact pathways (\(P < 0.01, \text{impact value}>0.1\)) in lactation group. Extremely upregulated function of the TCA cycle pathway (\(P < 0.0001\)) in lactating cows was identified along with 70% substrates increased in the mammary gland cell. These results provide the first integrated insight into better understanding of lactation-related overall and partial metabolic mechanisms and will be beneficial in developing regulated strategies for lactating dairy cows. More importantly, novel systematic investigation can be obtained from this study to address complex biological questions.

**Key Words**: dairy cow, lactation, metabolomics

**M174** Conjugated linoleic acid (CLA) reduces milk fat content in sows without altering litter performance. E. C. Sandri, P. C. Carraro, and D. E. Oliveira*, Santa Catarina State University, Lages, Santa Catarina, Brazil.

In lactating sows, a great proportion of the energy consumed is prioritized to milk production and synthesis of its components, resulting in an intense catabolism of body stores. As shown in dairy cows, ewes and goats, C18:2 trans-10,cis-12 conjugated linoleic acid (CLA) decreases milk fat synthesis and it may be an option to minimize the energy costs of lactation without compromising the piglet performance. This study evaluated the effect of CLA on sow milk yield and composition, and on piglet performance. Twenty multiparous sows from a commercial lineage, with a mean body weight (BW) of 200 ± 10 kg were randomly assigned to one of the 2 treatments (n = 10/treatment) for 18 d: (1) Control (no fat) and; (2) 1% of CLA (29,9% of trans-10,cis-12 and 29,8% of cis-9,trans-11) mixed in the ration. The diet was formulated to meet the nutritional requirements for the breed. Sows were kept in a controlled environment (temperature, humidity, and ventilation) and the CLA treatment was administered from d 7 through d 25 of lactation. Milk samples were collected from all sows from d 0 to d 25 to evaluate milk concentrations of fat, protein, lactose, and total solids. Data were analyzed as a complete randomized design using the Mixed Procedure of SAS. The model included the random effect of sow, and the fixed effects of treatment and d 0 measurements, the latter used as a covariate and removed if not significant. Compared with Control, CLA treatment decreased milk fat content by 20% (Control = 6.2 vs. CLA = 4.9%, \(P = 0.004\)). In addition, CLA reduced milk protein content by 13.7% (Control = 3.0 vs. CLA = 2.6%, \(P = 0.08\)). Despite the reduction in fat content, the weight of piglets at weaning was not different between treatments (Control = 7.8 vs. CLA = 7.9 kg, \(P = 0.60\)). These results indicate that CLA reduces the milk fat content without negatively affecting litter performance.

**Key Words**: milk fat synthesis, milk fat depression, piglet performance

**M175** The gene expression of fatty acid transporters and triglyceride codifying genes changes according the stage of lactation in dairy ewes. M. Camêra1, E. Ticiani1, K. J. Harvatine2, E. C. Sandri1, and D. E. Oliveira1,1, Santa Catarina State University, Lages, SC, Brazil, 2Penn State University, State College, PA.

During lactation the mammary gland produces a substantial amount of triglycerides using fatty acids synthesized in the mammary gland and from the plasma, prioritizing milk fat synthesis over adipose tissue,
especially at the beginning of lactation. Specific fatty acid transporter proteins and enzymes are involved in fatty acid uptake by mammary cells and triglyceride synthesis. The objective of this study was evaluate gene expression of long chain acyl-CoA synthetase (ACSL1), lipoxygenase family fatty acid transporter (SLC27A6), fatty acid binding proteins (FABP3 and FABP4), fatty acid translocator CD36 (FATCD36), lipoprotein lipase (LPL), acylglycerol phosphatidyltransferase (AGPAT6), lipin (LIPIN1), diacylglycerol acyltransferase (DGAT1), and peroxisome proliferator-activated receptor gamma (PPARγ) at different stages of lactation in dairy ewes. Mammary gland biopsies were taken from 6 lactating ewes at 15, 70, and 120 DIM, to represent early, mid, and late lactation. Total RNA was extracted, cDNA synthesized and quantitative real-time PCR analysis conducted. Data were analyzed by PROC MIXED (SAS Institute) procedure using stage of lactation as a fixed effect, animal as random, and the geometric mean of the housekeeping genes (ribosomal protein S18 and β-actin) as a covariate. Data points with Studentized residuals outside of ± 2.5 were considered outliers and excluded from analysis. There was no effect of stage of lactation for ACSL1, SLC27A6, and FABP4 transcripts (P > 0.05). The expression of FABP3 and FATCD36 was higher in early lactation and decreased as lactation progressed (P < 0.05). Similarly, the transcripts of AGPAT6 and DGAT1 tended to be increased in early lactation (P < 0.05) and LIPIN1 increased to be in early lactation (P = 0.09). In addition, LPL and PPARγ were increased in early lactation compared with mid and late lactation (LPL P = 0.01 and P = 0.02 and PPARγ P = 0.03 and P = 0.03, respectively). Our results show a higher expression of fatty acid transporters and key enzymes in mammary tissue at early stages of lactation prioritizing milk fat synthesis.

Key Words: fatty acid synthesis, mammary gland, milk fat

M176 Milk yield differences between xanthosine treated and control glands are associated with changes in milk protein gene expression. R. K. Choudhary1, S. Choudhary1, D. Pathak2, R. Udehiya2, R. Verma3, S. Kaswan3, A. Sharma3, M. Honparkhe3, and A. Capuco6, 1School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab, India, 2Department of Veterinary Anatomy, GADVASU, Ludhiana, Punjab, India, 3Department of Livestock Production & Management, GADVASU, Ludhiana, Punjab, India, 4Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana, Punjab, India, 5Department of Veterinary Gynaecology & Obstetrics, GADVASU, Ludhiana, Punjab, India, 6Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD.

In vivo and in vitro treatment of mammary glands with xanthosine has been shown to increase mammary stem/progenitor cell population in heifers. Inosine, a ribonucleoside that is related to xanthosine, has been reported to increase milk production in transgenic goats. However, the underlying mechanisms of these effects are poorly understood. The goal of this study was to examine the effects of xanthosine on the mammary stem cell population and milk production in dairy goats. Primiparous Beetle goats (n = 7) were assigned to the study. Five d after kidding, one gland (either left or right) was infused xanthosine (TRT) twice daily (2×) for 3 d and the other gland served as control (CON). Mammary biopsies were collected at 10 d and RNA was isolated. Daily milk yield per gland was recorded 10.5 ± 1.3 d after biopsies for 7 wk. Average milk yield in TRT glands was increased 2% (P = 0.04, paired t-test) relative to CON glands until 7 wk. After 7 wk, milk yield of TRT and CON glands did not differ. Analysis of milk composition revealed that protein, lactose, fat and solids-not-fat percentages remained the same in TRT and CON glands. Expression of transcripts for β-lactoglobulin (BLG4), β-casein (CSN2), estrogen receptor-α (ESR1) and aldehyde dehydrogenase 1 (ALDH1, a mammary stem cell marker) was significantly increased and α-lactalbumin (LALBA) and casein α-S2 (CSN1S2) tended to be increased in TRT glands. These results support the hypothesis that xanthosine increases milk production and the mammary stem cell population.

Key Words: goat lactation, xanthosine, mammmary stem cell

M177 Peroxisome proliferator-activated receptor gamma (PPARy) agonist and conjugated linoleic acid (CLA) have different effects on expression of milk protein genes in lactating ewes. M. Camerla1, E. C. Sandri1, K. J. Harvatine2, and D. E. Oliveira1, 1Santa Catarina State University, Lages, Santa Catarina, Brazil, 2Penn State University, State College, PA.

Milk protein is very important to the dairy industry. Milk protein synthesis is impacted by animal genetics, but is less responsive to nutrition. In a previous study using thiazolidinedione (TZD) trying to overcome the milk fat depression effect of trans-10,cis-12 CLA we observed an increase in milk protein content in lactating ewes treated with TZD. This study used a specific chemical PPARγ agonist and CLA to evaluate their effect on expression of milk protein genes (caseins and whey) and their interaction. Twenty-four crossbred lactating ewes (60 ± 0.45 kg body weight) 70 ± 3 DIM, producing 1.2 ± 0.34 kg of milk/d were randomly assigned to one of the 4 treatments (n = 6/treatment) for 7 d. Treatments were: 1) Control (intravenous infusion of 100 mL/d of saline); 2) TZD (Rosiglitazone, intravenous infusion of 4 mg/kg of BW per d in 100 mL of saline); 3) CLA (27 g/d orally dosed methyl ester containing 29.9% of trans-10,cis-12 CLA and 29.8% of cis-9,trans-11); and 4) TZD+CLA. Mammary biopsies were taken, RNA was extracted, cDNA synthesized and qRT-PCR analysis conducted for casein genes (CSN1S1, CSN1S2, CSN2, CSN3) and whey proteins genes (β-lactoglobulin (BLACTO) and α-lactalbumin (LALBA)). Compared with control, TZD increased milk protein concentration 18.7% and expression of CSN1S1 (P = 0.05), CSN1S2 (P = 0.01), CSN2 (P = 0.01), CSN3 (P = 0.03) and BLACTO (P = 0.02) by 4, 8.6, 5, 4.9 and 4.7 fold, respectively. CLA increased the expression of CSN1S2 (P = 0.03), CSN2 (P = 0.001), CSN3 (P = 0.01) compared with control in 5.5, 5.3 and 3.9 fold, respectively. TZD+CLA tended (P = 0.06) to increase milk protein concentration 11.5% compared with control, but decreased expression (P = 0.05) of all genes studied. Overall, TZD positively affected mammary expression of genes encoding the major milk proteins, while CLA had a partial effect.

Key Words: gene expression, milk protein synthesis, thiazolidinedione

M178 Strategies to ameliorate the negative impact of heat stress on immune status of cows during the dry period. T. F. Fabris1, J. Laporta1, D. J. McLean2, D. J. Kirk2, J. D. Chapman2, F. N. Corra1, Y. M. Torres1, and G. E. Dahl1, 1University of Florida, Gainesville, FL, 2Phibro Animal Health Corp., Teaneck, NJ.

Heat stress (HT) of cows in the dry period (DP) decreases immune function and lowers milk yield in the next lactation compared with cooled dry cows. The objective of this study was to evaluate the effects of a dietary treatment (OmniGen-AF) fed to HT cows before, during and after the DP on immune function, hematology and immune related gene expression. Sixty days before dry-off, cows were cooled (i.e., shade, fans and coolers) and divided into 2 groups: control (fed 56 g/d of AB20; CON) and OmniGen-AF (fed 56 g/d of OmniGen-AF; OG). Cows were dried-off 45 d before parturition and further split into cooling (shade, fans and
soakers; CL) or HT (only shade) pens, which resulted in 4 treatments: HT (n = 17), CL (n = 16), HT + OG (HTOG, n = 19) and CL + OG (CLOG, n = 14). In the DP, rectal temperature (RT; °C), respiration rate (RR; breaths per min) and temperature humidity index (THI) were recorded to evaluate heat strain. Blood samples were collected before dry-off, during the DP and lactation from a subset of cows (HT, n = 12; CL, n = 12; HTOG, n = 11 and CLOG, n = 9) to evaluate L-selectin (CD62L, copies per ng of total mRNA) and CXCR2 mRNA (a.k.a. IL8-R) gene expression in immune cells. Other samples were used before dry-off and in the DP to evaluate neutrophil function and blood hematology (HT, n = 8; CL, n = 7; HTOG, n = 8 and CLOG, n = 6). HT increased RR (45.2 vs. 77.2 ± 1.6 bpm) and RT (38.9 vs. 39.3 ± 0.05 °C) versus CL (P < 0.01). OG increased L-selectin expression versus CON before dry-off (10229 vs. 5893 ± 2353; P = 0.09). L-selectin expression did not differ during the DP, but after calving there was an interaction of DP heat stress and dietary treatment (P = 0.05); CLOG cows had increased L-selectin expression versus CL cows (24,951 vs. 7,198 ± 5,061). Expression of CXCR2 and neutrophil function did not differ among groups. OG tended to increase neutrophil (10³/µL) count versus CON (3.6 vs. 3.3 ± 0.17; P = 0.13) and HT cows had lower hematocrit % versus CL (29.4 vs. 31.6 ± 0.6; P = 0.12). OG supplementation increased L-selectin expression before dry-off, and that may be related to improved immune status of cows during the DP and in the next lactation.

Key Words: immunity, heat stress, OmniGen-AF

M179 Thiazolidinedione (TZD) does not modify the milk protein synthesis in lactating ewes. E. C. Sandri*, M. Camera, W. B. Junior, P. C. Carraro, E. D. Silva, and D. E. Oliveira, Santa Catarina State University, Lages, Santa Catarina, Brazil.

Thiazolidinedione (TZD) can stimulate insulin sensitivity by binding to transcription factors and indirectly acting on protein synthesis in mammary gland. In a previous study using ewes in early lactation (70 d in milk, DIM) we observed a positive effect of TZD on milk protein content. This study evaluated the effect of TZD on protein synthesis in late lactating ewes. Twenty-three lactating ewes with a mean body weight of 60 ± 0.45 kg, producing 0.98 ± 0.20 kg milk/day, 120 ± 3 d in milk and fed with a TMR of corn silage plus concentrate were randomly assigned to 1 of 2 treatments in a complete randomized block design: 1) Control (iv. infusion of 5 mL/d of saline solution); 2) TZD (iv. infusion of 4 mg/kg of BW/day in 5 mL of saline solution). The experimental period lasted 15d (5 of adaptation and 10 of measurements). Milk samples were collected from all ewes on d 1, 3, 4, 6, 7, 10 and pooled by ewe to evaluate the concentrations of milk fat, protein, lactose, casein and total solids. Data were analyzed using the PROC MIXED of SAS with ewe as random effect, and treatment, block and their interaction as fixed effects. There was no effect of treatment, block and their interaction for milk yield or for the concentrations and yields of protein, fat, lactose, and total solids. However, TZD reduced milk casein concentration (Control = 5.65 vs. TZD = 5.17%, P = 0.02). These results indicate that TZD does not stimulate the milk protein content in lactating ewes in late lactation.

Key Words: dairy ewe, milk composition, milk protein content
The objectives were to evaluate effects of high mastitis and ketosis risks (MRisk and KRisk) on commencement of luteal activity (CLA), luteal and follicular phase (LP and FP) lengths and cumulative pregnancy at 120 DIM (P/120) using data from an in-line milk analysis system (Herd Navigator, DeLaval). Starting at ~5 DIM and repeating every ~2d, lactate dehydrogenase and β-hydroxybutyrate were quantified by the system to generate MRisk and KRisk indicators (0–100%) based on a bio-model that accounted for individual cow variation in values. Milk progesterone (mP4) measures started at ~20 DIM. Records (n = 328,649) of 910 Holstein cows (2,278 lactations) were obtained from 4 Alberta herds. As indicators of potential clinical cases, only MRisk ≥ 70% and KRisk ≥ 90% were considered. Data of mP4 was used to determine CLA (first mP4 ≥ 5ng/mL), lengths of LP (period of mP4 ≥ 5ng/mL) and FP (period of mP4 < 5ng/mL between consecutive LP) and pregnancy (uninterrupted LP at ≥ 50d after AI). Effects of MRisk and KRisk on LP and FP lengths were evaluated on 2,891 cycles, while CLA and P/120 were evaluated on 1,823 lactations. Analyses used PROC GLIMMIX of SAS including parity, milk yield and DIM in the models. Incidences of MRisk and KRisk were 22.2 and 3.6% and occurred at (μ ± SD) 106 ± 56 and 36 ± 10 DIM. Overall LP and FP lengths (μ ± SEM) were 14.1 ± 0.2 and 13.9 ± 0.2d, and P/120 was 38% (range 30–51% among farms). The odds of having MRisk was increased (odds ratio [OR] = 5.5) in multiparous than in primiparous cows. The odds of MRisk was greater (OR = 3.8) in cows yielding ≥ 31kg/d than in those yielding < 31kg/d. MRisk did not affect CLA, but increased the occurrence of LP ≥ 16d (OR = 4.4) and FP ≥ 12d (OR = 1.5). MRisk during first 120 DIM decreased P/120 (OR = 0.5). The occurrence of KRisk tended (P = 0.09) to be greater in multiparous than in primiparous cows (OR = 8.9), but did not affect reproductive parameters. Cows experiencing high mastitis risk were more likely to have abnormal (i.e., prolonged) luteal and follicular phases during estrous cycles, and less likely to be pregnant at 120 DIM.

Key Words: dairy herd, estrous cycle, fertility

M181 Development of a model to study mammary gland function during heat stress. Ri. O. Rodrigues*1, T. Leiva1,2, Ro. O. Rodrigues1, and T. B. McFadden1,1University of Missouri, Columbia, MO, 2Sao Paulo State University, Botucatu, Brazil.

The aim was to develop a half-udder model for quantifying the local effects of elevated udder temperature on mammary gland (MG) function in dairy cows. Heating pads were used to heat the MG. Four settings were tested and were found to be stable after 45 min at significantly different temperatures (P < 0.001; Warm = 43.4, Low = 45.8, Medium = 48.6, and High = 50.4 ± 0.5°C). Heating pads were then applied to half-udders of individual cows. Two cows were used to test each temperature setting; for each cow, one half-udder served as heated treatment and the other half-udder was the unheated control. During each 4-h test period, cows were monitored every 20 min. There was no evidence of blisters, inflammation, redness, sensitivity to touch or any sign of discomfort. Udder skin temperature increased in a heat-setting dependent manner (P ≤ 0.01). Milk temperature was higher in heated than in unheated halves (P < 0.001; 37.4 vs. 36.9 ± 0.04°C, respectively), but no differences over time or between heat-settings were observed. Next, heat was applied to half-udders of 4 cows for 48 h, and individual halves were milked every 12 h. Udder skin temperature increased in the heated compared with unheated halves (P < 0.001; 38.6 vs 36.4 ± 0.08°C, respectively). Somatic cell counts and milk composition were similar between udder halves throughout the trial. At the 48 h milking, yield was reduced by 0.8 ± 0.2 kg in heated halves compared with unheated halves (P < 0.05). RNA from milk somatic cells collected from each udder-half at 48 h was sequenced, and a total of 151 annotated genes were differentially expressed (P ≤ 0.001 and FDR ≤ 0.10) between the heated and unheated halves. These genes represented several functional clusters, including cell membrane, signaling, inflammatory and stress response, apoptotic processes, and mammary gland development. Pathways found to be differentially expressed between groups included cytokine-cytokine receptor integration, chemokine and TNF signaling, coagulation and complement cascade, and others. These experiments established a half-udder model for investigating local effects of heat stress on mammary function.

Key Words: lactation, RNA-sequencing, thermal stress

M180 Evaluating effects of mastitis and ketosis risks on reproductive parameters using indicators from an automated in-line milk analysis system. T. C. Bruinje*1 and D. J. Ambrose1,2, 1Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, 2Livestock Research Section, Alberta Agriculture and Forestry, Edmonton, AB, Canada.

Our objective was to evaluate the relationship between blood urea nitrogen near the time of AI and fertility of lactating Holstein cows. Lactating Holstein cows (n = 541) were submitted to a Double-Ovsynch protocol to receive their first timed artificial insemination (TAI). Body condition score (BCS) was evaluated and a blood sample was collected immediately before the last PGF2α treatment of the Double-Ovsynch protocol. Blood samples were assayed for progesterone (P4) by RIA and BUN concentrations by ELISA. All cows were fed the same TMR diet containing 17.3% CP/kg DM (RDP - 11.72% and RUP - 5.61%) and 1.65 Mcal/kg DM of NEI formulated to meet NRC requirements. Only synchronized cows (P4 < 0.5 ng/mL at TAI) were included in the analyses. Milk production and components were recorded in the first 4 official milk tests. Cows were divided into quartiles based on BUN (Q1 = least BUN; Q4 = greatest BUN). Data were analyzed by logistic regression and ANOVA using the GLIMMIX and MIXED procedures of SAS. Overall, 95% of all cows were considered synchronized. At 32 d after TAI, there was a quadratic effect (P = 0.03) of BUN on P/AI (59%, 58%, 59%, and 59% for Q1, Q2, Q3, and Q4, respectively). Similarly, at 67 d after AI there was a quadratic effect (P < 0.01) of BUN on P/AI (57%, 45%, 45%, and 56% for Q1, Q2, Q3, and Q4, respectively). Pregnancy loss from 32 to 67 d after TAI did not differ (P = 0.27) among quartiles (4%, 7%, 12%, and 15% for Q1, Q2, Q3, and Q4, respectively). Similarly, at 32 d after TAI there was a quadratic effect (P < 0.01) of BUN on P/AI (57%, 45%, 45%, and 56% for Q1, Q2, Q3, and Q4, respectively). Pregnancy loss from 32 to 67 d after TAI did not differ (P = 0.38) among quartiles (48.8, 48.1, 48.5, and 48.3 kg/d for Q1, Q2, Q3, and Q4, respectively); however, milk protein % was greater (P < 0.01) for Q1 and Q2 cows and least for Q3 and Q4 cows (3.09, 3.06, 2.99, and 2.98, for Q1, Q2, Q3, and Q4, respectively). Thus, there was a quadratic effect of BUN near TAI on P/AI at 32 and 67 d after TAI, and
M183  **Post-weaning calving cattle gene expression in response to maternal feeding with methyl donors pre-partum.** C. Bespalhok Jacometo*, P. Montagner, Z. Zhou, F. Lopes, D. Luchini, M. Nunes Corrêa, and J. Loor, 1Universidade de La Salle, Bogotá, DC, Colombia, 2Universidade Federal de Pelotas, Pelotas, RS, Brazil, 3University of Illinois, Urbana, IL, 4Adisseo SA., São Paulo, SP, Brasil, 5Adisseo NA., Alpharetta, GA.

The aim of this study was to assess the effect of feeding a methionine (MET) or choline (CHO) source to dams on post-weaning calf liver expression of genes related to methyl-donor pathways and energy metabolism. The experiment was conducted as a randomized complete block design with 2 × 2 factorial arrangement of MET (Smartamine M, Adisseo NA) and CHO (ReaShure, Balchem Inc.) level (with or without). Eighty Holstein calves born to cows receiving during the last ~4 wk of pregnancy MET (0.08% DMI; n = 20), CHO (60 g/d; n = 20), MIX (MET-CHO; n = 20) or control (CON; n = 20) were evaluated. Immediately after birth calves were separated from the dam, fed first colostrum within 6 h, housed individually and fed a common milk replacer twice daily. Liver biopsies were harvested (n = 8/group) at 50 d of age (~1 wk after weaning) for qPCR analysis. Data were analyzed using the MIXED procedure of SAS, with MET and CHO as a fixed effect, and also a methyl donor contrast effect was tested. Regarding methionine cys cleavage and transulfuration pathway, maternal feeding with methyl donors downregulated (P < 0.05) the expression of PEMT and GCLC, and the expression of other genes associated with this pathway (MTR, CHDH, MAT1A, MAT2A, BHMT and SAHH) were not affected by maternal diet. Maternal methyl donor feeding reduced (P < 0.05) CSAD (taurine metabolism) and GSR (glutathione metabolism) expression, while no effect was observed on CDO and GCLC expression. Additionally, MET maternal feeding downregulated (P < 0.01) GSR expression. Expression of PPARA was downregulated (P = 0.01) when the maternal diet was supplemented with methyl donors, primarily to feeding MET (P = 0.02), and GR and PCK1 tended (P = 0.08 for both) to be lower when cows were fed methyl donors. Other genes related to carbohydrate metabolism and hepatokines (SLC2A2, PC and FGF21) were not affected by maternal diet. Maternal methyl donor feeding reduced (P < 0.05) CSAD (taurine metabolism) and GSR (glutathione metabolism) expression, while no effect was observed on CDO and GCLC expression. Additionally, MET maternal feeding downregulated (P < 0.01) GSR expression. Expression of PPARA was downregulated (P = 0.01) when the maternal diet was supplemented with methyl donors, primarily to feeding MET (P = 0.02), and GR and PCK1 tended (P = 0.08 for both) to be lower when cows were fed methyl donors. Other genes related to carbohydrate metabolism and hepatokines (SLC2A2, PC and FGF21) were not affected by maternal diet. Overall, the data suggest that maternal feeding with methyl donors during the last ~4 wk of gestation was associated with differences in calf hepatic gene expression in the post-weaning period and the response is different according to methyl donor source.

**Key Words:** amino acid, intrauterine nutrition, nutrigenomics

M185  **Effects of feeding a rumen-protected methionine on plasma amino acid concentrations, glandular morphology, and immunolabeling in the bovine endometrium.** S. L. Stella, D. A. V. Acosta, C. Skenandore, B. Q. Pinto, Z. Zheng, D. Luchini, and F. C. Cardoso, 1University of Illinois, Urbana, IL, 2The Colombian Corporation for Agricultural Research (CORPOICA), Bogotá, Colombia, 3Texas A&M College of Veterinary Medicine, College Station, TX, 4Adisseo NACA, Alpharetta, GA.

Glandular and immune function of the uterus are required for reproductive success in dairy cows. The objective of this study was to evaluate the impact of feeding a rumen-protected methionine, Smartamine M (RPM), on amino acid (AA) concentrations, glandular morphology, and immunolabeling of glutathione peroxidase 1 antibody (GPX) and superoxide dismutase 1 antibody (SOD). Multiparous Holstein cows (n = 20) were randomly assigned to 2 treatments starting at 21 d before calving until 73 DIM. Treatments were: CON (n = 9, fed the close-up and lactation diets with a Lys:Met = 3.5:1) and MET (n = 11, fed the basal diet+RPM to a Lys:Met = 2.8:1). Uterine endometrial biopsies and blood samples from the cecoygeal artery or vein were collected at 15, 30, and 73 DIM. Images were captured using whole image scanning and quantification of glandular area, epithelial height, number of cells per gland, and percentage of positively immunolabeled cells were obtained. Median values were used as cutoff values for high/low scoring during frequency analysis. Statistical analysis were performed using the MIXED and FREQ procedures of SAS. CON had lower (P < 0.01) methionine plasma concentrations (18.05 ± 2.0 μmol/mL) than MET (30.39 ± 1.6 μmol/mL). CON had higher (P < 0.01) cystine plasma concentrations (3.62 ± 0.3 μmol/mL) than MET (2.8 ± 0.3 μmol/mL). An overall treatment by DIM interaction was observed for glandular epithelial height and number of cells per gland: CON (11.76 ± 2.0 μm) had higher (P = 0.03) glandular epithelial height than MET (10.45 ± 1.7 μm) and CON (16.0 ± 2.8) had higher (P = 0.03) number of cells per gland than MET (13.81 ± 2.5). Statistical differences were not observed for glandular area (P > 0.19), GPX (P > 0.18), or SOD (P > 0.89). Frequency analysis for samples having a high or low score
of positively immunolabeled cells for GPX revealed a tendency ($P = 0.08$) at 15DIM for CON ($n = 4$) having increased chances of having a high score as compared with MET ($n = 1$). Supplementation of RPM altered the plasma AA concentrations, uterine glandular morphology, and results in increased immunolabeling of GPX at 15DIM for dairy cows.

Key Words: methionine, uterus, immunolabeling

M186  **Effects of *Saccharomyces cerevisiae* fermentation products on ovarian and uterine characteristics.** S. L. Stella*1, K. Glosson1, I. Yoon2, and F. C. Cardoso1, 1University of Illinois, Urbana, IL, 2Diamond V, Cedar Rapids, IA.

Improving uterine environment and ovarian function may lead to improved reproductive efficiency in dairy cattle. The objective of this study was to observe the effects of *Saccharomyces cerevisiae* fermentation products (SCFP) on ovarian dynamics and uterine environment. Multiparous Holstein cows ($n = 101$) were supplemented from 30 d before calving to 65DIM. Treatments were CON (control: no supplement; $n = 32$), XPC (14 g/d Diamond V Original XPC; $n = 23$), NTL (19g/d NutriTek; $n = 21$), and NTH (38 g/d NutriTek; $n = 24$). Ultrasound (US) was performed in the reproductive tract of cows daily from 7 DIM until the dominant follicle reached 16 mm in diameter. The total follicular growth was measured from 1st US until aspiration and the follicle size at aspiration. The US continued biweekly until 65DIM. Uterine fluid was harvested at 30 DIM via Foley catheter that was inserted into the uterine body and 20 mL of saline was flushed into the uterus and extracted. Specific gravity and protein content values were obtained via refractometer. Swabs of the endometrium were obtained at 15 and 30DIM, streaked onto slides, stained, and scanned using whole image scanning. Polymorphonuclear neutrophils (PMN) were counted and a percentage was calculated. Statistical analysis was performed using the MIXED procedure of SAS. Contrast statements were CON vs XPC, CON vs NTL, and CON vs NTH. No differences ($P > 0.15$) were observed for total follicular growth and follicular size at aspiration. Follicles from cows fed CON (20.09 ± 1.2 mm) were smaller ($P = 0.01$) at 65 DIM than cows fed NTL (24.87 ± 1.4 mm), and tended to be larger than cows fed XPC (17.04 ± 1.4 mm; $P = 0.11$), and NTH (17.08 ± 1.4 mm; $P = 0.11$). Cows fed CON (0.25 ± 0.08 g/dL) had higher ($P = 0.05$) uterine protein content than cows fed XPC (0.022 ± 0.1 g/dL). Statistical differences were not observed for lavage specific gravity ($P > 0.89$). Cows fed CON (19.3 ± 3.4%) had lower ($P = 0.05$) PMN than cows fed NTL (30.1 ± 4.4%) and XPC (30.76 ± 4.3%, $P = 0.04$). Supplementation of SCFP increased follicular size at 65 DIM and the PMN content in the uterus, possibly leading to better reproduction and immunity.

Key Words: *Saccharomyces cerevisiae* fermentation product, Metri- tis, PMN


The aim of the study was to determine metabolic responses to a short-term period of negative energy balance induced by feed restriction (FR). Seven multiparous Holstein cows (93 ± 15 DIM) were randomly assigned to 7 treatments in a 7 × 4 incomplete Latin square design with 5-d periods. In 6 treatments including a restricted control (RC), daily DMI was restricted to provide 60% of energy requirements; the 7th treatment consisted of ad libitum (AL) intake. Feed was provided once daily at 0900 h. Effects of FR (AL vs RC), day, time within day, and interactions were evaluated using the MIXED procedure of SAS. Milk yield ($P < 0.01$), milk protein concentration ($P = 0.03$) and yield ($P < 0.01$), and lactose yield ($P < 0.01$) were lower for RC, whereas milk fat ($P < 0.01$) and urea N concentrations were higher ($P < 0.01$). Treatment RC induced lower plasma insulin ($P = 0.01$) and glucose ($P = 0.04$) concentrations, with quadratic ($P < 0.01$ for both) decreasing trends reaching nadir on d 3. Concentration of NEFA was higher ($P < 0.01$) and increased quadratically ($P < 0.01$) with its maximum on d 3 during FR. Serum BHBA increased linearly ($P = 0.04$) for RC ($RC \times d; P = 0.16$) with its peak at d 4. Catabolism of amino acids (AA) increased early during FR as indicated by plasma urea N increasing ($P < 0.01$) quadratically ($P < 0.01$), with its peak on d 2 and decreasing afterward. Plasma 3-methylhistidine increased linearly ($P < 0.01$) denoting tissue mobilization. A group of AA (Glu, Val, Leu, Tyr, Phe, Ser, His, Thr, Asn, Ala, Pro, Met) decreased in a quadratic manner with the nadir at d 2 and 3, while Asp, Trp and Ile decreased linearly. Concentrations of other AA increased (Gln, Gly, Cys) or did not vary (Lys, Arg) during FR. Plasma AA concentrations decreased after feed delivery in both diets, coinciding with the increase of insulin, except for Glu and Gln that increased after feeding only during FR. Metabolic adaptations to lower insulin during FR seemed to select catabolism of AA as the first energy source before later relying more on fatty acids. Based on responses of plasma AA and insulin to feeding, protein synthesis in tissues likely remained sensitive to insulin within day.

Key Words: negative energy balance, lipid mobilization, protein metabolism

M188  **Effects of rain exposure on environmental conditions and vaginal temperature of Criollo dairy cows in Dominican Republic.** H. L. Sánchez-Rodríguez*1, K. Domenech-Pérez1, R. C. Youngblood3, L. Björk-Magnúsdóttir2, P. Iglesias-Estévez2, I. I. Suero-Pérez2, G. Muñiz-Colón1, and C. Cabrera-Cabrera2, 1University of Puerto Rico at Mayaguez, Mayaguez, Puerto Rico, 2ISA University, Santiago, Dominican Republic, 3Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University, Mississippi State, MS.

This study evaluated rain exposure effects on relative humidity (RH), air temperature (AT), and vaginal temperature (VT) of Criollo dairy cows in Dominican Republic ($n = 22$; 3.24 ± 1.43 lactations; 129.38 ± 62.13 d in milk). The dairy farm RH and AT were collected by 4 environmental data loggers and each cow had an implanted waterproof data logger for VT collection. Data were collected every 5 min for 3 consecutive days (and averaged by hour) as part of a larger trial affected by rain. A rainy day (RAIN; from 1115 to 1540 h) was compared with the day before (PRE-RAIN) and the day after (POST-RAIN), both without rain (PROC GLIMMIX, SAS). Day and hour interacted to affect RH ($P < 0.0001$), AT ($P < 0.0001$), and VT ($P < 0.0001$). During RAIN greater RH values were observed than in PRE-RAIN and POST-RAIN from 1300 to 2400 h (81.08 ± 1.23, 62.22 ± 0.50, and 63.14 ± 0.64%, respectively; $P < 0.0001$). However, from 0100 to 0600 h, RH was higher in POST-RAIN than in PRE-RAIN and RAIN (98.08 ± 0.12, 93.47 ± 0.28, and 93.68 ± 0.17%, respectively; $P < 0.0001$). The AT followed an opposite trend, from 1300 to 2300 h its values were lower during RAIN than in PRE-RAIN and POST-RAIN (28.07 ± 0.12, 32.10 ± 0.15, and 32.30 ± 0.16°C, respectively; $P < 0.0001$). During RAIN the VT was lower than in PRE-RAIN from 1500 to 2400 h (38.77 ± 0.05 and 39.11 ± 0.06°C, respectively; $P < 0.0001$), and lower than in...
POST-RAIN during 1500 to 1900 h (39.15 ± 0.06 and 39.70 ± 0.06°C, respectively; P < 0.0001). However, during POST-RAIN the VT values were greater than in PRE-RAIN from 1500 to 1700 h (39.6 ± 0.05 and 39.6 ± 0.06°C, respectively; P < 0.0001). Then POST-RAIN VT values sharply decreased from 1600 to 2400 h (40.08 ± 0.05 to 38.21 ± 0.03°C, respectively; P < 0.0001), reaching lower values than in the PRE-RAIN from 1900 to 0100 h (38.46 ± 0.04 and 38.71 ± 0.05°C, respectively; P < 0.0001). Rain exposure facilitated immediate heat dissipation in grazing cattle. However, it also increased relative humidity (RH), which may have later limited body heat dissipation through evaporation.

Key Words: Criollo cows, rain exposure, heat dissipation


Study objectives were to evaluate effects of chronic lipopolysaccharide (LPS) infusion on the acute phase response and immune cell dynamics in lactating cows. Following acclimation (3d), cows (164 ± 22 DIM; 676 ± 16 kg BW; parity 3±1.4) were enrolled in a study composed of 2 experimental periods (P); during P1 (3d), cows consumed feed ad libitum and baseline values were obtained. At the initiation of P2 (7d) cows were assigned to 1 of 2 treatments: 1) saline-infused and pair-fed (CON-PF; 40 mL/h saline; n = 6) or 2) continuous LPS-infused and ad libitum-fed (LPS-AL; E. coli O55:B5; 0.017, 0.020, 0.026, 0.036, 0.055, 0.088, and 0.148 µg/kg BW/h for d 1–7, respectively; n = 6). Blood samples for analysis of acute phase proteins were collected on 0.055, 0.088, and 0.148 µg/kg BW/h for d 1–7, respectively; n = 6).

In LPS-AL cows 1 d postbolus and steadily declined with time (P < 0.05). Then POST-RAIN VT values sharply decreased from 1600 to 2400 h (40.08 ± 0.05 to 38.21 ± 0.03°C, respectively; P < 0.0001), reaching lower values than in the PRE-RAIN from 1900 to 0100 h (38.46 ± 0.04 and 38.71 ± 0.05°C, respectively; P < 0.0001). Rain exposure facilitated immediate heat dissipation in grazing cattle. However, it also increased relative humidity (RH), which may have later limited body heat dissipation through evaporation.

Key Words: bovine adipose tissue, steroidogenic enzyme, mRNA abundance

M191 Additional small dose of prostaglandin F2α at timed AI fails to improve pregnancy per AI in lactating dairy cows. J. A. Sauls*, B. E. Voelz, L. G. D. Mendonca, and J. S. Stevenson, Kansas State University, Manhattan, KS.

Two experiments were performed to test the hypothesis that administering PGF2α concurrent with timed artificial insemination (AI) in lactating dairy cows would enhance pregnancy per AI (P/AI). In experiment 1, lactating Holstein cows (n = 297) in 1 herd were enrolled at non-pregnancy diagnosis (30 to 36 d after AI) to synchronize subsequent ovulation before AI. Cows were assigned randomly to receive (i.m.) 10 mg PGF2α concurrent with AI (d 0; treatment) or no injection (control). Blood samples were collected on d −3, 0 and 13 to determine serum concentrations of progesterone. Ovaries were scanned via transrectal ultrasonography to determine follicle diameters (d −3), subsequent ovulation risk (d 13), and total volume of luteal tissue (d 13). Diagnosis of pregnancy occurred 32 and 80 d after AI. In experiment 1, ovulation risk post-AI exceeded 91% and did not differ (P = 0.96) between treatments. Treatment with PGF2α at AI decreased (P = 0.05) double ovulation risk. In contrast, PGF2α treatment numerically increased progesterone (5.4 ± 0.4 vs. 5.8 ± 0.4 ng/mL) and luteal tissue volume (7.8 ± 0.8 vs. 8.6 ± 0.8 ng/mL) on d 13 by 7.4% (P = 0.27) or 10.3% (P = 0.16) in control and treated cows, respectively. Pregnancy per AI at d

Key Words: lipopolysaccharide, acute phase proteins

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32 and 80 was numerically increased by 16.7% \( (P = 0.47) \) and 22.3% \( (P = 0.30) \), respectively. Cows with progesterone >0.5 ng/mL at timed AI had reduced \( (P < 0.001) \) ovulation risk and tended \( (P < 0.10) \) to have poorer P/AI; both unaffected by treatment. In experiment 2, lactating dairy cows \( (n = 1,066) \) in 2 other herds were enrolled after unsuccessful first or later inseminations, and assigned randomly to treatment or control as described in experiment 1. Initial \( (d \ 35) \) and confirmed \( (d \ 65) \) pregnancy diagnosis revealed no differences in P/AI or embryonic survival. Pregnancy per AI was greater \( (P < 0.05) \) in primiparous cows \( (21 \% \pm 2 \%) \) than crossbred cows \( (22 \% \pm 2 \%) \), and between herds \( (28 \% \pm 3 \%) \). Pregnancy loss was greater \( (P = 0.04) \) for cows inseminated at first \( (10 \%) \) vs. later services \( (5 \%) \). We conclude that treatment with PGF2α concurrent with timed AI did not improve pregnancy per AI or embryo survival in lactating dairy cows.

**Key Words:** progstaglandin, artificial insemination, pregnancy per AI

M192  **Effect of addition of l-carnitine during culture on pregnancy rate obtained after transfer of cryopreserved bovine embryos produced in vitro.** A. Zolini*1, P. J. Hansen1, and J. Block1,2, 1University of Florida, Gainesville, FL, 2OvaTech LLC, Gainesville, FL.

The aim of this study was to determine the effect of culture media supplementation with l-carnitine on embryo development and pregnancy rate following cryopreservation. Embryos were produced in vitro using cumulus-oocyte complexes collected by ovum pick-up (OPU) from pregnant, Holstein heifers \( (n = 24) \) following superstimulation. Superstimulation was induced 48 h after dominant follicle removal with 2 intramuscular injections of 90 mg of follicle-stimulating hormone (FSH; Folltropin-V) diluted in hyaluronic acid (MAP-5) given 48 h apart. OPU was performed 32 h after the second FSH injection. After fertilization with X-sorted semen, presumptive zygotes \( (n = 417) \) were randomly assigned in a crossover design to culture in SOF-BE1 supplemented with 0 or 0.75 mM l-carnitine at 38.5°C in a humidified atmosphere of 5% \( O_2 \), 5% \( CO_2 \) and 90% \( N_2 \). The proportion of oocytes that cleaved was assessed on d 3 after insemination and the proportion of oocytes that developed to the blastocyst stage was determined on d 7. Grade 1 and 2 morula and blastocyst stage (early, blastocyst, expanded and hatched) embryos were harvested on d 7 and subjected to controlled-rate freezing following equilibration in 1.5 M ethylene glycol. Lactating Holstein cows were synchronized for timed embryo transfer using the OvSynch-56 protocol (Carvalho et al., 2014). At d 7 after presumptive ovulation, a single embryo \( (n = 102) \) was randomly thawed and transferred into cows having a corpus luteum confirmed by ultrasonography. Pregnancy was diagnosed at d 33, 45, and 72 of gestation. Data were analyzed using the GLIMMIX procedure of SAS \( (P < 0.05) \). There was no effect of l-carnitine on cleavage rate, blastocyst rate or on the proportion of embryos selected for freezing. Pregnancy rates on d 33, 45 and 72 was not affected by l-carnitine \( (33.3 \% \pm 0.06 \text{ vs. } 27.7 \% \pm 0.06, 31.2 \% \pm 0.06 \text{ vs. } 27.7 \% \pm 0.06, 22.9 \% \pm 0.06 \text{ vs. } 22.2 \% \pm 0.06 \text{ respectively}) \). l-Carnitine also had no effect on pregnancy loss between d 33 and 45 and d 45 and 72 \( (6.0 \% \pm 0.1 \% \text{ vs. } 0.0 \% \text{ and } 26.6 \% \pm 0.1 \% \text{ vs. } 20.0 \% \pm 0.1 \% \text{ respectively). In conclusion, supplementation of embryo culture media with l-carnitine had no effect on embryo development or pregnancy rate after cryopreservation.**

**Key Words:** l-carnitine, IVF, transference

M194  **Adipose tissue remodeling in transition dairy cows is affected by body condition score and lipolysis intensity.** G. A. Contreras*1, C. S. Barboza1, K. Thelen1, J. de Souza2, J. De Koster1, and A. L. Lock2, 1Department of Large Animal Clinical Sciences, East Lansing, MI, 2Department of Animal Science, East Lansing, MI.

Lipopysis induces a remodeling process in adipose tissue (AT) that is characterized by an inflammatory response with immune cell migration, proliferation of cellular components of the stromal vascular fraction (SVF), and changes in the extracellular matrix. This study evaluated the effect of body condition score (BCS) and lipolysis intensity on markers of AT remodeling in transition dairy cows. Blood and subcutaneous AT samples were collected from multiparous Holstein cows with high (HB; \( n = 12 \)) and low \( (L) \) body condition scores \( (BSC > 3.75 \) or moderate (MB; \( n = 9 \)) BCS < 3.5) BCS at 27 ± 7 (FO) and 10 ± 5 (CU) d prepartum and at 8 ± 3 (PP) d postpartum. Expression of genes related to AT remodeling was analyzed by RT-PCR, and cell immuno staining in AT SVF was evaluated by flow cytometry. Lipolysis increased at CU and reached its peak at PP compared with FO as reflected in calculating free fatty acid (FFA) concentrations \( (FO: 0.27 \pm 0.05, CU: 0.39 \pm 0.05, PP: 0.99 \pm 0.05 \text{ mEq/L}) \). FFA were higher in HB \( (0.63 \pm 0.02 \text{ mEq/L}) \) compared with MB \( (0.47 \pm 0.02 \text{ mEq/L}) \) cows reflecting an effect of BCS on lipolysis rate during gestation and after parturition. Gene expression indicated that osteopontin
Mobilization of triacylglycerols (TAG) during the transition period is crucial to mitigate negative energy balance (NEB). Lipolysis is mediated by lipases; however, known regulators of lipolysis in nonruminants are not upregulated during the transition period in dairy cows. The objective of this study was to determine the coordinated response of bovine adipose lipases to mobilize TAG during the transition period. Multiparous pregnant dairy cows were blocked by anticipated calving date and randomly assigned to either the control group (n = 3), fed an ad libitum diet, or fatty liver induction (FLI) group (n = 8; overfed prepartum and feed restricted postpartum) until clinical ketosis onset. Adipose tissue and blood samples were collected at −14, +1, and +14 d relative to calving (DRTC) for NEFA and lipase quantification. Additional samples (n = 3) were taken once during a period of positive energy balance (PEB) to utilize as PEB controls. Protein abundance of adipose tissue, PNPLA3 may be a key lipase during the transition period; however, the mechanistic relationship between these lipases needs to be further explored. Key Words: lipolysis, adipose tissue macrophage, remodeling

M195 Coordination of adipose tissue lipolysis during the transition period in dairy cows. S. J. Erb*, R. S. Pralle, and H. M. White, University of Wisconsin-Madison, Madison, WI.

Mobilization of triacylglycerols (TAG) during the transition period is crucial to mitigate negative energy balance (NEB). Lipolysis is mediated by lipases; however, known regulators of lipolysis in nonruminants are not upregulated during the transition period in dairy cows. The objective of this study was to determine the coordinated response of bovine adipose lipases to mobilize TAG during the transition period. Multiparous pregnant dairy cows were blocked by anticipated calving date and randomly assigned to either the control group (n = 3), fed an ad libitum diet, or fatty liver induction (FLI) group (n = 8; overfed prepartum and feed restricted postpartum) until clinical ketosis onset. Adipose tissue and blood samples were collected at −14, +1, and +14 d relative to calving (DRTC) for NEFA and lipase quantification. Additional samples (n = 3) were taken once during a period of positive energy balance (PEB) to utilize as PEB controls. Protein abundance of adipose tissue, PNPLA3 may be a key lipase during the transition period; however, the mechanistic relationship between these lipases needs to be further explored. Key Words: lipolysis, adipose tissue macrophage, remodeling

M196 Expression of corticosteroidogenic metabolizing enzymes in adipose tissue of dairy cows with high and normal body condition score. K. Schuh*,1,2, S. Häussler1, C. Koch1, D. Frieten2, G. Duse1, H. Sadri1, and H. Sauerwein1, 1University of Bonn, Institute for Animal Science Physiology & Hygiene, Bonn, North-Rhine-Westphalia, Germany, 2University of Applied Sciences Bingen, Animal Nutrition and Health, Bingen am Rhein, Rhineland Palatinate, Germany, Educational and Research Centre for Animal Husbandry, Hofgut Neumühle, Münchweiler a.d. Alsenz, Rhineland Palatinate, Germany.

Adipose tissue (AT) is known to express genes involved in steroid synthesis and metabolism. Due to the ability of dairy cows to mobilize large amounts of body fat, elevated mobilization of AT might increase the release of steroidogenic compounds into the circulation, affecting the physiological steroid hormone balance. In the present study, we tested the mRNA expression of enzymes catalyzing the conversion of cholesterol to 11-deoxycorticosterone i.e., steroidogenic acute regulatory protein (StAR), 3b-hydroxysteroid dehydrogenase (3bHSD), and P450–21-hydroxylase (CYP21) in subcutaneous (sc)AT of dairy cows with different body condition score (BCS). German Holstein cows (n = 38) were preselected and allocated to groups 15 wk before calving, based on either a high (HBCS; BCS >3.75) or normal (NBCS; BCS <3.5) BCS. In addition, back fat thickness (BFT) of both groups was measured (HBCS: BFT >1.4; NBCS: BFT <1.2). Biopsies from scAT (tail head region) were sampled on d −49, 3, 21, and 84 relative to calving. The mRNA abundance of the target genes was assessed by qPCR and normalized by using the 4 most stable reference genes. Data were analyzed using the mixed-model procedure followed by Bonferroni correction (SPSS 21). Differences in BCS and BFT (P < 0.001) due to early grouping of cows were maintained throughout the whole study. From calving until d 84 after calving, HBCS cows lost twice as much BFT compared with NBCS cows (P < 0.001). From d −49 to +84, in both groups the mRNA abundance of CYP21 and StAR increased by 65% (P < 0.01) and 275% (P < 0.001), respectively. The abundance of CYP21 mRNA tended to be 48% higher in NBCS cows on d 21 (P = 0.068) compared with HBCS cows, whereas that of 3bHSD was consistently low over all time points. Increased mRNA abundance of StAR over time, revealed the increased availability of cholesterol for the steroidogenic response. The post-partal increase of CYP21 abundance indicated an enhanced conversion of progesterone into corticosterone in bovine AT. However, the low and unchanged expression of 3bHSD over time gives no indication for a major role of bovine AT in the synthesis of androgens, estrogens and corticosteroids. Key Words: corticosteroidogenesis, bovine adipose tissue, mRNA abundance


Immunoaivation via a lipopolysaccharide [LPS] bolus negatively affects multiple productive parameters in farm animals. However, the paradigm of acute LPS bolus likely does not resemble chronic on-farm in vivo immunoaivation. Therefore, study objectives were to characterize the effects of chronic inflammation on production in lactating Holstein cows. Eleven cows (164 ± 22 DIM; 676 ± 16 kg BW; parity 3.1 ± 0.4) were acclimated for 3 d, and subjected to 2 periods (P); during P1 (3 d), cows consumed feed ad libitum and baseline samples were obtained. During P2 (7 d), cows were assigned to 1 of 2 treatments: (1) saline-infused and pair-fed (CON-PF; 40 mL/h saline; n = 5) or (2) LPS-infused and ad libitum-fed (LPS-AL; E. coli O55:B5; 0.017, 0.020, 0.026, 0.036, 0.055, 0.088, and 0.148 μg/kg BW/d 1–7, respectively;
n = 6). CON-PF cows were pair-fed to eliminate confounding effects of dissimilar nutrient intake. LPS induced a febrile response in LPS-AL cows on d 1 (+0.49°C) relative to baseline and LPS-AL cows remained hyperthermic for the remainder of P2 (+0.26°C; P < 0.01). Relative to CON-PF, respiration rate and heart rates increased 17 breathes per min and 11 beats per min, respectively, during P2 (P < 0.01) for LPS-AL cows.DMI decreased (28%; P < 0.05) on d 1 as a result of LPS, but DMI progressively increased with time (P < 0.01) returning to baseline levels after 6 d; by design, the CON-PF cows DMI pattern mirrored LPS-AL cows. Relative to baseline and CON-PF cows, milk yield from LPS-AL cows decreased 17% on P2D1 (P = 0.01). From P2D2 onwards, milk yield from both treatments were similar but decreased (12%; P < 0.01) relative to P1. Except for increased milk fat concentration (19%; P = 0.03), LPS infusion had little or no effect on SCC, milk protein, and lactose content. Based upon body temperature and production variables, this data supports that lactating Holstein dairy cows become incredibly tolerant to chronic and exponentially increasing amounts of infused LPS.

Key Words: lipopolysaccharide, production, immunooactivation


The development of insulin resistance during the peripartum can promote adipose tissue lipolysis. Considering that adiposity can exacerbate free fatty acid (FFA) mobilization, our objective was to characterize insulin action in lean and overconditioned peripartal dairy cows. Twenty-five pregnant, multiparous Holstein dairy cows were enrolled 28 d before expected parturition. Cows were allocated into one of 2 adiposity groups, lean (LEAN; BCS 3.0 ± 0.16) or overconditioned (OVER, BCS 3.9 ± 0.30). Blood samples were collected routinely. Subcutaneous adipose tissue (SubqAT) biopsies were performed on d −12 and 10, relative to parturition. The hyperinsulinemic-euglycemic clamp (HEC) procedure was performed on d −10 and 12, relative to parturition. Plasma and serum metabolites were analyzed using colorimetry. Circulating insulin levels and SubqAT protein kinase B (PKB) phosphorylation were determined using immunoassays. SubqAT adipocyte volume and number were determined. Relative to LEAN, OVER lost more body weight, BCS units, and back fat thickness during the peripartum (P < 0.01). Adipocyte volume was 48% greater in OVER prepartum (P < 0.01); however, adipocyte number was not influenced by adiposity. Adipocyte volume and number decreased from pre- to postpartum (P < 0.01). Plasma FFA levels were consistently elevated in OVER including a 74% surge at parturition (P < 0.01). Plasma β-hydroxybutyrate levels were greater in OVER (P < 0.05). Although plasma glucose or insulin were not modified by adiposity, basal serum insulin concentrations decreased postpartum (P < 0.05). During the HEC, adiposity had no effect on steady state serum glucose, insulin, or FFA levels. Although adiposity did not modify HEC glucose infusion rate (P = 0.18), the insulin sensitivity index (SIclamp) was 49% lower for OVER postpartum (P < 0.01). Insulin-stimulated phosphorylation of SubqAT PKB was not affected by adiposity; however, PKB phosphorylation decreased 18% from pre- to postpartum (P < 0.05). We conclude that observed elevations in circulating FFA in overconditioned cows cannot be attributed to impaired SubqAT PKB signaling, albeit adiposity reduced systemic insulin sensitivity.

Key Words: adiposity, insulin resistance, hyperinsulinemia


Hair accumulates cortisol over time and concentrations during intervals (IT) are used to assess chronic exposure of animals to situations that increase cortisol secretion. Our objectives were to assess impact of milk yield genotype on hair cortisol when unique unselected (stable milk yield since 1964; n = 12) and contemporary (n = 12) Holsteins that differed in milk yield by more than 4,500 kg milk/305 d were subjected to stressors during IT of the periparturient period and early lactation. Cows were blocked by expected calving date and hair removed from both shoulders and left side 4 wk before expected calving. Shoulder hair was collected at 2 d (IT1), 7 wk (IT2) and 14 wk (IT3) and left side hair at 14 wk (IT4) of lactation. Liver, mammary and tailhead adipose samples were collected at −12 ± 1 DIM (during IT1) and at 4, 14, and 42 ± 1 DIM (during IT2). For IT3, saline or LPS (0.25 μg/kg BW, Escherichia coli 055:B5) was administered twice by iv injections 4 d apart and liver sampled at 0, 4, and 24 h after each injection. Left side samples represented the entire 18 wk study (IT4). Cortisol in washed, ground hair was extracted with methanol and measured by a validated ELISA. Right and left shoulder values were averaged. The IT1 and IT2 samples were assessed for effect of genotype, interval, and interaction by repeated measures using PROC MIXED (SAS) with IT as the repeated effect. The IT3 and IT4 samples were analyzed separately to assess effect of genotype, treatment (saline, LPS) and interaction. Means differed when P < 0.05. There was no interaction in either model. Results indicate hair cortisol accumulation did not differ between genotypes (2.90 ± 0.24 pg/mg) but was increased when cows were in early lactation and subjected to 3 multiple biopsies (IT1 vs. IT2; 2.41 vs. 3.39 ± 0.26 pg/mg; P = 0.002). During IT3 and IT4, there was no effect of genotype and the short duration of the LPS-induced response was insufficient to elicit a change in hair cortisol. Although there is concern that increased milk yield increases stress in cows, it had no effect on hair cortisol concentrations in this study.

Key Words: hair cortisol, Holstein genotype, milk yield

M200 Effect of the intrauterine dextrose infusion at non-pregnancy diagnosis on fertility of lactating dairy cows. S. Bas*, A. A. Barragan, J. M. Piñeiro, B. T. Menichetti, and G. M. Schuenemann, Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH.

The dairy industry relies on having appropriate reproductive performance to ensure milk production and replacement heifers. Intrauterine infusion of dextrose (i.e., 50% dilution) has been proven to cure clinical endometritis and increase pregnancy rates in dairy cows. The objectives of the present study were to (1) assess the effects of the intrauterine infusion of a 50% dextrose solution on non-pregnant cows, and (2) to estimate the prevalence of subclinical endometritis (SCE) at the time of non-pregnancy diagnosis in lactating dairy cows. Holstein lactating cows (n = 711) from 3 commercial dairy herds were enrolled at non-pregnancy diagnosis, blocked by parity (primiparous and multiparous) and repeat breeder (RB) status (RB ≥ 3 services; noRB < 3 services), and were randomly allocated to receive the intrauterine infusion with a dextrose solution (DEX; n = 356) or remain as controls cows (CON; n = 355). In addition, SCE was assessed on a subset of cows (DEX = 100; CON = 100). Immediately after non-pregnancy diagnosis, DEX cows were infused with 120 mL of a 50% dextrose solution using individually wrapped, single-use sterile infusion pipettes; while CON cows received no treatment. In animals were SCE was evaluated, samples for
assessment of polymorphonuclear cells were collected from the uterine body using the cytobrush technique immediately before treatment administration. All cows followed the established on-farm reproductive program for subsequent AI. Pregnancy (PAI) was determined by the herd veterinarian at 40 ± 5 d post-AI. No differences (P ≥ 0.05) in the prevalence of SCE were observed between DEX and CON cows. Overall, no difference (P ≥ 0.05) in PAI were observed between cows in the DEX (31.3 ± 2.5%) and CON (26.6 ± 2.3%) groups. However, analysis by RB status showed that noRB cows within the DEX group had higher (P < 0.05) PAI when compared with cows in the CON group (36.6 ± 3.6% and 26.9 ± 3.4% respectively). Interestingly, PAI in RB cows treated with DEX (24.8 ± 3.4%) was not different (P ≥ 0.05) from CON (26.1 ± 3.3%). In conclusion, the intrauterine infusion with 120 mL of a 50% dextrose solution may alter the uterine environment early in lactation as reflected by the improved PAI in noRB dairy cows.

Key Words: dextrose, fertility, repeat breeder


Whole-body insulin sensitivity and metabolic responses to epinephrine are reduced during immunoinflammation in several animal models, but effects of a chronic LPS infusion on these metabolic changes in lactating cows are not well-known. Therefore, experimental objectives were to characterize the metabolic response to an i.v. glucose tolerance test (GTT; 250 mg/kg BW) and epinephrine challenge (EC; 1.4 μg/kg BW) on d 8 of continuous LPS infusion in mid-lactation Holstein cows (164 ± 22 DIM; 676 ± 16 kg BW; parity 3.1 ± 0.4). Following a 3 d acclimation, cows were enrolled in 2 experimental periods (P). During P1 (3 d), cows were fed ad libitum and baseline data were collected. Starting P2 (8 d), cows were assigned to 1 of 2 treatments: (1) saline-infused and pair-fed (CON-PF; i.v. 40 mL/h saline; n = 5) or (2) LPS-infused and ad libitum-fed (LPS-AL; E. coli O55:B5; 0.017, 0.020, 0.026, 0.036, 0.055, 0.088, 0.148, and 0.148 μg/kg BW/hr for d 1–8, respectively; n = 6). LPS reduced DMI (28%) on d 1 but DMI gradually returned to pre-infusion levels by the end of P2, and overall milk yield was reduced similarly (17%) between treatments. Following the d 8 0600h milking, feed was removed and all cows received a GTT. Feed was returned following the challenge. Four hours following the GTT feed was again removed and cows received an EC. Blood samples for both challenges were collected −30, −20, −10, 0, 5, 10, 20, 30, 45, 60, and 90 min relative to bolus. An additional 2.5 min postbolus sample was collected for the EC. For the GTT, area under the curve (AUC) was calculated through the 90 and 45 min sample for glucose and insulin, respectively. For the EC, AUC was calculated through the 60 min sample for glucose and nonesterified fatty acids. In response to the GTT, glucose and insulin AUC were increased 33 and 56%, respectively in LPS-AL relative to CON-PF cows (P = 0.01). There were no differences in the glucose or nonesterified fatty acid response to the EC. In summary, chronic LPS exposure induces peripheral insulin resistance but does not affect adipocyte and hepatocyte sensitivity to an epinephrine challenge.

Key Words: epinephrine, insulin, lipopolysaccharide

M202 Slick-haired Puerto Rican Holstein cows have larger sweat glands than their wild type-haired counterparts. Z. E. Contreras-Correa*, N. Peña-Alvarado2, W. Torres-Ruiz2, J. R. Almodóvar-Rivera2, K. I. Domenech-Pérez1, C. Youngblood4, M. Pagán-Morales1, A. Mesonero-Morales1, J. Curbelo-Rodriguez1, P. F. Randal-Follin1, G. C. Muñiz-Colón1, V. Colón-González1, A. L. Jiménez-Arroyo1, G. M. Jiménez-Arroyo1, H. L. Sánchez-Rodríguez1, University of Puerto Rico at Mayaguez Campus, Department of Animal Science, Mayaguez, Puerto Rico, 1Laboratorio de Investigaciones Pesqueras, Departamento de Recursos Naturales y Ambientales, Cabo Rojo, Puerto Rico, 2University of Puerto Rico at Mayaguez Campus, Department of Biology, Mayaguez, Puerto Rico, 4Institute of Genomics, Biocomputing and Biotechnology, Mississippi State University, Mississippi State, MS.

In the Puerto Rican Holstein cow population, the presence of a shorter and sleek hair coat (SLICK) has previously been associated with a superior tolerance to heat stress in terms of lower body temperature and respiratory rate, and a greater milk yield in comparison with wild type-haired (WT) cattle. However, besides a shorter hair coat, this superior thermoregulatory capacity must be of a multifactorial nature. Therefore, the present study aimed to evaluate other factors that may help to better understand these differences, including skin thickness (ST; from the epidermis to the deep layer of the dermis), and cross-sectional sweat gland area (SGA) and perimeter (SGP). Groups of SLICK (n = 7) and WT (n = 7) cows with similar milk yields (P = 0.8025), were first phenotypically selected and then genonomically confirmed. From each cow, 2 skin biopsies (6 mm in diameter) were collected immediately cranial to the right shoulder after local anesthesia infiltration. Samples were fixed in 10% formalin, embedded in paraffin, sectioned perpendicular to the skin surface (7 μm thickness), and stained with hematoxylin and eosin. From each biopsy, one microscopic slide was prepared containing 3 histological sections. Microscopic images (4×) were analyzed by the NIS Element D software (Nikon, Melville, NY), evaluating all the cross-sectional cuts of sweat glands per histological section (39.01 ± 11.97 cuts / section) for SGA and SGP determination. The ST was measured in triplicate from each histological section. Data were averaged by microscopic slide (PROC MEANS) and analyzed using the GLIMMIX procedure of SAS. The SLICK cows presented greater values of ST (1,006 ± 34 vs. 808 ± 23 μm; P = 0.0027), SGA (6,009 ± 335 vs. 3,169 ± 306 μm²; P = 0.0008), and SGP (315 ± 9 vs. 232 ± 12 μm; P = 0.0024) than their WT counterparts. Our results demonstrate a greater size of the sweat glands in the shoulder region of the SLICK cows. Future studies should evaluate if such differences are associated with increased sweat secretion, thus facilitating body heat dissipation through evaporation.

Key Words: slick-haired Puerto Rican Holstein cow, sweat glands, thermoregulation

M203 Interaction of pre-calving DCAD diet and serotonin infusions on pre and post calving energy markers in multiparous Holstein cows. A. A. Cheng*, C. J. Slater, E. L. Endres, and L. L. Hernandez, University of Wisconsin-Madison, Madison, WI.

The periparturient period places extreme metabolic stress on cows. Our lab has previously demonstrated that infusions with a serotonin precursor, 5-hydroxy-L-tryptophan (5-HTP), increases circulating glucose and nonesterified fatty acids (NEFAs) while concurrently decreasing β-hydroxybutyrate (b-HB). These results indicate that treatment with 5-HTP may be able to increase the cow’s ability to deal with negative energy balance due to calving. It is unknown whether feeding a negative DCAD diet before calving in conjunction with 5-HTP treatment could mitigate metabolic stress due to calving. This study
utilized a randomized complete block design with a 2 × 2 factorial arrangement. Holsteins were fed either a positive (+130 mEq/kg) or negative (−130 mEq/kg) DCAD diet 21 d before calving and were also treated daily with saline or 5-HTP (1 mg/kg) IV beginning 7 d before estimated calving date. 32 multiparous Holstein cows, blocked by parity, were randomly assigned to 1 of 4 treatment groups in a 2 × 2 factorial arrangement: (1) positive DCAD plus saline; (2) positive DCAD diet plus 5-HTP; (3) negative DCAD plus saline; and (4) negative DCAD plus 5-HTP. This resulted in an n = 8 per group. Plasma glucose, blood urea nitrogen (BUN), insulin, and adiponectin were measured. Serum was used to measure NEFAs and BHB. Preliminary results suggest that neither pre or post-calving insulin, leptin, or cortisol were significantly affected by either DCAD or 5-HTP treatment. However, both pre and post calving BUN were significantly elevated in cows treated with saline versus 5-HTP (P = 0.0184 and 0.005, respectively). This suggests that serotonin may have a significant impact on protein turnover during the transition period, regardless of DCAD level. Future experiments should be targeted at understanding the effects serotonin may have on protein turnover in the transition period.

Key Words: serotonin, energy, transition

M204 Some factors affecting the response of treatment with novel hormonal protocols in anestrus water buffaloes. S. Sah and B. Devkota*, Department of Theriogenology, Faculty of Animal Science, Veterinary Science and Fisheries, Agriculture and Forestry University, Rampur, Chitwan, Nepal.

Buffalo rearing is important for rural economy of many countries. Buffaloes are reared under subsistence to semi-intensive system exposing them to a variety of managemental, nutritional and environmental stress that adversely affects on their reproductive performance. The present study was conducted in Southern Nepal where post monsoon to early winter is a good breeding season for buffaloes where as late winter to dry early summer is poor breeding season. Recently, attempts using novel hormones have been made for round the year breeding of buffaloes, however, obtaining pregnancy during poor season is difficult. The objective of the study was to understand factors limiting the response of treatment with novel hormonal protocols in anestrus water buffaloes during different seasons. Total of 42 anestrus buffaloes, 14 in each group during good season, transition season and poor season were used. In good season, ovsynch protocol was applied whereas CIDR co-synch protocol was used during transition to poor season. Blood samples were collected before the treatment to analyze nutritional parameters (glucose, total protein, cholesterol) and stress factor (cortisol). Body condition score was recorded. Transrectal ultrasonography was used to determine pregnancy. Differences in nutritional parameters and cortisol level between different seasons and between pregnant and non-pregnant animals were analyzed by Student t-test. Pregnancy rate between different seasons and for different protocols were analyzed by Chi-Square test. Ovsynch treatment during good season produced 42.9% (6/14) pregnancy whereas CIDR co-synch protocol produced 50.0% (7/14) during transition and 28.6% (4/14) during poor season. Body condition score affected (P < 0.05) on pregnancy after treatment. Blood cholesterol and cortisol levels were different (P < 0.05) among 3 seasons. When compared with pregnant group, levels of glucose and total protein were lower (P < 0.05) and cholesterol level tended to be lower (P = 0.07) in non-pregnant group. In conclusion, progestin based hormonal protocols can be successfully applied in anestrus buffaloes during transition to poor season. However, the outcome depends largely on nutritional and stress factors.

Key Words: anestrus buffalo, reproductive performance, stress

M205 Arteriovenous blood metabolomics: An efficient method to determine the key metabolic pathway for milk synthesis in intra-mammary gland. B. Wang*1, 2, L. S. Jiang3, L. L. Guan4, and J. X. Liu1, 1Institute of Dairy Science, College of Animal Sciences; MoE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou, Zhejiang, China, 2Beijing Key Laboratory for Dairy Cow Nutrition, College of Animal Science and Technology, Beijing University of Agriculture, Beijing, China, 3Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada.

This study sought to identify changes in the metabolic signature of arterial blood and milk from dairy cows during response to 3 forage diets [23% of alfalfa hay (AH, n = 10), 30% of corn stover (CS, n = 10), and 30% of rice straw (RS, n = 10), % of DM basis] of 30 Holstein dairy cows (milk yield = 30.0 ± 3.53 kg/d, day in milk = 160 ± 27.8 d, parity = 3.4 ± 1.57; mean ± SD). Diets were isonitrogenous but not isoenergetic, with 31.1 and 18.9%, 36.3 and 19.5%, 36.9 and 21.9% for NDF and ADF in diets of AH, CS, and RS, respectively. The GC-TOF/MS analysis was performed. Multivariate statistical analysis with orthogonal partial least-square discriminant analysis was used. If P-value <0.05, with log2(fold change) > 0.2 or <−0.2 and q-value <0.15 as cut-off, the metabolites were considered as different. The metabolomics profiling identified a total of 216 and 219 metabolites in the arterial and venous plasma, respectively. Among them, 1 and 4 metabolites were unique to arterial and venous plasma with 25 of them having significantly different abundance between arterial and venous plasma. The KEGG pathway analysis revealed the differential abundant metabolites mainly involved in nutritional transport, amino acid metabolism, and protein translation. Moreover, pathways of aminoacyl-tRNA biosynthesis, phenylalanine metabolism involving glutamate, phenylalanine and tyrosine, could be selected as the actual pathways to reflect the metabolic progress for milk synthesis. In addition, the significant changes of phenylpropanoate in cows fed CS and RS and prostaglandin A2 in cows fed RS compared with AH, suggesting they are probable biomarker when cows fed low quality forage. The comprehensive assessment of metabolic changes in the arteriovenous metabolome during lactating and under different diets provide some new and fundamental understandings of the important metabolic pathways such as phenylalanine metabolism and aminoacyl-tRNA biosynthesis, and key metabolites such as 3-hydroxybutyric acid, glutamate, phenylpropanoate, and prostaglandin A2 and for improving milk production.

Key Words: arteriovenous, metabolomics, milk synthesis
Most metabolic hydrogen ([2H]) produced in the rumen during microbial fermentation is directed toward methanogenesis. Because a decrease in methane (CH$_4$) production does not always result in better animal performance, the aim of this study was to understand the fate of [2H] when rumen methanogenesis is inhibited. Three chemical inhibitors with different modes of action (nitrate, NIT; 3-nitrooxypropanol, NOP; anthraquinone, AQ) were evaluated with the Rumen Simulation Technique (RUSITEC). The microbial community was adapted to the system for one week before measurements. Treatments and days fixed effects, and their interaction, were analyzed with the MIXED procedure. Effects, and their interaction, were analyzed with the MIXED procedure.

In vitro modification of metabolic hydrogen production and consumption with methanogenesis inhibitors. J. Guyader*1, E. M. Ungerfeld2, and K. A. Beauchemin1, 1Leithbridge Research and Development Centre, Agriculture and Agri-Food Canada, Leithbridge, AB, Canada, 2Instituto de Investigaciones Agropecuarias INIA Carilanca, Temuco, Chile.

Key Words: N use efficiency, phenotypic residual feed intake, feed efficiency

Is phenotypic residual feed intake associated with feed efficiency, nitrogen use efficiency, urinary nitrogen and methane losses in lactating dairy cows? F. Sun*1, M. Aguerre2, and M. Wattiaux1, 1Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, 2Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC.

Our objective was to determine whether phenotypic residual feed intake (pRFI) was associated feed efficiency (fat-and-protein-corrected milk/dry matter intake (DMI)), N use efficiency (milk N/N intake), and metabolic energy losses: urinary N and enteric methane (CH$_4$) in dairy cows. Data for this study was obtained from a 12-week trial in which 18 multiparous Holstein cows in mid-lactation were fed total mixed rations with 16.5% crude protein, approximately 29.0% neutral detergent fiber and 45.5% nonfiber carbohydrates (dry matter basis). Nitrogen balance was from urine and fecal spot sampling and CH$_4$ emission was from a GreenFeed unit (C-Lock Inc., Rapid City, SD). First, an equation was developed to predict DMI for the cohort of 18 cows based on the multiple linear regression between actual DMI and actual energy expenditures (NRC-predicted energy in milk, change in body weight, and maintenance) for each cow over 12 weeks. A pRFI was then calculated for each cow as the difference between actual and predicted DMI (cohort pRFI ranging from -2.35 to 2.13 kg/d). Pearson correlations indicated that pRFI was positively correlated with actual DMI (20.8 to 28.1 kg/d, r = 0.66, P < 0.01) and fecal N excretion (140 to 200 g/d, r = 0.61, P < 0.01) but negatively correlated with feed efficiency (1.34 to 1.77, r = -0.54, P = 0.02) and N use efficiency (25.1 to 32.1%, r = -0.76, P < 0.01). In contrast, pRFI was not correlated with milk urea nitrogen (12.3 to 16.0 mg/dL, r = -0.11, P = 0.67), urinary N (210 to 400 g/d, r = 0.22, P = 0.38), urinary urea-N (162 to 239 g/d, r = 0.32, P = 0.20), enteric CH$_4$ emission (401 to 566 g/d, r = 0.15, P = 0.54), CH$_4$/DMI (16.4 to 24.3 g/kg, r = -0.34, P = 0.17), CH$_4$/milk yield (10.6 to 18.2 g/kg, r = 0.06, P = 0.80) and milk yield (29.5 to 42.7 kg/d, r = 0.12, P = 0.63). Results of this study suggested that pRFI of dairy cows reflected feed efficiency and was associated with fecal N excretion and N use efficiency but not with urinary-N and enteric CH$_4$ (metabolic energy losses).

Key Words: metabolic hydrogen, methane, rumen
interest in modifying their practices to face climate change, they are aware of the vulnerability of their crops and they feel that their barn is adequate to keep their cows comfortable.

**Key Words:** survey, forage, cows

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**M209** Variation in carbon footprint of milk production due to management differences: A whole-farm analysis of 142 dairy farms in Ontario, Canada. S. Jayasundara**, W. T. Wright**, A. Weersink**, A. VanderZaag**, R. Gordon**, and C. Wagner-Riddle**, University of Guelph, Guelph, ON, Canada, **Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada, **Agriculture and Agri-Food Canada, Ottawa, ON, Canada, **Wilfrid Laurier University, Waterloo, ON, Canada.

Dairy farming is a significant contributor of agricultural greenhouse gas (GHG) emissions in Canada. Quantification of inter-farm variation in carbon footprint (CF) of milk (kg CO\(_2\) eq kg\(^{-1}\)) fat and protein corrected milk (FPCM) and identification of management variables influencing this variation may be helpful in developing feasible strategies for reducing the CF of milk. This study estimated the cradle to farm-gate CF of milk production in 142 dairy farms across Ontario, a province that contributes about one third of total Canadian milk production. The objectives of the study were to: (i) quantify the inter-farm variation in CF of milk production using commercial farm data derived from an annual cost of production survey conducted by Dairy Farmers of Ontario and Canadian Dairy Commission, and (ii) identify key management attributes that significantly influence the CF of milk production. The analysis was conducted according to the methods recommended by International Dairy Federation. Results indicated that the CF of milk production on commercial dairy farms ranged from 0.44 to 1.73 kg CO\(_2\) eq kg\(^{-1}\) FPCM (mean ± standard deviation = 1.02 ± 0.24). The lower quartile of farms produced milk with mean CF of 0.77 ± 0.10 kg CO\(_2\) eq kg\(^{-1}\), while the quartile of high emitting farms produced milk with mean CF of 1.32 ± 0.18 kg CO\(_2\) eq kg\(^{-1}\). Low emitting farms produced milk more efficiently as indicated by significantly (P < 0.05) lower calving interval (months), age at first calving (months), whole farm feed-use (kg feed dry matter kg\(^{-1}\) FPCM), land-use (m\(^2\) kg\(^{-1}\) FPCM), and direct energy-use (MJ kg\(^{-1}\) FPCM) compared with high emitting farms. Results suggest that, at provincial scale, closing the productivity gap between low and high performing dairy farms may reduce the CF of milk production.

**Key Words:** dairy cattle, milk, greenhouse gases

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**M210** Effects of feeding oscillating dietary rumen-degradable protein (RDP) levels on feed intake, milk production, and nitrogen utilization in dairy cows. A. Y. Makurumure*, T. Mutsangwa, Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.

The objective was to delineate the interactions between partial replacement of barley starch with lactose (as dried whey permeate, DWP) and dietary rumen-degradable protein (RDP) levels on milk production, ruminal pH, and nitrogen (N) utilization in cows. Eight Holstein cows (738 ± 98 kg BW; 93 ± 39 DIM) were used in a replicated 4 × 4 Latin square with a 2 × 2 factorial arrangement of dietary treatments. One square had 4 ruminally-cannulated cows for ruminal pH measurements. The treatment factors were levels of DWP (0 vs. 12.5% of DM) and RDP (9.5 vs. 11.5%). Diets contained ~17.7% CP, and the 0% and 12.5% DWP diets contained 23.7 and 17.8% starch, respectively. The dietary addition of DWP tended (P = 0.08) to increase DM; however, actual and energy-corrected milk yields were not affected by the dietary addition of DWP or level of RDP. Milk fat content and yield were not affected by diet. The dietary addition of DWP increased milk protein content (P = 0.01) and yield (P = 0.01). Milk lactose content was similar in cows fed 9.5 and 11.5% RDP without added DWP, but milk lactose content was greater in cows fed 9.5% RDP compared with those fed 11.5% RDP with added DWP (interaction, P < 0.01). Milk urea-N content was similar in cows fed 9.5 and 11.5% RDP with added DWP, but milk urea-N content was greater in cows fed 11.5% RDP compared with those fed 9.5% RDP with added DWP (interaction, P < 0.01). Total-tract CP digestibility was greater (P = 0.02) in cows fed 11.5% RDP compared with those fed 9.5% RDP. Urinary N excretion was unaffected by diet; however, adding DWP increased (P = 0.03) fecal N excretion (g/d). Feeding DWP increased milk N secretion (P < 0.01). Total N excretion was unaffected by diet; however, apparent N balance tended to be greater (P = 0.10) and productive N (i.e., milk N + apparent N balance) was greater (P = 0.04) in cows fed 11.5% RDP compared with 9.5% RDP. Dietary treatments had no effect (P > 0.05) on mean ruminal pH, and area or duration when ruminal pH was less than 5.8 and 5.5. Our results indicate that partially replacing barley starch with DWP had no negative effects on ruminal acidosis, but it increased milk N secretion.

**Key Words:** starch, ruminal acidosis, nitrogen utilization

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**M211** Interactions between partial replacement of barley starch with sugars and dietary rumen-degradable protein level on nitrogen utilization and ruminal acidosis in dairy cows. T. Chambwe*, G. B. Penner, and T. Mutsangwa, Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.

The objective was to delineate the interactions between partial replacement of barley starch with lactose (as dried whey permeate, DWP) and dietary rumen-degradable protein (RDP) levels on milk production, ruminal pH, and nitrogen (N) utilization in cows. Eight Holstein cows (738 ± 98 kg BW; 93 ± 39 DIM) were used in a replicated 4 × 4 Latin square had 4 ruminally-cannulated cows for ruminal pH measurements. The treatment factors were levels of DWP (0 vs. 12.5% of DM) and RDP (9.5 vs. 11.5%). Diets contained ~17.7% CP, and the 0% and 12.5% DWP diets contained 23.7 and 17.8% starch, respectively. The dietary addition of DWP tended (P = 0.08) to increase DM; however, actual and energy-corrected milk yields were not affected by the dietary addition of DWP or level of RDP. Milk fat content and yield were not affected by diet. The dietary addition of DWP increased milk protein content (P = 0.01) and yield (P = 0.01). Milk lactose content was similar in cows fed 9.5 and 11.5% RDP without added DWP, but milk lactose content was greater in cows fed 9.5% RDP compared with those fed 11.5% RDP with added DWP (interaction, P < 0.01). Milk urea-N content was similar in cows fed 9.5 and 11.5% RDP with added DWP, but milk urea-N content was greater in cows fed 11.5% RDP compared with those fed 9.5% RDP with added DWP (interaction, P < 0.01). Total-tract CP digestibility was greater (P = 0.02) in cows fed 11.5% RDP compared with those fed 9.5% RDP. Urinary N excretion was unaffected by diet; however, adding DWP increased (P = 0.03) fecal N excretion (g/d). Feeding DWP increased milk N secretion (P < 0.01). Total N excretion was unaffected by diet; however, apparent N balance tended to be greater (P = 0.10) and productive N (i.e., milk N + apparent N balance) was greater (P = 0.04) in cows fed 11.5% RDP compared with 9.5% RDP. Dietary treatments had no effect (P > 0.05) on mean ruminal pH, and area or duration when ruminal pH was less than 5.8 and 5.5. Our results indicate that partially replacing barley starch with DWP had no negative effects on ruminal acidosis, but it increased milk N secretion.

**Key Words:** starch, ruminal acidosis, nitrogen utilization

Due to the complexity of the dairy system, estimates of N flows and loss require the use of models to ensure all pathways and forms through which N is lost are accounted for and to assess how changes to one part of the system affect another. The objective of this study was to use a whole farm model to compare N losses from 3 regions in the US under different manure management settings. The Integrated Farm Systems Model was used to simulate a large dairy farm in the Western, Midwestern, and Eastern regions of the US. Simulated farms had the same number of animals (2000 lactating Holsteins; 800 heifers >1 yr; 800 heifers <1 yr) with varying stocking density based on regional practices (5, 2, and 1.7 head/ha respectively). A target milk production of 11,000 l FPCM/animal/yr was set for all simulations. Four manure management scenarios were simulated for each region: A) uncovered basin storage with broadcast application; B) covered basin storage with broadcast application; C) uncovered basin storage with manure injection; and D) covered basin storage with manure injection. Model outputs were averaged over 25 weather years. Whole farm N efficiency, estimated as the proportion of N imports exported in milk and meat, was 0.29, 0.35, and 0.37 for Western, Midwestern and Eastern farms under scenario A. The location and form of major N losses and response to manure management scenarios varied with region. Results highlight that stemming N loss from one location often increases loss in another. Holistic assessment of N management is required to find solutions that increase farm level N efficiency rather than simply diverting N losses.

**Key Words:** dairy, nitrogen, environment

M213 Economic and environmental impacts of revised amino acid recommendations on Canadian dairy farms. S. Binggeli*, H. Lapierre2, E. Charbonneau1, D. Ouellet2, and D. Pellerin1, 1Université Laval, Quebec, QC, Canada, 2Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.

To ensure dairy farms sustainability, milk production must be cost effective and manure nitrogen (N) reduced. The main objective of this research was to evaluate the economic and environmental impacts of.

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### Table 1 (abstract M212). Size and percent change in nitrogen losses under different management scenarios in three regions of the US

<table>
<thead>
<tr>
<th>Management strategy</th>
<th>Unit</th>
<th>Barn NH3 (g N loss/kg FPCM)</th>
<th>Storage NH3 (g N loss/kg FPCM)</th>
<th>Soil N2O (g N loss/kg FPCM)</th>
<th>Soil NH3 (g N loss/kg FPCM)</th>
<th>Leachate N (g N loss/kg FPCM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>5.35</td>
<td>1.83</td>
<td>0.0328</td>
<td>0.887</td>
<td>0.0535</td>
</tr>
<tr>
<td>B</td>
<td>% change from A</td>
<td>+2.1</td>
<td>-41.2</td>
<td>+52.3</td>
<td>+34.1</td>
<td>+83</td>
</tr>
<tr>
<td>C</td>
<td>% change from A</td>
<td>+1.5</td>
<td>1.0</td>
<td>+19.6</td>
<td>-92.6</td>
<td>+48.9</td>
</tr>
<tr>
<td>D</td>
<td>% change from A</td>
<td>+2.8</td>
<td>-41.1</td>
<td>+101</td>
<td>-90.3</td>
<td>+329</td>
</tr>
<tr>
<td>Midwestern</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>1.99</td>
<td>0.787</td>
<td>0.0647</td>
<td>0.471</td>
<td>2.49</td>
</tr>
<tr>
<td>B</td>
<td>% change from A</td>
<td>0</td>
<td>-74.1</td>
<td>+22.5</td>
<td>+9.7</td>
<td>+39.6</td>
</tr>
<tr>
<td>C</td>
<td>% change from A</td>
<td>0</td>
<td>0</td>
<td>+0.5</td>
<td>-53.2</td>
<td>0.8</td>
</tr>
<tr>
<td>D</td>
<td>% change from A</td>
<td>0</td>
<td>-74.3</td>
<td>+23.4</td>
<td>-48.8</td>
<td>+43.3</td>
</tr>
<tr>
<td>Eastern</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
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<td>0.131</td>
<td>1.91</td>
<td>2.79</td>
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<tr>
<td>B</td>
<td>% change from A</td>
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<td>+81.9</td>
<td>+19.2</td>
</tr>
<tr>
<td>C</td>
<td>% change from A</td>
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<td>+31.7</td>
</tr>
<tr>
<td>D</td>
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<td>-83.3</td>
<td>+42.7</td>
<td>+18.2</td>
<td>+71.6</td>
</tr>
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</table>

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### Table 1 (abstract M213). Farm economic and environmental impacts of RevRec by region

<table>
<thead>
<tr>
<th>The Maritimes</th>
<th>The Prairies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk/cow/yr, kg</td>
<td>8,608</td>
</tr>
<tr>
<td>NRC 2001</td>
<td>0.079</td>
</tr>
<tr>
<td>RevRec</td>
<td>0.102</td>
</tr>
<tr>
<td>Net income, $/kg FPCM</td>
<td>12.20</td>
</tr>
<tr>
<td>RevRec</td>
<td>11.90</td>
</tr>
<tr>
<td>MP avg rqt, g/cow/d</td>
<td>2,232</td>
</tr>
<tr>
<td>RevRec</td>
<td>2,159</td>
</tr>
<tr>
<td>Lys avg rqt, g/cow/d</td>
<td>155</td>
</tr>
<tr>
<td>RevRec</td>
<td>131</td>
</tr>
<tr>
<td>Met avg rqt, g/cow/d</td>
<td>53</td>
</tr>
<tr>
<td>RevRec</td>
<td>43</td>
</tr>
<tr>
<td>His avg rqt, g/cow/d</td>
<td>—</td>
</tr>
<tr>
<td>RevRec</td>
<td>—</td>
</tr>
<tr>
<td>MP avg sup, g/cow/d</td>
<td>2,232</td>
</tr>
<tr>
<td>RevRec</td>
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</tr>
<tr>
<td>Lys avg sup, g/cow/d</td>
<td>155</td>
</tr>
<tr>
<td>RevRec</td>
<td>143</td>
</tr>
<tr>
<td>Met avg sup, g/cow/d</td>
<td>53</td>
</tr>
<tr>
<td>RevRec</td>
<td>43</td>
</tr>
<tr>
<td>His avg sup, g/cow/d</td>
<td>—</td>
</tr>
<tr>
<td>RevRec</td>
<td>—</td>
</tr>
</tbody>
</table>

1Fat- and protein-corrected milk.
balancing dairy rations with a revised AA recommendations (RevRec) on Canadian dairy farms compared with NRC (2001). N-CyCLES, an Excel-based linear programming whole farm model, built using NRC (2001) recommendations, was updated with rev AA requirements (rqt; maintenance and lactation: Lapierre et al., 2016 [CNC]; growth and gestation: NRC beef cattle, 2016; efficiency: Doepel et al., 2004 [JDS:1279] and supply (sup) (microbial CP; White et al., 2016 [JDS:7932]; RUP: NRC, 2001). Data used for simulations were 2010–2014 means from DHI and management group databases for the Maritimes and Central Canada, and from Alberta agriculture websites for the Prairies. Essential AA rqt needed to be filled, with no constraint to cover metabolizable protein (MP) rqt. The results, as average (avg; Table 1), point to an economic gain when balancing rations with RevRec, compared with NRC (2001) MP and AA. The main change is a reduction in purchased feeds with a reduced need of rumen-protected AA to balance Lys and Met ratio. Farm N balance was lower Using RevRec, implying better used of N. An AA factorisation model seems to have global positive impacts on Canadian dairy farms and may help to reduce total CP sup and N excretion, assuming no impact on milk production.

Key Words: dairy cow, amino acid, profitability

M214 The effect of adding zeolites to dairy manure compost on ammonia emissions and nitrogen specification. M. E. de Haro Martí1, M. Chahine2, H. Neibling2, and L. Chen1, 1University of Idaho, Gooding, ID, 2University of Idaho, Twin Falls, ID.

Composting of manures and other agricultural wastes is an acceptable and wide spread used waste management technique. As a waste management practice, composting reduces the volume of composted wastes between 35 and 50%, which allows the materials to be significantly more affordable to transport than raw wastes. Most manures, including dairy manure, don’t have the proper carbon to nitrogen ratio (C:N) for composting without the loss of nitrogen as ammonia during the composting process. A zeolite is a crystalline, hydrated aluminosilicate of alkali and alkaline earth cations having an infinite, open 3 dimensional structure. Zeolites are able to further lose or gain water reversibly and to exchange cations with and without crystal structure. The objective of this study was to demonstrate the effects of adding zeolites to the dairy manure compost mix on ammonia emissions and the quality of the final compost product. The study was conducted on a commercial dairy in Southern Idaho. Manure stockpiled during the winter and piled after the corral cleaning was mixed with fresh pushed up manure from daily operations, and with straw from bedding and old straw bales, in similar proportions for each windrow. Windrows were mixed and mechanically turned using a tractor bucket. Three replications of control and treatment were made. The control consisted of the manure and straw mix as described. The treatment consisted of the same mix as the control plus the addition of 8% of zeolites by weight during the initial mix. Windrows were actively composted for 120 d or more with 5 turns per windrow including the initial mix preparation. Data were analyzed using ANOVA. Cumulative ammonia emissions were reduced by 11% in the zeolite treated compost vs. control (2.76 mg NH₃-N/m³ versus 3.09 mg NH₃-N/m³; P < 0.05) during the first 3 turns which occurred in the first 45 to 55 d of composting. Nitrates concentration in the amended compost (702 mg/kg) was 3 times greater than in the control (223 mg/kg; P = 0.05). The project demonstrated the feasibility of using the addition of zeolites into the composting process as a Best Management Practice to reduce ammonia emissions and to change the nitrogen speciation during the composting process.

Key Words: compost, zeolites, ammonia emission


The objective was to examine effects of environment, dietary Zn source and an intra-mammary lipopolysaccharide (LPS) challenge on plasma concentration of heat shock protein (Hsp) 70, and Hsp gene expression in the mammary gland (MG). Seventy-two multiparous lactating cows were randomly assigned to 4 treatments (n = 18/treatment) in a 2 × 2 factorial arrangement, during summer. Cows were either cooled (CL) or not cooled (NC) and fed diets supplemented with 75 ppm Zn hydroxychloride or 35 ppm Zn hydroxychloride + 40 ppm Zn-Met complex. During the first 84 d of the experiment, all cows were cooled with fans and misters (temperature-humidity index ≥ 73) and fed respective dietary treatments. Starting at 85 d, NC cows were deprived of cooling (temperature-humidity index = 78) for the following 84 d (environmental challenge). Plasma was collected at 0, 12, 24, 48, 72, 85, 96, 110, 125, 138, 152, 165 d of the experiment. A subset of 16 cows was subjected to an intra-mammary LPS challenge at 118 d. Plasma was collected at −4, 0, 3, 6, 12, 24, 48, 96, and 144 h relative to LPS infusion. Plasma concentrations of Hsp70 were analyzed by ELISA. Mammary biopsies were collected from another subset of 30 cows at 0, 91, and 140 d of the trial to analyze gene expression of Hsp90, 70, and 27. Data were analyzed using the mixed procedure of SAS. There were no (P > 0.15) differences due to dietary Zn source or Zn source × environment interaction for any parameters examined. During environmental challenge, NC cows had higher plasma Hsp70 at 165 d and tended to have higher values at 110 and 125 d compared with CL (environment × d, P < 0.01). After LPS challenge, NC cows tended to have higher plasma concentration of Hsp70 at 12 h, but lower at 48 h (environment × h, P < 0.11) than CL. Relative to CL, MG of NC cows had greater (P < 0.05) gene expression of Hsp90, 70, and 27. In conclusion, deprivation of cooling during summer increased gene expression of Hsp of MG and plasma concentration of Hsp70. However, dietary Zn source had no impact on Hsp expression in blood or MG.

Key Words: cooling, zinc, heat shock protein

M216 A case study to evaluate cooling options in Georgia grazing dairies. R. M. Orellana*, J. K. Bernard, and S. Tao, University of Georgia, Tifton, GA.

Heat audits were performed on 3 grazing dairies in Georgia to evaluate cooling systems during summer. Farms were managed similarly and used rotary parlors with open holding pens with misters. When grazing during the day, cows were cooled by misters attached to pivots without access to shade. Cows were milked twice daily (0400 and 1500 h) and fed a partial TMR. Feeding schedules, facilities, and cooling settings varied among farms. In farm A, cows were fed in an open area equipped with misters for 3.5 h after each milking. In farm B, cows were fed 2 h before and 2 h after each milking in an open ridge barn equipped with overhead sprinklers which operated before PM milking. In farm C, cows were fed for 3.5 h after milking in an open ridge barn installed with fans and soakers over feed bunks. Ten cows per farm were randomly selected and vaginal temperature (VT) was measured every 10 min for 3 d. Cow’s genotype (Holstein or Holstein × Jersey) was determined by phenotype. Environmental data were obtained from local weather stations. Average air temperature and relative humidity during heat audits were 29°C and 70%. Data were analyzed using the mixed procedure of SAS. A farm by hour interaction (P < 0.01) was observed for VT.
Around midnight, cows from all farms had similar VT. From AM milking to departure from feedlots, all cows showed a gradual decrease in VT, but cows in farm C decreased the most rapidly. After leaving the feedlots, cows from farm B and C had a small increase in VT, but cows on farm A maintained VT. All cows had similar VT during PM milking, but cows in farm A maintained lower VT than cows in farms B and C in feedlots. All cows showed an increase in VT after leaving feeding pens, but the highest VT was observed in cows from farm B, which gradually decreased and remained constant until next day. Regardless of farms, Holstein had lower VT than Holstein × Jersey during PM milking, but higher VT after leaving feedlots (genotype × hour, \( P < 0.01 \)). In summary, holding pen misting was effective to reduce body temperature and pivot cooling helped to maintain it. Holstein and crossbred cows responded differently to cooling and solar radiation.

**Key Words:** grazing, cooling, vaginal temperature

M217  **Effects of an evaporative cooling system on reducing heat stress in dairy cattle.** J. R. Johnson\(^1,2\), M. J. Wolf\(^2\), J. McBride\(^2\), and M. J. Brouk\(^1\), \(^1\)Kansas State University, Manhattan, KS, \(^2\)VES Environmental Solutions, Chippewa Falls, WI.

A study was conducted to evaluate the effect of 2 cooling systems on barn temperature, core body temperature (CBT), respiration rate (RR), rear udder temperature, and lying time in lactating Holstein dairy cows. A switchback design was used where cows were moved between barns for 6 d, therefore exposing treatment groups to each barn for 3 d. Twenty lactating Holstein dairy cows were randomly assigned to 1 of 2 treatment groups: CONV refers to cows housed in a conventional, open-sided freestall barn using feedline coolers and fans located over the feedline and stalls as its main source of cow cooling, and TUN + EVAP, which refers to cows housed in a tunnel-ventilated freestall barn using an evaporative cooling system provided by VES Environmental Solutions (Chippewa Falls, WI). The cooling system in the tunnel-ventilated barn (TUN + EVAP) was effective at reducing barn temperature and temperature humidity index (THI), while relative humidity (RH) was increased in comparison CONV. Lower THI of the cow environment for TUN + EVAP failed to result in treatment differences for CBT, however, with CONV and TUN + EVAP having similar CBT of 38.6 ± 0.04°C (\( P = 0.79 \)). TUN + EVAP cows had reduced RR (\( P < 0.01 \)) compared with CONV (52.0 vs 57.9 ± 2.2, respectively) and this difference was greater during the afternoon (1600 h) with average RR of 55.4 and 63.0 ± 2.6 for TUN + EVAP and CONV, respectively (\( P < 0.01 \)). Similar results were found for rear udder temperatures where TUN + EVAP cows had reduced rear udder temperatures overall (33.2 vs 34.5 ± 0.3°C; \( P < 0.01 \)) and during the afternoon period (34.0 vs 34.9 ± 0.4°C; \( P < 0.01 \)) compared with CONV. Cows housed in the TUN + EVAP barn had increased lying time by 1 h/d (\( P < 0.01 \)) compared with CONV (11.8 vs 10.8 ± 0.3 h/d for TUN + EVAP and CONV, respectively). Overall, the evaporative cooling system was effective in reducing the barn THI leading to reduced RR and rear udder temperature and increased daily lying time. No treatment differences were detected for CBT, however, likely a result of the cooler ambient conditions under which the study took place.

**Key Words:** heat stress, core body temperature, lying behavior

M218  **Circulating insulin resistance biomarker lignoceroyl sphingosine is not elevated in Holstein dairy cows in response to heat stress.** J. E. Rico\(^1\), Z. C. Phipps\(^1\), Q. Zeng\(^1\), A. M. Shall\(^1\), J. D. Kaufman\(^2\), A. G. Rius\(^2\), and J. W. McFadden\(^1\), \(^1\)West Virginia University, Morgantown, WV, \(^2\)University of Tennessee, Knoxville, TN.

The sphingolipid ceramide (Cer) mediates the development of insulin resistance. Lipidomics has revealed that lignoceroyl sphingosine (C24:0-Cer) is a circulating biomarker for insulin resistance in dairy cattle. Environmental heat stress conditions compromise milk production, a response that may involve enhanced insulin action. Our objective was to investigate the effects of heat stress on circulating ceramide concentrations. Twelve multiparous, lactating Holstein dairy cows were assigned to 2 environmental conditions [thermonutral (TN) or heat stress (HS)] for 7 d in a crossover design. Temperature-humidity index was maintained below 66 for TN treatment, and above 68 (peaking at 76) for HS treatment. Blood was collected at 0800 (AM) and 1900 h (PM) on d 6 and 7 of conditioning, and plasma samples pooled to reflect AM and PM metabolic status. Plasma concentrations of Cer, monohexosylceramide (GlcCer), and lactosylceramide were determined using mass spectrometry. Data were analyzed using a mixed model with fixed effects of treatment (HS and TN) and time (AM and PM). As previously established, heat stress increased rectal temperature and respiration rate, and reduced DM intake and milk production (\( P < 0.05 \)). Circulating free fatty acids were elevated during AM, relative to PM (\( P < 0.05 \)). Circulating β-hydroxybutyrate was increased by HS, relative to TN (\( P < 0.05 \)). Relative to TN, HS did not increase C24:0-Cer or C24:0-dihydroceramide. Mild reductions in GlcCer levels were observed in response to HS treatment (e.g., 20% C20:0-GlcCer, \( P < 0.05 \)), while lactosylceramide levels were unchanged. In contrast, C16:0-Cer and C16:0-dihydroceramide levels increased 14 and 19%, respectively. Plasma fatty acid levels were moderately associated with the majority of Cer quantified (\( r = 0.3 - 0.4 \), \( P < 0.05 \)). For instance, C24:0-Cer was positively associated with circulating fatty acids (\( r = 0.38 \), \( P < 0.05 \)). We conclude that short-term heat stress conditioning did not increase the insulin resistance biomarker C24:0-Cer. Our results suggest insulin resistance likely did not develop in heat-stressed cows.

**Key Words:** ceramide, heat stress, insulin resistance

M219  **Seasonality of calving on dairy farms across the United States.** F. C. Ferreira\(^*1,2\) and A. De Vries\(^1\), \(^1\)University of Florida, Gainesville, FL, \(^2\)Embrapa Gado de Leite, Juiz de Fora, MG, Brazil.

Seasonality is present in reproductive parameters in dairy farms in the United States but detailed information about the magnitude of it is limited. The objective of this study was to describe the seasonality in calving patterns of dairy farms across the United States. Lactation records were obtained from USDA. The focus was calvings in 2010. We used a model with sine and cosine functions with a period of 14 d to describe seasonality of the year as proposed by Stolwijk et al. (1999). The coefficient of cyclic variation (CCV, %) was chosen to measure the amount of seasonality per farm because it allows the standard error of the measurement to be taken into consideration and it is a very intuitive measure (Fulford, 2014). Proc GENMOD on SAS 9.4 was used to run the model per farm. The number of calving was adjusted by the number of cows present. States with fewer than 20 farms in the data set, farms with fewer than 50 cows present on average and farms that were greatly expanding or shrinking during 2010 were excluded. We calculated average and the 20, 50 and 80 percentiles of CCV per state (mean, P20, P50 and P80, respectively). The states analyzed (and number of farms) were PA (1,210), NY (860), MN (1,368), OH (667), IA (427), WI (216), MI (85), KY (44), KS (86), TX (80), SD (50), GA (74), NE (52), MA (30), ME (48), FL (33), OK (19), TN (67) and NC (72). Seasonality in calving was observed in all states studied. Among those, the greater average seasonality for calving was seen in Ohio (Mean = 54%, P20 = 41%, P50 = 53%, and P80 = 67%) followed by Florida (46%, 23%, 42%, 67%). The ones with
the least amount of seasonality were Maine (17%, 8%, 14%, 23%) and Wisconsin (15%, 7%, 14%, 21%). On average, the weighted CCV of calving for the US of the farms available in our data set was 25% (18%, 24%, 30%) with the nadir point happening in early summer and the peak in mid-late fall. In conclusion, seasonality in calving was present in all states. Models with sine and cosine functions are able to smooth seasonal patterns, identify the extreme points and allow for inclusion of covariates if needed.

Key Words: calving pattern, sine and cosine functions, coefficient of cyclic variation

**M220**  
1H NMR-based blood metabolomics in cold-stressed dairy goats. N. Mehaba, W. Coloma-García, A. A. K. Salama*, and G. Caja, *Group of Ruminant Research (G2R), Universitat Autonoma de Barcelona, Bellaterra, Spain.*

The objective was to identify possible biomarkers of cold stress in blood of dairy goats. Eight lactating Murciano-Granadina dairy goats (2.13 ± 0.36 L/d; 70 ± 2 DIM; 41.75 ± 2.02 kg body weight) were maintained under 2 environmental conditions varying in ambient temperature: 1) 4 goats under thermoneutral (TN; 15 to 20°C), and 2) 4 goats under cold stress (CS; −4 to 8°C). In both environments, humidity averaged 60 ± 5% with 12–12h light-dark cycles. The experimental design was crossover with 2 treatments in 2 periods (21d each). Blood samples were collected weekly and analyzed by 1H nuclear magnetic resonance (H NMR) spectroscopy operating at 600 MHz. Multivariate analyses of data were carried out by the ChemoSpec package of R program and further analyzed by the web-based MetaboAnalyst program. Principal component and partial least square–discriminant analyses were used to identify possible metabolite markers. Goats under CS conditions had lower (P < 0.05) rectal temperature (−0.32°C), water consumption (−1.25 ± 0.24 L/d), and milk yield (−0.19 L/d) than TN goats. These results indicate that the low temperatures used in this experiment caused significant cold stress in goats. Metabolomics analysis revealed that CS goats had higher α- and β-glucose in plasma. This is in agreement with greater (P < 0.05) blood glucose in CS (66.7 mg/dL) than TN goats (64.1 mg/dL). There was also an increment in blood phosphatidylcholine, which could be related to lipid metabolism as CS goats mobilized body fat reserves and had greater (P < 0.05) blood nonesterified fatty acids concentrations (0.215 mmol/L) than TN goats (0.107 mmol/L). Tyrosine levels were greater in CS goats, which could be used for the synthesis of catecholamines. In conclusion, the H-NMR was a useful technique to define differences in blood metabolome by cold stress. The metabolic changes detected were mainly related to the increment in glucose, lipid metabolism, and neurotransmitters synthesis. Study funded by Project AGL2013–44061-R (Plan Nacional, MINECO, Spain).

Key Words: NMR metabolomics, cold stress, metabolism

**M222** Interaction between level of production and dry period length on subsequent milking performance. A. Bach*,1,2 and J. M. Pont3,1, ICREA, Institució Catalana de Recerca i Estudis Avançats, Spain; 2Department of Ruminant Production, IRTA, Spain; 3Granja San José, Spain.

A total of 28,637 lactation records from 5,793 Holstein cows milked in a commercial herd (Granja San José, Huesca, Spain) between 2000 and early 2016 were used to assess the potential impact of dry period length (DPL) and level of milk production at dry-off (MPD) on milking performance during the first 100 DIM of the subsequent lactation. Cows were milked in a parlor equipped with electronic meters. All data were recorded and saved into a database on a daily basis. The database stored information about parity, fresh date, daily milk production, and date of dry-off. Milk production of each cow and lactation during the last 3 d preceding dry-off was averaged. Also, milk production during the first 100 DIM for each lactation was summed (cumulative daily milk) within cow. Both DPL and MPD were categorized following the quartiles of their distributions (1: ≥68 d, 2: between 64 and 68 d, 3: between 60 and 64 d, and 4: ≤60 d for DPL; and 1: ≥27.8 kg/d, 2: from 23.5 to 27.8 kg/d, 3: from 18.9 to 23.5 kg/d, and 4: ≤18.9 kg/d for MPD). A mixed-effects model was used to evaluate the fixed effects of lactation number, DPL, MPD, and their interactions, and the random effect of cow. Cows with a DPL ≤60 d produced (4,608 ± 59 kg) less (P < 0.05) than cows with DPL >60 d (4,734 ± 61.9 kg), regardless of lactation number. Milk production before dry-off was positively correlated (P < 0.05) with milk production in the subsequent lactation, and this effect was more (P < 0.05) important in cows with an MPD ≤27.8 kg/d and a DPL ≤60 d (4,514 ± 78 kg/d) than in cows with an MPD ≥27.8 kg/d and a DPL ≥60 d (4,887 ± 81 kg/d), whereas the impact of production before dry-off was lower when cows had a DLP ≥268 d, with cows with an MPD ≥23.5 kg/d producing more milk (4,942 ± 87 kg) than those with an MPD <23.5 kg/d (4,605 ± 97 kg). Furthermore, a triple interaction was found between parity, DPL, and MPD, with milk production from cold stress (CS; −3 to 6°C). The experimental design was a crossover with 2 treatments in 2 periods (21 d each). Goats were fed ad libitum and machine-milked twice daily (0800 and 1700h). Feed intake, water consumption, rectal temperature, and respiration rate were recorded daily. Milk samples for composition were collected weekly. Insulin, glucose, nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), cholesterol and triglycerides were measured in blood samples taken weekly. Body weight (BW) was recorded at the start and end of each period. Compared with TN goats, CS goats had similar feed intake, but lower (P < 0.05) water intake (~23%), milk yield (~8%), respiratory rate (~6 breaths/min) and rectal temperature (~0.32°C). Furthermore, milk of CS goats contained greater (P < 0.05) protein (~10%), fat (~12%) and lactose (~4%). The CS goats lost ~0.45 kg BW, whereas TN goats gained 2.2 kg (P < 0.05). Insulin and cholesterol blood levels were not affected by CS. However, values of blood glucose (64.1 vs. 66.7 mg/dL), NEFA (0.107 vs. 0.215 mmol/L) increased (P < 0.05) by CS, whereas BHBA (0.528 vs. 0.400 mmol/L) and triglycerides (22.0 vs. 18.2 mg/dL) decreased (P < 0.05). In conclusion, dairy goats were sensitive to low ambient temperatures with marked productive and metabolic responses. These responses included decreased milk production, increased milk fat and protein contents, and incremented blood NEFA and glucose levels despite similar insulin values. It seems that NEFA was directly used by the mammary gland (increased milk fat content) rather than metabolism in liver (lower BHBA and triglycerides). Study funded by Project AGL2013–44061-R (Plan Nacional, MINECO, Spain).

Key Words: cold stress, lactation, metabolism


Low winter temperatures in some regions combined with increasingly frequent extreme cold waves have negative impact on animal performance, behavior and welfare. The objective of this study was to evaluate the physiological and lactational responses of dairy goats to cold stress. Eight Murciano-Granadina dairy goats (2.13 ± 0.36 L/d; 70 ± 2 DIM; 41.75 ± 2.02 kg body weight) were maintained in metabolic cages and randomly divided into 2 groups: thermoneutral (TN; 15 to 20°C) and cold stress (CS; −3 to 6°C). The experimental design was a crossover with 2 treatments in 2 periods (21 d each). Goats were fed ad libitum and machine-milked twice daily (0800 and 1700h). Feed intake, water consumption, rectal temperature, and respiration rate were recorded daily. Milk samples for composition were collected weekly. Insulin, glucose, nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), cholesterol and triglycerides were measured in blood samples taken weekly. Body weight (BW) was recorded at the start and end of each period. Compared with TN goats, CS goats had similar feed intake, but lower (P < 0.05) water intake (~23%), milk yield (~8%), respiratory rate (~6 breaths/min) and rectal temperature (~0.32°C). Furthermore, milk of CS goats contained greater (P < 0.05) protein (~10%), fat (~12%) and lactose (~4%). The CS goats lost ~0.45 kg BW, whereas TN goats gained 2.2 kg (P < 0.05). Insulin and cholesterol blood levels were not affected by CS. However, values of blood glucose (64.1 vs. 66.7 mg/dL), NEFA (0.107 vs. 0.215 mmol/L) increased (P < 0.05) by CS, whereas BHBA (0.528 vs. 0.400 mmol/L) and triglycerides (22.0 vs. 18.2 mg/dL) decreased (P < 0.05). In conclusion, dairy goats were sensitive to low ambient temperatures with marked productive and metabolic responses. These responses included decreased milk production, increased milk fat and protein contents, and incremented blood NEFA and glucose levels despite similar insulin values. It seems that NEFA was directly used by the mammary gland (increased milk fat content) rather than metabolism in liver (lower BHBA and triglycerides). Study funded by Project AGL2013–44061-R (Plan Nacional, MINECO, Spain).

Key Words: NMR metabolomics, cold stress, metabolism
cows with >5 lactations being barely affected by MPD and DPL. It is concluded that shortening DPL below 60 d compromises milk yield in the following lactation and this effect is exacerbated when milk production at dry-off is low, especially between lactations 2 and 5 (both included). 

**Key Words:** dry-off, management, milk yield

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**M223**  **Evaluation of the economically optimal dry period length under four herd constraints.** P. Pattamantorn*1 and A. De Vries, University of Florida, Gainesville, FL.

Evaluations of the economically optimal dry period length (DPL) are scarce. Our hypothesis was that optimal DPL depends milk and feed prices as well as on the most limiting herd constraint such as the number of milking cows (MLK), total cows (TOT), milk quota (QUO), or total milking time (TIM). The objective of this study was to quantify the optimal DPL under these 4 herd constraints, as well as provide a sensitivity analysis based on milk and feed prices. A model with 2 adjacent lactations with 31 weekly categories of DPL (from 0 d to 210 d) between the 2 lactations was build. Standard Wood’s lactation curves were used. Milk loss and days open in the subsequent lactation as a result from varying DPL were predicted by polynomial regression equations fitted on data from the literature. Income over feed cost (IOFC) per herd per day was the measure of interest. In the default case, all constraints were met simultaneously. Milk price was $0.35/kg and feed costs were $0.26/kg DMI for lactating and $0.08/kg DMI for dry cows, respectively. Default days open was 120 in each parity. Culling was not included. These inputs allowed for the calculation of the number of lactating and dry cows in both parities from which IOFC were calculated. Default results showed that the optimal DPL under 4 constraints were 98, 77, 98, and 91 d for MLK, TOT, TIM, and QUO, respectively. MLK and TIM constraints resulted in 19% of all cows dry whereas TOT had 16% of cows dry. A 56 d DPL led to losses of $0.40/milk cow per d (MLK), $0.09/cow per d (TOT), $0.56/hour of milking time per d (TIM), and $0.29/100 kg milk (QUO) compared with the optimum DPL. Greater milk price and lower dry cow feed costs increased the optimal DPL. Adding other variable costs reduced the optimal DPL for MLK. In conclusion, the optimal DPL depended on herd constraint as well as milk and feed prices. Longer DPL for otherwise low producing cows allows for milking more higher producing cows when MLK and TIM were herd constraints. Cow performance responses to varying DPL as well as inclusion of other costs may need to be refined for final results.

**Key Words:** optimization, income over feed cost, dry cow

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**M224**  **The association of blood calcium concentration shortly after parturition with metritis and milk production in Holstein dairy cows.** R. C. Neves*1, B. M. Leno2, M. D. Curler3, M. J. Thomas2, T. R. Overton2, and J. A. A. McArt1, 1Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY, 2Department of Animal Science, Cornell University, Ithaca, NY, 3Dairy Health and Management Services LLC, Lovell, NY.

Our objectives were to evaluate the association of immediate postpartum blood Ca concentration with metritis and Dairy Herd Improvement Association (DHIA) first test milk production. A total of 314 primiparous (PP) and 1,111 multiparous (MP) cows from 6 herds in New York State were enrolled in a cohort study, and had a blood sample collected within 12 h of parturition. Diagnosis of metritis (defined as a watery, fetid, and reddish-brownish uterine discharge up to 14 d in milk) was performed by farm personnel. The effect of blood Ca concentration on metritis and first test milk yield for PP and MP were analyzed with Poisson regression and linear mixed models, respectively. Herd was included as a random effect in all models. Postpartum Ca concentration was not associated with metritis (10 and 8.5% prevalence in PP and MP, respectively) diagnosis in PP [P = 0.76; while controlling for the effect of retained placenta (RP)] or MP cows [P = 0.21; controlling for RP and parity (2nd versus 3rd or higher)]. Primiparous and MP animals with lower postpartum Ca concentrations produced more milk at first DHIA test than animals with higher Ca concentrations. After dichotomization of the Ca variable using previously reported subclinical hypocalcemia cut-points of 8.0 and 8.4 mg/dL, PP cows with Ca concentrations ≤ 8.0 (8%) and 8.4 (17.5%) mg/dL (controlling for metritis and d in milk at DHIA test) produced 4.0 kg (P = 0.006) and 2.4 kg (P = 0.01) more milk, respectively, than PP animals with Ca concentrations > 8.0 and 8.4 mg/dL at first DHIA test. Multiparous cows with Ca concentrations ≤ 8.0 (51%) and 8.4 (68%) mg/dL (controlling for metritis, displaced abomasum, parity, and d in milk at DHIA test) produced 1.8 kg (P = 0.002) and 1.5 kg more milk (P = 0.01), respectively, than MP animals with Ca > 8.0 and 8.4 mg/dL at first DHIA test. In our study, immediate postpartum Ca concentration had no association with metritis diagnosis, and cows with lower blood Ca concentration produced more milk in early lactation compared with cows with higher blood Ca concentrations.

**Key Words:** calcium, subclinical hypocalcemia, transition cow

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**M225**  **Increased serum calcium in dairy cows with oral calcium formate supplementation in the postpartum period.** E. W. Carneiro1, S. H. Honorato2, E. Ichikawa2, and R. Almeida*1, 1Universidade Federal do Paraná, Curitiba, PR, Brazil, 2Bayer Animal Health, São Paulo, SP, Brazil.

The objective of the study was to evaluate the effects of oral calcium formate supplementation on serum total calcium (tCa), ionized calcium (iCa), NEFA, BHBA, cholesterol, AST, albumin, P and Mg minerals in early lactation cows. In a commercial dairy farm with 950 lactating cows in Southern Brazil, 129 Holsteins (45 primiparous and 84 multiparous) were blocked by parity and by tCa status (Idexx VetTest Chemistry Analyzer) 6 h after calving. Blood samples were analyzed for group allocation (normal and hypocalcemia groups) using 8.2 mg/dL (2.05 mM) as the cutpoint. Within each block, fresh cows were randomly allocated to 3 groups; control (C), group 1 (G1), and group 2 (G2), respectively supplemented with 0, 350 and 700 mL of 14.3% (wt/wt) calcium as a 48.6% aqueous suspension of calcium formate (Calfon Oral, Bayer Animal Health), 6 h after parturition. Eight blood samples were collected on evacuated tubes without anticoagulant, 6 h before the oral calcium supplementation, 6.5 h (t2), 7.5 h (t3), 8.0 (t4), 10 (t5), 14 (t6), 24 (t8) h after calving. Serum samples were frozen at −80°C for 90 d and then analyzed for tCa using colorimetric test (Arsenazo) and by ion selective electrode (iCa). Data were analyzed using the MIXED procedure of SAS with a model containing the effects of block, treatment, time, and treatment × time interaction as fixed effects and cow within treatment as a random effect. Hypocalcemia incidence rates were 37% using on-farm tCa from VetTest (≥2.05 mM), 40% using tCa (≥2.0 mM) and 71% using iCa (<1.0 mM). The lowest tCa and iCa values were observed at 24 h postpartum. It has shown an increase (P < 0.05) in serum levels of tCa and iCa between t2 to t7 for G1 and G2 compared with group C. Oral Ca supplemented cows have shown higher (P < 0.05) AST concentrations at t4 to t8 and lower (P < 0.05) Mg levels at t5 to t8. No differences were detected (P > 0.05) for the remaining analytes. The oral calcium formate supplementation has shown beneficial effects on the increment of ionic and total Ca on early
M226  Association of milk fatty acids and β-hydroxybutyrate concentrations in postpartum dairy cows.  J. K. Poncheki1, P. M. Souza1, J. A. Horst2, D. P. D. Lanna3, and R. Almeida*,1 1Universidade Federal do Paraná, Curitiba, PR, Brazil, 2Associação Paranaense de Criadores de Bovinos da Raça Holandesa, Curitiba, PR, Brazil, 3Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, SP, Brazil.

The aim of this study was to correlate serum β-hydroxybutyrate (BHB) concentrations with milk fatty acids in early lactation cows. Six hundred and 80 Holstein cows (306 primiparous and 374 multiparous) calving from September, 2015 to August, 2016 were evaluated in a commercial dairy farm located in Palmeira, Paraná State, Southern Brazil. A milk sample was collected from each cow between the 5th and the 15th day after calving, and they were frozen and stored in bottles at −20°C. Later, 90 (44 from primiparous and 46 from multiparous) samples were analyzed by gas chromatography to determine 52 fatty acids profile (Finnigan Focus CG, Thermo Fisher Scientific). On the same day of milk sampling, a blood sample was collected to analyze serum BHB in automatic biochemical analyzer (BS-200, Mindray, Shenzhen, China). On day 5 (D5) and 10 (D10) after calving, BHB was also measured using a blood drop in ketone test strips (FreeStyle Optium Ketone Monitoring System, Abbot). Averages for milk fat and milk protein percentages were 4.56 ± 1.12% and 3.65 ± 0.43%, respectively, with 9.2 DIM. Averages for BHBA were 1.03 ± 0.75 and 1.14 ± 0.90 mmol/L on D5 and D10, respectively. Cows categorized as subclinical ketotic (BHB ≥1.2 mmol/L) were 26% (D5) and 32% (D10). Moderate negative correlations were detected between BHBA and C4:0, C6:0, C8:0, C10:0, C10:1, C11:0, C12:0, C13:0 iso, C13:0, C14:0, C15:0 iso, C15:0 anteiso, C15:0 and C15:0 anteiso/C17:0 ratio (r = −0.49, −0.61, −0.56, −0.55, −0.43, −0.45, −0.52, −0.57, −0.53, −0.56, −0.44, −0.47, −0.51, and −0.51, respectively), showing that cows with intense negative energy balance (NEB) have impaired de novo fatty acids synthesis in mammary gland. Moderate positive correlations were observed between BHBA and C18:1 cis-9, C18:1 cis-11, C18:1 cis-12, C18:1 cis-13 and C16:1 cis-9/C15:0 ratio (r = 0.51, 0.51, 0.51, 0.43, and 0.54, respectively), being that C18:1 cis-9 has been suggested in other studies as an important biomarker for NEB. In summary, several fatty acids and their ratios can be used to identify postpartum dairy cows with intense NEB.

Key Words: ketosis, negative energy balance, transition period

M227  Feeding incremental levels of nicotinic acid to prepartum dairy cows increases colostral immunoglobulin concentration.  K. Aragona*,1, E. Rice1, M. Engstrom2, and P. Erickson1, 1University of New Hampshire, Durham, NH, 2DSM Nutritional Products Inc.

In the United States, >60% of colostrum does not meet the industry standards of ≥50 g of IgG/L. Ingestion of high quality colostrum is imperative for survival of the newborn calf, as it has little to no circulating immunoglobulins (Ig) at birth. Calves that do not absorb enough Ig to obtain successful passive transfer have an increased risk of morbidity and mortality, decreased average daily gains, and produce less milk in the first and second lactations. Previously, supplementing 48 g/d of nicotinic acid (NA) to prepartum dairy cows wk before expected calving date increased Ig concentration of colostrum by 18%. The objective of this study was to determine the effects of incremental levels of NA (0, 16, 32 or 48 g/d) on Ig concentration in colostrum and subsequent effects on the pre-weaned calf. Thirty-six multiparous Holstein dairy cows were blocked by expected calving date and randomly assigned to 1 of 4 treatments 4 wk prepartum. The NA was mixed with 40 g of corn meal and top dressed onto the total mixed ration (TMR), fed once daily. Within 90 min of parturition, the calf was removed, colostrum was harvested and weighed and an aliquot was taken for IgG determination via radial immunodiffusion (RID) assay. Calves were weighed and fed 8 pints of maternal colostrum. Blood samples were collected from calves via jugular vein at 0 and 24 h of age for analysis of IgG concentration via RID. Calves remained on study until weaning at 6 w old. Results are shown in Table 1. These results indicated that supplementing NA to prepartum dairy cows improves the overall quality of colostrum.

Key Words: prepartum dairy cow, colostrum, nicotinic acid

M228  Effects of supplemental β-carotene to prepartum dairy cows on colostrum quality and the pre-weaned calf.  K. Aragona*,1, E. Rice1, M. Engstrom2, and P. Erickson1, 1University of New Hampshire, Durham, NH, 2DSM Nutritional Products Inc.

Effects of supplemental β-carotene to prepartum dairy cows on colostrum quality and the pre-weaned calf.  K. Aragona*,1, E. Rice1, M. Engstrom2, and P. Erickson1, 1University of New Hampshire, Durham, NH, 2DSM Nutritional Products Inc.

Table 1 (abstract M227). Effects of supplementing incremental levels (0, 16, 32 or 48 g/d) of nicotinic acid to prepartum dairy cows

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment (g/d)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>IgG, g/L</td>
<td>72.7</td>
<td>87.4</td>
<td>81.1</td>
</tr>
<tr>
<td>Colostrum yield, L</td>
<td>11.4</td>
<td>11.6</td>
<td>12.3</td>
</tr>
<tr>
<td>IgG yield, g</td>
<td>822.1</td>
<td>919.1</td>
<td>1004.2</td>
</tr>
<tr>
<td>Protein, %</td>
<td>13.5</td>
<td>15.1</td>
<td>14.8</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.5</td>
<td>7.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Solids, %</td>
<td>22.5</td>
<td>26.4</td>
<td>25.8</td>
</tr>
<tr>
<td>Ash, %</td>
<td>1.03</td>
<td>1.15</td>
<td>1.22</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>3.3</td>
<td>2.9</td>
<td>3</td>
</tr>
<tr>
<td>24-h calf serum IgG, g/L</td>
<td>24.9</td>
<td>27.4</td>
<td>28.3</td>
</tr>
<tr>
<td>Apparent efficiency of absorption</td>
<td>37.5</td>
<td>35.6</td>
<td>38.2</td>
</tr>
</tbody>
</table>
The vitamin A precursor β-carotene acts as an antioxidant, able to effectively scavenge free radicals to prevent the formation of secondary reactive oxygen species that can destroy cells and cell components. In vitro incubation of β-carotene resulted in lower NH₃-N concentrations and higher microbial protein concentrations, compared with control, indicating β-carotene may increase utilization of NH₃-N, leading to increased growth of microbes, thereby providing more protein to the cow. The objective of this study was to determine the effects of supplemental β-carotene on immunoglobulin (Ig) concentration of colostrum and subsequent effects on the pre-weaned calf. Eighteen multiparous Holstein dairy cows were blocked by expected calving date and randomly assigned to 1 of 2 treatments: Control or 7 g/d of a 10% β-carotene product. The β-carotene was mixed with 40 g of corn meal and top dressed onto the total mixed ration (TMR), fed once daily. Within 90 min of parturition, the calf was removed, colostrum was harvested and weighed and an aliquot was taken for IgG determination via radial immunodiffusion (RID) assay. Calves were weighed and fed 8 pints of maternal colostrum. Blood samples were collected from calves via jugular vein at 0 (before colostrum feeding) and 24 h of age for analysis of IgG concentration via RID and calculation of apparent efficiency of absorption (AEA). Calves remained on study until weaning at 6 w old. Results are shown in Table 1. The results indicated that supplementing β-carotene to prepartum dairy cows increased colostral IgG concentration, tended to increase protein, fat and solids percentages, but lowered colostrum yield. Supplemental β-carotene also increased 24 h calf serum IgG concentrations in calves fed maternal colostrum.

Table 1 (abstract M228). Effects of supplementing β-carotene to prepartum cows on colostrum quality and the pre-weaned calf

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>β-carotene</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG, g/L</td>
<td>72.7</td>
<td>93.3</td>
<td>5.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Colostrum yield, L</td>
<td>11.4</td>
<td>6.9</td>
<td>1.5</td>
<td>0.04</td>
</tr>
<tr>
<td>IgG yield, g</td>
<td>822.1</td>
<td>670.7</td>
<td>127.2</td>
<td>0.41</td>
</tr>
<tr>
<td>Protein, %</td>
<td>13.5</td>
<td>15.9</td>
<td>0.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.5</td>
<td>6.9</td>
<td>0.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Solids, %</td>
<td>22.5</td>
<td>27.4</td>
<td>1.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Ash, %</td>
<td>1.03</td>
<td>1.06</td>
<td>0.04</td>
<td>0.66</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>3.3</td>
<td>2.9</td>
<td>0.3</td>
<td>0.21</td>
</tr>
<tr>
<td>24-h calf serum IgG (g/L)</td>
<td>22.3</td>
<td>31.4</td>
<td>2.3</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Key Words: prepartum dairy cow, β-carotene, colostrum

M229 Feeding green tea extracts has minor effects on antioxidant status of dairy cows during the transition period. V. Fischer¹, S. C. B. Stivanin¹, E. F. Vizzotto¹, M. de Paris¹, M. B. Zanela², C. Matte¹, C. Klei n¹, and V. Stone¹, ¹Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, ²Empresa Brasileira de Pesquisa Agropecuária, Pelotas, RS, Brazil.

This work aimed to investigate the effect on redox status in dairy cows fed Origanum vulgare or Camellia sinensis extracts during the transition period. Twenty-four Jersey cows with initial overall BW 430 ± 33kg were randomly assigned to one of the following treatments: control (CO, no plant extract), green tea extract (GTE, 5 g/cow/day) and oregano extract (OE, 10 g/cow/day). Cows received the plant extracts from −21 d to +21 d relative to parturition and were fed with the same basal diet, composed of corn silage, concentrate, and pasture. Blood samples were collected by jugular vein on d −21, −6, +2, and +21 relative to parturition and further analyzed for redox status parameters. Data were submitted to variance analysis, using the Mixed procedure of SAS according to a completely randomized design, considering the effect of diet (n = 3), day of measurement (n = 4), diet by day interaction, cows as the random effect and data collected at different days as repeated measures. Treatments did not affect milk production (overall mean 16.6 ± 3.3 L/d). Cows fed GTE had lower values for dichlorofluorescein oxidation in erythrocytes (DCFE) than cows of control and OE groups. There were no significant differences between groups for dichlorofluorescein oxidation in plasma (DCFP), carbonil, thiols contents as well as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione activities. Cows fed with green tea extract reduced DCFE, suggesting a slight positive effect on blood redox status.

Table 1 (abstract M229).

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Green Tea</th>
<th>Oregano</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCFE¹</td>
<td>12,246b</td>
<td>10,288a</td>
<td>16,433b</td>
<td>719</td>
</tr>
<tr>
<td>DCFP¹</td>
<td>4,006a</td>
<td>3,929a</td>
<td>3,749a</td>
<td>380</td>
</tr>
<tr>
<td>Carbonyl¹</td>
<td>1.6a</td>
<td>1.6a</td>
<td>1.7a</td>
<td>0.1</td>
</tr>
<tr>
<td>Thiols¹</td>
<td>0.20a</td>
<td>0.20a</td>
<td>0.20a</td>
<td>0.0</td>
</tr>
<tr>
<td>SOD¹</td>
<td>16.3a</td>
<td>15.3a</td>
<td>17.3a</td>
<td>0.7</td>
</tr>
<tr>
<td>CAT¹</td>
<td>1.2a</td>
<td>1.1a</td>
<td>1.2a</td>
<td>0.1</td>
</tr>
<tr>
<td>GPx²</td>
<td>0.8a</td>
<td>0.7a</td>
<td>0.8a</td>
<td>0.1</td>
</tr>
<tr>
<td>GSH²</td>
<td>0.1a</td>
<td>0.1a</td>
<td>0.01a</td>
<td>0.0</td>
</tr>
</tbody>
</table>

¹²Means in the same row followed by different letters are significantly different (Lsmeans, P < 0.05).

¹In nmol/mg of protein.

²In U/mg of protein.

Key Words: plant extract, dairy cow, redox status
Ruminant Nutrition I

M230  Ratio of dietary forage-to-concentrate affect liver and mammary tissue transcriptome in primiparous Holstein dairy cows. Z. Zhou1,2, L. Ma1,4, J. Q. Wang1, J. J. Loor1, M. Bionaz2, and D. P. Bu1,5,1, State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL, 3Animal and Rangeland Sciences, Oregon State University, Corvallis,OR, 4CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China, 5Hunan Co-Innovation Center of Safety Animal Production, CICSA, Changsha, Hunan, China.

A transcriptomics approach was used to evaluate the influence of forage-to-concentrate ratio (F:C) on liver (LV) and mammary (MG) tissue metabolism in mid-lactation dairy cows. Twenty-four primiparous lactating Holstein cows (body weight, 558 ± 10 kg; days in milk, 136 ± 37; daily milk yield, 21.12 ± 2.30 kg) were randomly allocated to 2 groups receiving either a high-forage diet (HF, F:C = 60:40) with Chinese wild rye, alfalfa hay and corn silage as forage source or a low-forage diet (LF, F:C = 40:60) with corn stover as forage source. A subgroup of cows (n = 5/diet) was used for analysis of liver and mammary transcriptome. Biopsies of LV and MG were collected at the end of 8-week feeding to measure mRNA profiles using a 4 × 44K Bovine Agilent microarray chip. Data were analyzed with SAS JMP Genomics using ANOVA with a false discovery rate correction (FDR <0.05). The Dynamic Impact Approach was used for pathway analysis. The analyses uncovered 759 and 225 differentially expressed genes (DEG) between LF and HF group in MG and LV, respectively. The greater number of DEG in MG underscored that mammary transcriptome was more responsive to changes in F:C compared with LV. Among the LV DEG, more genes were upregulated (135 vs. 90), whereas more DEG were downregulated (315 vs. 444) in MG, suggesting a potentially different response among changes in F:C. Pathway analysis revealed enhanced amino acid metabolism, such as taurine and hypotaurine metabolism, and decreased protein export in MG of LF-fed cows. Similarly, LF-fed cows also had enhanced lipid metabolism, such as steroid hormone biosynthesis in the MG compared with cows in HL. In contrast, compared with cows in HF, LF cows had overall lower folate biosynthesis which contributed to overall lower cofactor and vitamin metabolism. Although pathway analysis revealed that amino acid, lipid, energy, as well as cofactors and vitamin metabolism were among the most impacted biological processes in MG, similar alterations were not observed in LV. Overall, results indicate that MG and LV transcriptome in primiparous dairy cows was affected to a different extent by forage-to-concentrate ratio in mid-lactation.

Key Words: forage-to-concentrate ratio, liver and mammary tissue, transcriptome

M231  Maternal ethyl-cellulose rumen-protected methionine supplementation affects Holstein heifer calf development and growth. A. S. Alharthi1,4, F. Batistel1, C. Parys2, A. Helmbrecht2, and J. J. Loo1, 1University of Illinois at Urbana-Champaign, Urbana, IL, 2Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.

The objectives were to investigate if increasing supply of methionine during late-pregnancy in Holstein cows affects developmental parameters at birth and subsequent growth, and whether such effect is an utero-placenta or colostrum-dependent response. Thirty-nine Holstein heifers born to cows fed a basal control [CON; 1.47 Mcal/kg dry matter and 15.3% crude protein] diet with no added methionine or CON plus ethyl-cellulose rumen-protected methionine (MET; Mepron, Evonik Industries AG, Germany) were used. The MET was fed during the last 28 d of pregnancy at a rate of 0.09% of diet DM. Immediately after birth, heifers were randomly allocated considering dam treatment and colostrum as follows: 1) calves from CON cows and colostrum from CON cows (n = 9); 2) calves from CON cows and colostrum from MET cows (n = 9); 3) calves from MET cows and colostrum from MET cows (n = 11); and 4) calves from MET cows and colostrum from CON cows (n = 10). Body weight, hip and wither height, hip width and body length were measured at birth and weekly through weaning (42 d of age) and until 9 weeks of age. Calves from dams fed MET had greater hip height (P = 0.02; 81.0 vs 78.5 cm) at birth. However, body weight at birth (P = 0.67; 41.1 vs 41.7 kg), hip width (P = 0.15; 16.4 vs 15.8 cm), wither height (P = 0.11; 75.8 vs 77.3 cm) as well as colostrum quality (P = 0.95; 55.4 vs 52.4 IgG) and quantity (P = 0.80; 5.9 vs 5.7 kg) were not affected by maternal treatment. Over the first 9 wk of life, there was no colostrum effect for any of the growth variables measured. However, compared with CON, calves, from dams fed MET had greater body weight (P = 0.03; 60.8 vs 57.3 kg), hip height (P = 0.02; 87.7 vs 85.7 cm), wither height (P = 0.05; 83.5 vs 81.9 cm), and average daily gain (P = 0.01; 0.69 vs 0.60 kg/day). Hip width (P = 0.73; 19.7 vs 19.6 cm) and body length (P = 0.24; 122.8 vs 124.8 cm) were not affected by maternal MET. Overall, the data indicate that maternal supplementation with MET during late-gestation had a positive effect on neonatal heifer calf growth.

Key Words: fetal programming, amino acid, nutrition

M232  Effect of pasteurized and non-pasteurized colostrum on the growth performance and development of gastrointestinal tract of calves. G. T. Liu1,2, D. P. Bu1,5, S. C. Li3, K. Yang2, and Q. E. Zhang*,1, 1State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2Department of Animal Science, Ningxia University, Yinchuan, Ningxia, China, 3Department of Animal Science, University of Manitoba, Winnipeg, Canada, 4Dongying Austasia Modern Dairy Farm Co., Ltd., Dongying, Shandong, China, 5Hunan Co-Innovation Center of Safety Animal Production, CICSA, Changsha, Hunan, China.

The effects of colostrum pasteurization on the growth and gastrointestinal tract of calves were studied. Twenty newborn Holstein calves were randomly divided into 2 groups (n = 10) and were rasied in individual pens. Calves were either fed non- pasteurized colostrum (NPC) or pasteurized colostrum (PC) processed at 60°C for 60 min using a Dairy Tech colostrum pasteurizer (Dairy Tech Inc., Colorado). All calves were offered 4 L of colostrum within 1 h after birth and given a second feeding of 2 L of colostrum 6 h later. The experiment was carried out over 90 d. Measurements of the body weight, body length and metameral circumference of the calves were recorded every 30 d during the trial, while the dietary intake and diarrhea frequency were measured daily. Five calves from each group were randomly selected and slaughtered at 90 d of age. The dorsal sac and ventral sac of the rumen and small intestine were sampled to make paraffin sections and then observed its morphological changes. A repeated measures analysis was conducted by using the MIXED procedure of SAS while Initial body weight was included as a covariate in the model when appropriate. The calves in...
PC group had higher ($P < 0.05$) metatarsal circumference and tended to have longer ($P = 0.09$) body length at 90 d of age (Table 1). Colostrum type had no effect on the intake of starter feed and diarrhea frequency. However, the papilla width of rumen dorsal sac (1.25 vs. 1.02 mm) and rumen ventral sac (1.18 vs. 0.96 mm), the villus height of duodenum (0.55 vs. 0.43 mm) and jejunum (0.64 vs. 0.46 mm) in PC group were greater ($P < 0.05$) than that in NPC group, and the crypt depth of jejunum (0.39 vs. 0.49 mm) were smaller ($P < 0.05$). The results indicate that the pasteurized colostrum has a positive impact on the growth performance and development of gastrointestinal tract for calves in 90 d of age.

**Key Words:** calf, pasteurized colostrum, growth

**M233** Modulation of feeding behavior in lactating dairy cows by sweet sensory additives. M. Blanch*1, F. Bargo1, G. Tedó1, I. R. Ipharraguerre1,2, I. Guasch3, and A. Bach4,5, 1 Universitat Pompeu Fabra, Barcelona, Spain, 2 University of Kiel, Germany, 3 Blanca from the Pyrenees, Spain, 4 ICREA, Barcelona, Spain, 5 IRTA, Caldes de Montbui, Spain.

The aim of this study was to evaluate changes in feeding behavior and performance of lactating dairy cows in response to the addition of sensory additives into their TMR. A 90-d experiment was conducted involving 42 lactating cows (15 primiparous, 27 multiparous; BW = 690 ± 63 kg; DIM = 148 ± 73; milk yield = 38 ± 8 kg/d) randomly allocated to 3 treatments and fed a common TMR (15.4% CP, 29.2% NDF, 1.67 Mcal of NEl/kg). Treatments were either no supplementation and performance of lactating dairy cows in response to the addition of sensory additives into their TMR. A 90-d experiment was conducted involving 42 lactating cows (15 primiparous, 27 multiparous; BW = 690 ± 63 kg; DIM = 148 ± 73; milk yield = 38 ± 8 kg/d) randomly allocated to 3 treatments and fed a common TMR (15.4% CP, 29.2% NDF, 1.67 Mcal of NEl/kg). Treatments were either no supplementation (CON) or supplementation with sensory additive A (SAA - containing stevia glycosides) or B (SAB - the same formulation without stevia glycosides) at 30 g/d. Dry matter intake (DMI), milk production, milk composition, BW, feeding behavior, and feed efficiency (FE) were determined daily. Data were analyzed with a mixed-effect model that included the fixed effects of treatment, parity, week, and their 2-way interactions. Results are shown in Table 1. Feed intake was reduced ($P < 0.001$) when both sensory additives were added into the TMR; however, milk yield and composition did not differ among treatments. Regarding feeding behavior, SAB reduced ($P < 0.001$) the number of meals and eating time, and increased ($P < 0.001$) meal size compared with SAA and CON. In contrast, SAA reduced meal size ($P < 0.001$) and eating rate ($P < 0.001$) compared with SAB and CON. The use of sweet sensory additives may modulate feeding behavior in lactating dairy cows; nevertheless, such a response appears to be dictated by their composition (i.e., sweet ingredients).

**Key Words:** feeding behavior, lactating cows, sensory additives

### Table 1 (abstract M233). Performance and feeding behavior results

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatments</th>
<th>SE</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>SAA</td>
<td>SAB</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>T × wk</td>
<td>T × lact</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>34.6</td>
<td>34.5</td>
<td>35.2</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.97</td>
<td>3.84</td>
<td>3.78</td>
</tr>
<tr>
<td>Prot, %</td>
<td>3.32</td>
<td>3.31</td>
<td>3.28</td>
</tr>
<tr>
<td>DMI, kg</td>
<td>24.7a</td>
<td>23.3b</td>
<td>23.2b</td>
</tr>
<tr>
<td>FE, kg/kg</td>
<td>1.42</td>
<td>1.48</td>
<td>1.53</td>
</tr>
<tr>
<td>BW, kg</td>
<td>709.0</td>
<td>700.9</td>
<td>688.0</td>
</tr>
<tr>
<td>N meals, /d</td>
<td>9.28a</td>
<td>9.39a</td>
<td>8.27b</td>
</tr>
<tr>
<td>Meal duration, min</td>
<td>22.1</td>
<td>22.0</td>
<td>22.1</td>
</tr>
<tr>
<td>Meal size, kg</td>
<td>2.74b</td>
<td>2.56a</td>
<td>2.89a</td>
</tr>
<tr>
<td>Eating time, h/d</td>
<td>3.42a</td>
<td>3.46a</td>
<td>3.04b</td>
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<tr>
<td>Eating rate, g/min</td>
<td>123.9a</td>
<td>115.5b</td>
<td>130.4a</td>
</tr>
</tbody>
</table>

**Table 1 (abstract M232). Body weight (BW), body length (BL), and metatarsal circumference (MC) data**

<table>
<thead>
<tr>
<th>Item</th>
<th>NPC</th>
<th>0 d</th>
<th>30 d</th>
<th>60 d</th>
<th>90 d</th>
<th>SEM</th>
<th>Trt</th>
<th>Day</th>
<th>Trt × day</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>42.2</td>
<td>42.3</td>
<td>57.6</td>
<td>90.1</td>
<td>121.2</td>
<td>1.4</td>
<td>0.77</td>
<td>&lt;0.0001</td>
<td>0.75</td>
</tr>
<tr>
<td>BL (cm)</td>
<td>71.1</td>
<td>69.8</td>
<td>78.3</td>
<td>89.9</td>
<td>100.7</td>
<td>0.6</td>
<td>0.81</td>
<td>&lt;0.0001</td>
<td>0.09</td>
</tr>
<tr>
<td>MC (cm)</td>
<td>11.6</td>
<td>11.6</td>
<td>12.2</td>
<td>14.2</td>
<td>15.3</td>
<td>0.1</td>
<td>0.40</td>
<td>&lt;0.0001</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Heat stress (HS) decreases milk yield and deleteriously alters milk composition in dairy cows. This study examined the ruminal metabolic response of dairy cows to HS using a combination of LC-MS, GC-MS, and 1H NMR. Four multiparous Holstein dairy cows (101 ± 10 DIM; 574 ± 36 kg of BW, 38 ± 2 kg of milk/d) were randomly assigned to 4 environment chambers with a crossover design. Cows were either subjected to HS [HS: 36°C with light and 32°C without light; THI = 87.2 and 81.8] or kept under thermal neutral conditions [TN: 20°C; THI = 65.5] for 9 d for adaptation and then for another 9 d of pair-feeding to eliminate confounding effects of dissimilar feed intake. There was a 30-d washout period between periods. Rumen fluid was collected at 1000 h (after feeding) on d 9. All data were analyzed using R, SIMCA-P 13.0,
and SIEVE software. Metabolites were identified by m/z value matched to the NIST database. A student t-test was used to search for changed metabolic profiles with Variable Importance in the Projection (VIP) greater than 1 in an OPLS-DA model and a P-value less than 0.05. The OPLS-DA results showed that all the metabolic profiles in the rumen fluid changed were separated into 2 groups in response to HS and TN. Based on LC-MS, GC-MS and 1H NMR results, the metabolites of glucose, galactose, glycerol, butyrate, glucosamine, heptacosane and henricatane were increased by HS (VIP >1, P < 0.05, Fold change >0), while fatty acids and amino acids were decreased (VIP>1, P<0.05, Fold change <0). Most of the rumen metabolites affected by HS were related to several metabolic pathways, including urine cycle pathway, metabolism of amino acids, tryptophan metabolism and citrate cycle, which likely affect the precursor supply for milk component synthesis. These findings indicate that the use of multiple metabolomics platforms permits a far more detailed understanding of HS-induced metabolic changes in rumen digestion: potential mechanism by which HS decreases milk production and component synthesis.

**Key Words:** heat stress, metabolic, rumen fluid

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**M235** Impact of ad libitum milk feeding and butyrate supplementation on organ and epithelial growth in the gastrointestinal tract of dairy calves. C. Gerbert1, D. Frieten*2, C. Koch3, G. Dusef, K. Eder1, R. Zitan4, and H. M. Hammon5, 1Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Muenchweiler an der Alsenz, Germany; 2Department of Life Sciences and Engineering, University of Applied Sciences Bingen, Bingen, Germany; 3Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-University Giessen, Giessen, Germany; 4National Agricultural and Food Centre, Research Institute for Animal Production, Nitra, Slovakia; 5Institute of Nutritional Physiology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.

Ad libitum milk replacer (MR) feeding as well as butyrate (B) have independently shown that both stimulate postnatal growth and development in calves. We hypothesized that the combination of intensive MR feeding and B supplementation expedites the development of the gastrointestinal tract in preweaning calves in a synergistic manner. Holstein male calves were studied from birth until d 80 of age. All calves received colostrum and transition milk until d 3 of age and from d 4 onwards were fed MR (12.5% dry matter) in amounts of either 6 L/d (Res; n = 16) or ad libitum (Adl; n = 16) for 8 wk. In both feeding groups half of the calves were fed MR with 0.24% B (ResB+; AdlB+) or same MR with no B supplement (ResB−; AdlB−). From wk 9 to wk 10 MR was linearly reduced in all calves to 2 L/d. Hay, water, and concentrate were offered ad libitum. At d 80 calves were harvested and mucosal samples of the rumen and small intestine were taken for measurement of rumen papilla and intestinal villus and crypt size. Data (LSM) were analyzed by the Mixed Model of SAS with feeding regimen, B supplementation, and rumen/intestinal segment as fixed effects. Body weight (124 vs. 110 kg) and kidney fat weight (514 vs. 363 g) were greater (P < 0.05) but pancreas weight (92 vs. 106 g) was lower (P < 0.05) in Adl than in Res. The small intestine was longer (P < 0.05) in AdlB− than in ResB− (27.3 vs. 23.6 mm). Villus height in duodenum, proximal jejunum and ileum were greater (P < 0.05) in Adl than in Res (567, 637, 555 vs. 546, 611, 529 µm, resp.). Villus height was greater (P < 0.05) in B+ than in B− (385 vs. 554 µm). Crypt depth was reduced (P < 0.01) by B in ileum (262 vs. 253 mm). The villus height/crypt depth ratio increased by ad libitum feeding and by B and was greatest (P < 0.05) in AdlB− throughout the small intestine (2.27, 2.44, 2.24, 2.35 for AdlB−, AdlB+, ResB−, ResB+, respectively). Intensive MR feeding and B increased small intestinal mucosa growth.

These findings indicated a synergistic effect on small intestinal mucosa growth by intensive MR feeding and B supplementation.

**Key Words:** ad libitum milk replacer feeding, butyrate, intestine

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**M236** The effect of fermented ammoniated condensed whey supplementation on hyperketonemia incidence in transition dairy cows. R. C. Oliveira*1, K. J. Satler1, R. S. Pralle1, H. T. Holdt1, G. D. Poppy2, and H. M. White1, 1University of Wisconsin-Madison, Madison, WI; 2Fermented Nutrition Corporation, Luxemburg, WI.

Supplementation of glucoenogenic precursors in postpartum dairy cattle diets may improve production performance and decrease incidence of metabolic disorders related to negative energy balance. The objective of this trial was to examine the effect of fermented ammoniated condensed whey supplementation on hyperketonemia incidence in transition dairy cows. Holstein cows were individually housed in a tie-stall facility from 28 d prepartum to 45 d postpartum. Cows were blocked by expected calving date and randomly assigned to control (ctl; n = 20) or GlucoBoost (GB; Fermented Nutrition; n = 19). All cows received the same high-energy prepartum ration precalving to achieve postpartum hyperketonemia incidence consistent with industry averages. Cows began treatment rations (GB 0.9 kg AF/day replacing DM equivalent in ctl) on the day of calving. Hyperketonemia incidence was calculated from blood samples collected on d 1, 3, 5, 7, 9, 11, 14 and 17 after calving. Categorical data were analyzed by logistic regression (GLIMMIX, SAS) fitting a binary distribution response. Continuous variables were analyzed with the MIXED procedure of SAS. Models containing the fixed effect of treatment (GLIMMIX) or treatment, time, and the interaction of treatment x time (MIXED) and random effects of block and cow(block x treatment). Supplementation did not alter (P > 0.54) milk volume (41.1 vs. 42.2 ± 1.53 kg, ctl vs. GB), fat, protein, or lactose yield. Cows supplemented with GB had a numerically reduced hyperketonemia incidence (60.0 vs. 36.8 ± 11.28%, P = 0.17). Body weight change (−69.7 vs. −60.4 ± 8.78 kg, ctl vs. GB) and body condition score change (−0.39 vs. −0.30 ± 0.052 units, ctl vs. GB) from calving to 45 d did not differ (P > 0.25) by treatment. Concentration of NEFA differed over time but was not affected (P = 0.70) by treatment. Supplementation of GB did not alter body tissue mobilization or milk production, but reduced hyperketonemia incidence by 1.6 times. This suggest that GB may have altered hepatic metabolism to improve postpartum metabolic disease incidence.

**Key Words:** ketosis, GlucoBoost, transition cow

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**M237** Prepartum conjugated linoleic acid supplementation on lactation performance and metabolic health in dairy cows. R. C. Oliveira*1, R. S. Pralle1, L. C. de Resende2, C. H. P. C. Nova3, V. Caprarulo4, J. A. Jendza5, A. Troescher6, 1, 1University of Wisconsin-Madison, Madison, WI; 2Federal University of Lavras, Lavras, Brazil; 3State University of Northern Rio de Janeiro, RJ, Brazil; 4University of Milan, Milan, Italy; 5BASF, Florham, NJ; 6BASF, Lampwerk, Germany.

Prepartum supplementation with conjugated linoleic acid (CLA) may influence lipolysis and milk fat synthesis. The objective of this study was to examine the effect of prepartum CLA supplementation on lactation and metabolic health of dairy cows. Multiparous cows were enrolled in the study at ~18 d prepartum, and randomly assigned 20 g/d of a mixture of trans-10, cis-12 and cis-9, trans-11 CLA (CLA n = 143; 100 g/d of Luttrell Pure; BASF) or an equivalent amount of saturated lipids as Control (Ctl n = 141; 75 g of Energy Booster 100; Milk Specialties.

**Key Words:** conjugated linoleic acid, transitional cow
Global). Treatments were top dressed individually to cows daily from enrollment to calving and all cows were offered the same ration. Blood samples were collected on the first day of supplementation, −10 d prepartum, and d 1, 7, 14, and 30 of lactation. Milk yield was recorded daily until 60 DIM and averaged weekly. A milk sample was obtained prepartum, and d 1, 7, 14, and 30 of lactation. Milk yield was recorded weekly for component analysis. Categorical data were analyzed by logistic regression (GLIMMIX, SAS) fitting a binary distribution response. Continuous variables were analyzed with the MIXED procedure of SAS. Models containing the fixed effect of treatment (GLIMMIX) or treatment, time, and treatment x time (MIXED) and random effects of block and cow (block x treatment). Prepartum treatment period was 16.1 ± 4.5 and 16.4 ± 4.3 for Ctl and CLA, respectively. Cows supplemented with CLA had increased milk protein yield (1.38 vs. 1.43 ± 0.019 kg, P = 0.01), and tended to have increased milk fat (1.94 vs. 2.00 ± 0.025 kg, P = 0.07) and milk yield (46.6 ± 47.6 ± 0.45 kg, P = 0.09), that resulted in greater energy content of milk (35.35 ± 35.35 ± 0.379 Mcal/d, P = 0.03). CLA supplemented cows had a tendency for lower serum NEFA (0.02 vs. 0.02 ± 0.016 mM/L, P = 0.06) and serum BHBA (0.96 vs. 0.80 ± 0.067 mM, P = 0.09), which resulted in decreased prevalence of hyperketonemia on d 14 postpartum (23.5% vs. 3.5% ± 5.79, P = 0.05). Body condition score change was not affected (P > 0.51) by treatment. There were no significant differences in other health disorders (<0.05). Treatment period was 16.1 ± 4.5 and 16.4 ± 4.3 for Ctl and CLA, respectively. Cows supplemented with CLA had increased milk protein yield (1.38 vs. 1.43 ± 0.019 kg, P = 0.01), and tended to have increased milk fat (1.94 vs. 2.00 ± 0.025 kg, P = 0.07) and milk yield (46.6 ± 47.6 ± 0.45 kg, P = 0.09), that resulted in greater energy content of milk (35.35 ± 35.35 ± 0.379 Mcal/d, P = 0.03). CLA supplemented cows had a tendency for lower serum NEFA (0.02 vs. 0.02 ± 0.016 mM/L, P = 0.06) and serum BHBA (0.96 vs. 0.80 ± 0.067 mM, P = 0.09), which resulted in decreased prevalence of hyperketonemia on d 14 postpartum (23.5% vs. 3.5% ± 5.79, P = 0.05). Body condition score change was not affected (P > 0.51) by treatment. There were no significant differences in other health disorders (<0.05). Prepartum supplementation of CLA improved lactation performance and metabolic health of dairy cows.

Key Words: CLA, transition period, ketosis

**Table 1 (abstract M239).** Size of responses to rumen-protected methionine supplementation (n = 39) according to diet crude protein (CPdiet; > or <16% CP) and DIM

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<tr>
<th></th>
<th>0-59 DIM</th>
<th>60-109 DIM</th>
<th>110-140 DIM</th>
<th>SEM</th>
<th>CPdiet</th>
<th>DIM</th>
<th>CPdiet × DIM</th>
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<td>&lt;16%</td>
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<tr>
<td>DMI, kg/d</td>
<td>−0.73</td>
<td>0.15</td>
<td>0.59</td>
<td>−0.60</td>
<td>0.36</td>
<td>0.17</td>
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<td>Milk, kg/d</td>
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<td>1.26</td>
<td>1.17</td>
<td>−0.67</td>
<td>0.92</td>
<td>−0.26</td>
<td>0.60</td>
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<tr>
<td>Protein, g/d</td>
<td>21</td>
<td>31</td>
<td>73</td>
<td>−1</td>
<td>77</td>
<td>19</td>
<td>24 &lt;0.001</td>
</tr>
<tr>
<td>Fat, g/d</td>
<td>4</td>
<td>73</td>
<td>50</td>
<td>48</td>
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<td>72</td>
<td>51 0.94</td>
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</table>

J. Dairy Sci. Vol. 100, Suppl. 2

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M240 The use of H-nuclear magnetic resonance (H-NMR) in ewes suffering milk fat depression: Could blood metabolomic differences explain the individual variations? A. A. K. Salama1, P. G. Tora1, G. Hervás2, G. Caja1, and P. Frutos2, 1Group of Ruminant Research (G2R), Universitat Autonoma de Barcelona, Bellaterra, Spain, 2Instituto de Ganadería de Montaña (CSIC-ULE), Grulleros, León, Spain.

Dietary supplementation of dairy ewes with marine lipids improves milk fatty acid (FA) profile, but causes milk fat depression (MFD). A large individual variation in the MFD response has been observed, and factors causing this variation are not clear. This study was conducted in dairy sheep to test whether differences in the blood metabolomic profile would clarify causes of individual variations in terms of MFD when marine lipids were added to the diet. Assaf dairy ewes (n = 15) received a total mixed ration supplemented with 0 (control; n = 5) or 20 g of fish oil/kg DM [n = 10; with animals divided in those showing a strong (RESPO+, n = 5) or slight (RESPO-) MFD]. Blood samples collected before (d 0) and after (d 36) oil supplementation were analyzed by H-NMR spectroscopy operating at 600 MHz. For better metabolite detection, spectral editing was done based on spin-spin relaxation time (CPMG filter) and molecular diffusion (DOSY filter). Multivariate analyses of data were carried out by the ChemoSpec package of R program and the web-based MetaboAnalyst program. Principal component and partial least square–discriminant analyses were used to detect metabolite differences among treatment groups. Both RESPO- and RESPO+ ewes supplemented with fish oil had lower concentrations of proline, valine, isoleucine, keratin, lactate and β-glucose. The reductions in amino acid concentrations were in accordance with lower (P < 0.05) milk protein content in lipid-supplemented ewes (4.75%) compared with control ewes (5.03%). Additionally, some monounsaturated FA increased in RESPO- and RESPO+ ewes, which could include some potential antilipogenic FA that are supposed to be able to induce MFD. However, there was no clear discrimination between RESPO+ and RESPO- ewes. In conclusion, lipid supplementation caused significant differences in blood H-NMR metabolomics, but no clear discrimination was observed between animals showing slight and strong MFD. Study funded by Project AGL2014–54587 (Plan Nacional, MINECO, Spain).

Key Words: fish oil supplementation, H-NMR metabolomics, sheep


Previously we showed that supplementation of a corn silage-based diet with up to 3% of linseed oil (LO) reduced enteric CH4 production without adverse effects on DMI and milk production. However, a higher supplementation level (4%) impaired DMI and milk yield. In the same study, we also examined the effects of supplementing increasing amounts LO on N balance of dairy cows fed corn silage-based diet. For this purpose, 12 lactating, multiparous Holstein cows (DIM = 84 ± 28; milk yield = 42 ± 4.6 kg/d) were used in a replicated 4 × 4 Latin square design (35-d period, 14-d adaptation). Cows were fed for ad libitum (5% orts, on an as-fed basis) a corn silage-based TMR (61:39; forage:concentrate ratio) not supplemented (control) or supplemented with 2, 3 or 4% LO (on DM basis). Intake of N, N excretion (fecal and urinary) and milk N secretion were determined over 6 consecutive days, while ruminal NH3 concentration was determined over 2 consecutive days. Data were analyzed using the MIXED procedure (SAS) and differences between treatments and the control were declared significant (P ≤ 0.05) using Dunnett’s comparison test. Ruminal NH3 concentration was unaffected by adding LO at 2 and 3% to the diet, but decreased at 4% LO. A decline in N intake was observed when LO was included at 2, 3 and 4% in the diet (−6, −8 and −19%, respectively). Nitrogen excretion (g/d) in urine and feces also decreased with increasing inclusion of LO in the diet. However, when expressed as a proportion of N intake, excretion of N in feces and urine was not affected by adding LO to the diet. Milk N efficiency (g milk N/g N intake) was higher for cows fed LO-supplemented diets (averaged 31%) compared with cows fed the control diet (26%). Results show that diet supplementation with up to 4% of LO reduced N intake, had no effect on fecal and urinary N excretion (as a proportion of N intake) and improved milk N efficiency of dairy cows. It is concluded that supplementing a corn-silage based diet with up to 3% of LO reduced the amount of N excreted in the manure and increased milk N efficiency without negative effects on DMI or milk yield.

Key Words: linseed oil, corn silage, nitrogen utilization

M242 Effect of Lactobacillus animalis LA-51 and Propionibacterium freudenreichii PF-24 on the total tract digestibility of protein, starch, NDF and on fecal starch concentrations in high-producing cows. K. E. Nestor Jr.*, S. Lerner, and C. Jamison, Chr. Hansen Animal Health, Milwaukee, WI.

The effect of a combination of probiotic strains of Lactobacillus animalis (LA-51) and Propionibacterium freudenreichii (PF-24) fed at a total concentration of 3 × 109 on total-tract digestibility of NDF, protein, starch and on the concentration of starch in manure was assessed in a commercial study. Observations were gathered from 25 herds, ranging in total size from 80 to 6,000 cows. High groups within each herd were selected for testing. Groups ranged in size from 20 to 200 cows and were producing ≥80 lb average milk per day. Both Jerseys and Holsteins were included in the test. Group samples of manure and total mixed rations (TMR) were collected one week before and 3 weeks after the initiation of supplementation of feed with probiotics. Fecal and TMR samples were sent to Cumberland Valley Analytical Laboratory for analysis. Data were analyzed using 2-tailed paired t-test using herd as the experimental unit. There was no effect of probiotic feeding on total-tract digestibility of NDF or protein. Starch digestibility was increased (P < 0.01) and fecal starch was decreased (P < 0.01) with daily feeding of the combination of LA-51 and PF-24. When the data were limited to those herds where the initial concentration of fecal starch was ≥5%, total-tract digestibility of protein and starch increased (P < 0.05 and P < 0.001, respectively), fecal starch decreased (P < 0.001), and total-tract digestibility of NDF tended to increase (P < 0.12). The addition of an effective combination of probiotic organisms can improve the apparent digestibility of nutrients when fed to high-producing dairy cows.

Key Words: probiotic, digestibility, Lactobacillus

M243 Gaseous emissions from forages and rumen-cannulated steers: Do ruminants emit nitrous oxide? K. Gerlach*1, A. Sommer1, A. J. Schmithausen2, M. Trimborn2, W. Büscher2, and K.-H. Südekum1, 1Institute of Animal Science, University of Bonn, 2Research (G2R), Universitat Autonoma de Barcelona, Bellaterra, Spain, 3Instituto de Ganadería de Montaña (CSIC-ULE), Grulleros, León, Spain.

60 d. Greater responses in Mprot when diet CP is high may challenge the assumption that better AA profile of MP allows for diet CP reduction.

Key Words: meta-analysis, protein, days in milk

M244 Dietary supplementation of dairy ewes with marine lipids improves nitrogen utilization without negative effects on DMI or milk yield. A. A. K. Salama*1, P. G. Tora1, G. Hervás2, G. Caja1, and P. Frutos2, 1Group of Ruminant Research (G2R), Universitat Autonoma de Barcelona, Bellaterra, Spain, 2Instituto de Ganadería de Montaña (CSIC-ULE), Grulleros, León, Spain.

Nitrogen utilization in dairy cows fed a corn silage-based diet supplemented with increasing amounts of linseed oil. F. Hassanat*, C. Cherif, D. Warner, and C. Benchara, Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada.
This study aimed at assigning gaseous emissions from ruminants to animals or feeds they have consumed. Three adult rumen-cannulated German Holstein steers and 3 forage types were used in a 3x3 Latin square design. Forages were corn silage (CS, 366 g dry matter (DM)/kg, 70.7 g crude protein (CP)/kg DM), alfalfa silage (AS, 411 g DM/kg, 246 g CP/kg DM) and grass hay (GH, 881 g DM/kg, 79 g CP/kg DM). Each period consisted of 10 d where animals received 10 kg DM/d of one of the forages as sole feed and the last 3 d of each period were used for sampling. A defined amount of forage was put in a closed vessel and gas samples were obtained using evacuated headspace vials after 0, 10, 20, 30, and 40 min. Additional samples were taken 3 h after filling following the same procedure. Samples from the gaseous phase of the steers’ rumen were taken 3 h after offering feed in the morning. In 10-min intervals, 4 samples were obtained with a syringe through the closed lid of the rumen-cannula and filled into headspace vials. This was repeated on 3 consecutive d. Samples were analyzed for carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) using gas chromatography. Data were analyzed using SAS 9.4 and a mixed model taking forage type and period as fixed effect as well as their interaction. For the rumen samples, animal was added as random effect. Within each period, day was taken as repeated measurement. There were large differences in the amount of CO₂ and N₂O emitting from the forages. Most N₂O came from AS (9.9 µg/kg fresh matter (FM) · h; P < 0.05) and only small amounts from GH (0.20 µg/kg FM · h) and CS (0.04 µg/kg FM · h). Highest CO₂ concentrations were measured in CS (P < 0.05). After 3 h, emissions from forages were strongly reduced. Methane was not detected in any forage sample. Animals fed CS showed slightly lower (P < 0.05) CH₄ emissions in the rumen gas sample than when fed AS or GH (16.9% vs. 20.6% and 20.3%). Big differences were found for N₂O with 0.857 ppm for AS and 0.246 and 0.171 ppm for CS and GH (16.9% vs. 20.6% and 20.3%). Big differences were found for N₂O with 0.857 ppm for AS and 0.246 and 0.171 ppm for CS and GH (16.9% vs. 20.6% and 20.3%).

Key Words: emission, forage, ruminant

M244 In vitro post-ruminal digestion of rumen bypass emulsions encapsulated by interfacial crosslinking using polyphenol oxidase from potato tuber peels. F. Gadeyne*, N. De Neve1, B. Vlaeminck1, P. Van der Meeren2, and V. Fievez2, 1Laboratory for Animal Nutrition and Animal Product Quality, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Ghent, Belgium, 2Particle and Interfacial Technology Group, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Ghent, Belgium.

The protection of unsaturated fatty acids by emulsifying oil in a polyphenol oxidase (PPO) rich protein extract and cross-linking of interfacial protein upon 4-methylcatechol (4-MC) addition has been shown before as a promising technology to bypass rumen biohydrogenation (BH). An in vitro experiment was designed to assess the post-ruminal availability of linoleic acid (LNA) in rumen bypass emulsions. Therefore, emulsions (n = 3) were made with a protein extract of potato tuber peels, containing 20 mg triglycerides (TAG) of linseed oil per ml extract and a final 4-MC concentration of 0, 5, 10 or 20 mM, and were subjected to in vitro ruminal, abomasal and a time series of intestinal incubations. As TAG have to be hydrolyzed to nonesterified fatty acids (NEFA) before BH can occur, it was hypothesized protected LNA remained in the TAG fraction during ruminal incubation, but was released from its protective shell and concomitantly hydrolyzed to bio-available NEFA post-ruminally. Lipids were extracted from emulsions, after rumen or after the subsequent abomasal and intestinal incubation, separated in lipid fractions, methylated and analyzed using gas chromatography. Results were analyzed with the MIXED procedure of SAS. TAG LNA disappeared from the original emulsions after rumen incubation when no 4-MC was present (P < 0.001), but less in case of 5 (P = 0.006) or 10 (P = 0.024) mM 4-MC and no differences were observed anymore with 20 mM 4-MC (P = 0.879). All TAG LNA disappeared after abomasal and intestinal incubation compared with the original emulsions (P < 0.001), but LNA appeared partly as NEFA after abomasal and intestinal incubation when 4-MC was present (P < 0.004). Time series of intestinal incubations indicated, however, some level of overprotection, as LNA was least released in the NEFA fraction at the highest levels of rumen protection. In conclusion, dietary LNA in PPO-protected emulsions became bio-available for intestinal uptake, as a fraction of 0.223, 0.237, or 0.303 for emulsions with 5, 10 or 20 mM 4-MC, respectively, survived rumen BH and was released as NEFA after abomasal and intestinal incubations.

Key Words: polyphenol oxidase, biohydrogenation, rumen bypass


The dairy industry in Ireland is based on a spring-calving grazing system with the use of concentrate supplementation in the spring and autumn when there is a scarcity of grass. Problems with milk quality are more pronounced in such a system when the majority of the national herd reach late lactation at the same time. Therefore, the objective of this research was to investigate the effect of concentrate supplementation strategy on milk yield and composition and rumen fermentation parameters in late lactation dairy cows. Thirty-six Holsten Friesian dairy cows were blocked on days in milk (+185DIM) and balanced for parity, pre-experimental milk yield and milk composition, predicted 305-day milk yield and BCS. Cows were randomly assigned to one of 3 dietary treatments in a randomized complete block design (n = 12). The dietary treatments (T) were grass only (T1); grass + 2.6 kg DM barley based concentrate (T2); grass + 2.6 kg DM maize based concentrate (T3). The diets were fed for a 14-day acclimatization period and then for a further 63days. Cows offered T1 had a lower milk yield (14.54 kg) than T2 (17.15kg, P < 0.001) and T3 (16.73, P < 0.001). Similarly, T1 had lower milk solids (kg fat and protein; 1.47 kg) than T2 and T3 (1.51 and 1.48 kg, respectively; P < 0.001). The response to concentrates averaged 0.8:1 kg of milk per kg of concentrate (range of 0.5 to 1.2). Milk urea was higher in T1 (0.041%) than both T2 (0.038%, P < 0.001) and T3 (0.039%, P = 0.004) and rumen ammonia was significantly higher in T1 (5.63 mmol/L, P = 0.006) and T3 (5.77 mmol/L, P < 0.001) than T2 (5.28 mmol/L). Mean rumen pH of cows offered T2 (6.32) was lower than T1 (6.42, P = 0.002) and T3 (6.42, P = 0.003). In conclusion, concentrate supplementation increased milk yield and kilograms of milk solids and altered rumen fermentation parameters. Differences between barley and maize based concentrates were seen for rumen pH and ammonia.

Key Words: late-lactation, grazing, supplementation

M246 Reduction of aflatoxin transfer into milk of lactating dairy cows with the addition of a commercial clay. S. C. Allen1, Z. A. Mason1, B. J. Rude1, R. H. Bailey1, A. Hoang1, D. L. Sparks1,
Twenty-four Holstein cows were used in a randomized complete block design to test the efficacy of clay, MYCOAD, in reducing aflatoxin M₁ (AFM₁) in milk. Cows were blocked by parity and stage of lactation. Cows were housed in a freestall barn with sand bedding and were fed and milked twice daily. Cows were adapted to individual feed gates (Calan Broadbent Feeding System, American Calan, Northwood, NH) one week before the treatment period. The experiment consisted of a 7 d treatment period. Cows were randomly assigned one of 4 dietary treatments (n = 6): (1) control (CON) TMR; (2) aflatoxin (AF) TMR contaminated with 300ppb aflatoxin B₁ (AFB₁); (3) adsorbent diet (MYC) TMR containing 50 g of clay/cow/day; (4) AF diet with clay (AF+MYC) contaminated with 300ppb AFB₁ containing 50 g of clay/cow/day. All additions to TMR were topdressed. Data were analyzed using the MIXED procedure of SAS (Cary, NC). Main effects were treatment, days in milk, parity, and day. All interactions were tested and backward stepwise elimination was used to remove nonsignificant interactions. Significance was declared at P < 0.05. Dry matter intake (DMI) was similar across treatments. Cows consuming CON produced less milk compared with cows on other treatments (29.0 v. 36.1 kg/d; P < 0.01). Cows consuming CON were less efficient compared with cows on other treatments (0.81 v. 1.71 kg DMI/kg milk). This decrease in efficiency of CON cows was likely due to the decrease in MY. Although milk components (%) were similar across treatments, yields (kg) of protein and solids were less in cows fed CON diets. Protein yield averaged 0.85, 1.12, 1.08, and 1.09 kg/d (P < 0.02), and solids yield averaged 2.53, 3.13, 3.06, and 3.24 kg/d for CON, AF, MYC, and MYC+AF respectively. Milk from cows fed AF had the greatest concentration of AFM₁ (P < 0.001), and concentrations averaged 0.24, 2.26, 0.15, and 0.83 for CON, AF, MYC, and MYC+AF respectively. Cows fed AF+MYC averaged 1.43 ± 0.30 ppb AFM₁ less than cows fed AF, resulting in a 63% reduction. Results from this study show that adding MYCOAD to contaminated diets was effective at reducing AFM₁ concentrations in the milk of cows fed AFB₁ without negatively affecting DMI, milk yield or feed efficiency.

Key Words: aflatoxin, lactating cow, adsorbent

M247 Revised representation of urea recycling and ruminal nitrogen metabolism for the Molly cow model. M. Li*, R. R. White1, and M. D. Hanigan1, 1Department of Dairy Science, Virginia Tech, Blacksburg, VA, 2Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA.

Accurately predicting nitrogen (N) digestion and utilization will allow diet optimization to achieve improved N efficiency. The objectives of this study were to revise the representation of urea recycling in Molly cow model and evaluate the revisions. The work included 1) modification of the existing urinary urea excretion equation to include BW as a scalar; 2) supplement of urea gut entry rate to derive parameters related to urea return to rumen; and 3) reparameterization of equations related to urea N recycling and ruminal N metabolism. Model parameters were changed from initial values to optimized values. Model predictions were compared with a data set from 12 published studies with 54 treatment means before and after the revisions. Mean squared errors were assessed to characterize model performance. Residual analyses demonstrated that the modifications improved the accuracy of predictions of ruminal N digestion, absorption and recycling. After the modifications, prediction errors for duodenal flows of total N, microbial N, non-ammonia N, and non-ammonia non-microbial N were 14.8, 22.4, 17.8 and 28.2%, respectively, which were all approximately 2% units less than observed with the initial model due primarily to the decreased mean bias. Compared with the initial model, predictions of ruminal ammonia and blood urea concentrations were greatly improved with substantial decreases in mean and slope bias. Prediction errors for gut entry rate were 19.2% with 0.93% mean bias and 1.73% slope bias, which indicated that urea N recycling mechanisms were properly represented in the model. Although the accuracy of urinary urea flow was improved, it still had 81.7% prediction error, which implies high variation of urinary urea N secretion may exist in collected studies, and therefore the reparameterization is not necessarily more accurate even though it fits the observed data. In summary, the model modifications led to a robust representation of urea N recycling and ruminal N metabolism which enabled more accurate and precise predictions of the effects of feeding and management decisions on N efficiency, thus contributing to sustainability of the dairy industry.

Key Words: model, recycling, urea

M248 Value of pulp from green protein extraction of grass clover as forage for dairy cows. V. K. Damborg*, S. K. Jensen, and M. R. Weisbjerg, Department of Animal Science, Aarhus University, Aarhus, Foulum, Denmark.

The increasing demands for animal protein necessitate alternative feed protein sources e.g., protein from green biomass. Pulp of a grass-clover mixture produced by screw-pressing is macerated to release protein, and potentially improve accessibility of fiber and fiber-bound protein. The object of the study was to compare the pulp to grass-clover as forage for dairy cows. Freshly harvested grass-clover was separated into pulp and juice in an industrial scale Vincent twin-screw press (TSP-12). The juice was acidified by lactic acid fermentation precipitating the protein, followed by a decanting into paste, which was spin flash dried into green protein (GP). Pulp and grass-clover from the same field was separately ensiled without additives. The in vitro organic matter (OM) digestibility was 70.7 and 67.6% (n = 4) and crude protein (CP) concentrations were 16.8 and 13.6% of dry matter (DM), for pulp (P) and grass-clover (GC) silage, respectively. GP had a CP concentration of 33.5%DM. A production trial including 12 primiparous and 24 multiparous lactating cows was designed to compare P and GC, and to compare protein deficient rations (~14% CP of DM) to GP or to soybean meal (S) supplemented rations (~16% CP of DM). The trial design was an incomplete Latin square (4x6) consisting of 3 week periods and 6 treatments (GC, P, GC+GP, P+GP, GC+S, P+S) where rations without protein supplement (CG and P) were designed to be protein deficient. The forage:concentrate ratio was 55:45 for protein deficient TMRs and 50:50 for supplemented. The P or GC silage composed 68%DM of the forage. The DM intake (DMI) of P silage TMRs was 23.1 (~0.3) kg/d, which was higher than GC silage TMRs, 22.6 (~0.3) kg/d (P < 0.001). Milk yield for P silage TMRs was 37.4 (~0.9) kg/d, and lower for GC silage, 34.6 (~0.9) kg/d (P < 0.001). The results indicate that pulp from protein extraction of green biomass was a valuable forage for dairy cows compared with grass clover, even though part of the digestible soluble matter had been removed.

Key Words: protein extraction, pulp, forage

M249 Rumen degradability and intestinal digestibility of dry matter and crude protein of wheat and corn dry distillers grains with or without solubles. K. Nedelkov*1, N. Todorov1, M. T. Harper2, D. Girginov1, and M. Simeonov3, 1Trakia University, Stara Zagora, Bulgaria, 2The Pennsylvania State University, University Park, PA, 3Agricultural Institute, Stara Zagora, Bulgaria.
The objective of this study was to compare rumen degradation kinetics and intestinal digestibility of wheat and corn dry distillers grains with solubles (DDGS) or without solubles (DDG). Three Holstein and 12 Jersey cows with an average body weight of 436 ± 18 kg fitted with a rumen and T-type duodenal cannulas were used in the experiment. Six DDG products (3 from wheat, DDGSw1, DDGSw2, DDGSw3 and 3 from corn, DDGSc1, DDGSc2 and DDGc4) were collected from 4 ethanol plants. Feed samples were incubated in the rumen of the cows for 0, 2, 4, 8, 16, 24 and 48 h in 6 replications. The effective degradability of DM was higher (P < 0.05) for both wheat and corn DDG products from ethanol plants 1 and 2 compared with DDGSw from plant 3 and DDGc from plant 4. The soluble fraction of CP ranged from 23.9% for DDGSw2 to 12.4% for DDGc4 with higher (P < 0.05) values for wheat DDGs than those from corn. Effective degradability of CP at different outflow rates (kp = 0.045, 0.06, and 0.08) for DDGSw1 and DDGSw2 were higher (P < 0.05) compared with those for corn DDGs. Correlation between color parameters (L – lightness, a - yellowness and b - redness) of the wheat DDGs and their CP effective degradability was high (r = 0.82 to 0.95). Further research is needed to evaluate the possibility of using these easily estimated color parameters as a proxy of the protein nutritional value of DDGS. The intestinal digestibility of rumen degradable DM, measured by the mobile bag technique, ranged from 52.0% for DDGSw3 to 38.3% for DDGc4 and was higher (P ≤ 0.05) for wheat DDGs compared with DDGSc1 and DDGc4. Intestinal digestibility of CP was not different (P > 0.05) among corn DDGS. Intestinal digestibility of CP from wheat DDGS was also highly variable (79.7% to 93.6%). Although nutrient composition of DDGS from different ethanol plants is highly variable, the protein degradability and digestibility values obtained in this experiment can be used in formulating rations for ruminant animals.

Key Words: dry distillers grains, intestinal digestibility, rumen degradability

M250  Effects of combinations of prilled fatty acids with or without potassium carbonate on fermentation and biohydrogenation intermediates in continuous culture fermenters, L. E. Koch*1, B. M. Koch1, S. M. Hussein1, V. R. Trutwin1, T. C. Jenkins1, C. Soderholm2, J. Linn2, J. Albrecht2, and G. J. Lascano1, 1Koch*1, B. M. Koch1, S. M. Hussein1, V. R. Trutwin1, T. C. Jenkins1, 2Clemson University, Clemson, SC, 1Milk Specialties Global, Eden Prairie, MN.

The addition of buffers such as K2CO3 have been investigated in how they alter ruminal fermentation and reduce accumulation of milk fat inhibitors (MFI). Thus, we hypothesized that prilled saturated free fatty acids (FFA; C16:0 and C18:0) combined with K2CO3 would provide a slower, more prolonged release of K2CO3 than feeding it alone in reducing production of MFI. Four treatments were randomly assigned to 8 continuous culture fermenters for 2 periods of 10 d. Treatments included 4 combinations of FFA (Supplement A) and K2CO3 coated with prilled fatty acids (1:1; Supplement B) representing 1.25:0 (1), 0.83:0.83 (2), 0.42:1.66 (3), and 0.2:5.2% DM (4) of supplement A to B ratios. All treatments provided 1.25% DM of FA with K2CO3 increasing gradually from 0 to 1.25% DM. Data were analyzed using the MIXED procedure of SAS as a randomized complete block design with blocks of period and fermenters; where linear, quadratic and preplanned polynomial contrasts where evaluated. Addition of K2CO3 altered pH and biohydrogenation (Table 1). There was a quadratic increase in total VFA (P < 0.01) but butyrate was reduced linearly (P < 0.05), while valerate and isovalerate were increased quadratically with increasing K2CO3. Adding K2CO3 tended to increase the outflow of C18:1 (P = 0.09), C18:2 (P = 0.06) and reduce C18:3 (P = 0.06) linearly. Biohydrogenation intermediates, trans-10 and 12:1, were quadratically reduced with K2CO3. These results indicate that combinations of prilled fatty acids and K carbonate increase pH and reduce production of biohydrogenation intermediates linked to milk fat depression exhibiting a quadratic response.

Key Words: lipid, biohydrogenation, continuous culture


The aim of the study was to evaluate the pattern of undigested NDF (uNDF240h, %DM) excretion in feces and to compare the singular time points with a 24h composite. Four dairy cows, paired for lactation number (2), days in milk (184 ± 38d), milk production (35 ± 2kg), weight (664 ± 50kg), and daily rumination time (564 ± 50 min), were housed in tie-stalls for individual dry matter intake (DMI) determination. The feed was delivered once a day (0800h). After an adaptation period the feces collection started. The feces were collected every 2h (12 time points in total) for 3d; all samples were divided in 3 aliquots: one was kept separate, one was composited to create a 4 time points sample (c08–14h, c16–22, c00–06), representative for the morning, afternoon, and night excretion; the latter aliquot was used to create a composite of the whole day (c24h). During the study, the DMI, rumination time, milk production, were recorded daily, and the total mixed ration (TMR) sampled every day. The chemical composition of feeds and feces were determined. The data were analyzed using JMP-12 (SAS Institute Inc., Cary NC). A mixed model was used; the time points and the day were

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Table 1 (abstract M250). Biohydrogenation and pH profile of fermenters fed 4 combinations of supplements A and B
Research indicates that lactating dairy cows have a requirement for metabolizable lysine (MP Lys). Porcine blood meal (PBM) is a common, but highly inconsistent source of MP Lys in dairy diets. The study objective was to determine the efficacy of a rumen-protected lysine product (USA Lysine, Kemin Industries, Inc., Des Moines, IA) as a source of MP Lys in diets fed to early lactation Holstein cows compared with PBM. Thirty cows (8 primiparous and 22 multiparous; average 45–175 DIM) were utilized in a trial consisting of a 2-wk covariate period, a 4-wk treatment period (period 1), a 1-wk washout period and a 4-wk treatment period (period 2). At the end of the covariate, cows were blocked by parity, DIM and milk yield, then randomly assigned to 1 of 2 treatment sequences (n = 15) according to a crossover design. There were 2 diets: 1) Control (CON) - PBM as the main source of MP Lys; and 2) USA Lysine (USA-L) - USA Lysine with dried distillers grains w/ solubles (DDGS) replacing most the PBM. Cows were individually fed 1×/d and milked 2×/d. Milk yield and dry matter intake (DMI) were recorded daily and milk components were determined weekly. Data were analyzed with the Mixed procedure of SAS (SAS Institute, Inc. Cary, NC). The model included the fixed effects of treatment, period, week in period (as a repeated measure), sequence, parity, treatment by week in period, treatment by parity and the random effect of cow nested within sequence. Significance was declared at P < 0.05 and tendencies at 0.05 < P < 0.10. There were no differences (P > 0.25) between the CON and USA-L treatment groups in DMI (25.90 vs. 25.63 kg/d) or milk yield (41.71 vs. 41.19 kg/d), respectively. Milk components and component yields, as well as feed efficiency, were nearly identical across treatments. Nitrogen use efficiency in terms of milk urea nitrogen (MUN) was improved with the USA-L diet (9.98 vs. 10.74 mg/dL, P < 0.01) compared with the CON diet. In conclusion, USA Lysine in combination with DDGS effectively replaced PBM as the main source of MP Lys in a diet formulated for high-producing Holstein dairy cows.

Key Words: lysine, blood meal, milk yield

M253 Effects of a rumen-protected lysine product as a source of metabolizable lysine for high-producing dairy compared with porcine blood meal. S. Polukis1, A. Barnard1, T. Gressley1, 1University of Delaware, Newark, DE, 2Kemin Industries, Inc., Des Moines, IA.

Research indicates that lactating dairy cows have a requirement for metabolizable lysine (MP Lys). Porcine blood meal (PBM) is a common, but highly inconsistent source of MP Lys in dairy diets. The study objective was to determine the efficacy of a rumen-protected lysine product (USA Lysine, Kemin Industries, Inc., Des Moines, IA) as a source of MP Lys in diets fed to early lactation Holstein cows compared with PBM. Thirty cows (8 primiparous and 22 multiparous; average 45–175 DIM) were utilized in a trial consisting of a 2-wk covariate period, a 4-wk treatment period (period 1), a 1-wk washout period and a 4-wk treatment period (period 2). At the end of the covariate, cows were blocked by parity, DIM and milk yield, then randomly assigned to 1 of 2 treatment sequences (n = 15) according to a crossover design. There were 2 diets: 1) Control (CON) - PBM as the main source of MP Lys; and 2) USA Lysine (USA-L) - USA Lysine with dried distillers grains w/ solubles (DDGS) replacing most the PBM. Cows were individually fed 1×/d and milked 2×/d. Milk yield and dry matter intake (DMI) were recorded daily and milk components were determined weekly. Data were analyzed with the Mixed procedure of SAS (SAS Institute, Inc. Cary, NC). The model included the fixed effects of treatment, period, week in period (as a repeated measure), sequence, parity, treatment by week in period, treatment by parity and the random effect of cow nested within sequence. Significance was declared at P < 0.05 and tendencies at 0.05 < P < 0.10. There were no differences (P > 0.25) between the CON and USA-L treatment groups in DMI (25.90 vs. 25.63 kg/d) or milk yield (41.71 vs. 41.19 kg/d), respectively. Milk components and component yields, as well as feed efficiency, were nearly identical across treatments. Nitrogen use efficiency in terms of milk urea nitrogen (MUN) was improved with the USA-L diet (9.98 vs. 10.74 mg/dL, P < 0.01) compared with the CON diet. In conclusion, USA Lysine in combination with DDGS effectively replaced PBM as the main source of MP Lys in a diet formulated for high-producing Holstein dairy cows.

Key Words: lysine, blood meal, milk yield

M254 In silico prediction of miRNA activity in the hepatic response to prepartum body condition score and plane of nutrition during the transition period in grazing dairy cows. M. Vailati Riboni1, V. Palombo2, A. Agrawal1, M. J. Khan1, and J. J. Loor1, 1Università degli Studi del Molise, Campobasso, Italy, 2Università degli Studi del Molise, Campobasso, Italy, 3Università degli Studi del Molise, Campobasso, Italy, 4Università degli Studi del Molise, Campobasso, Italy, 5Università degli Studi del Molise, Campobasso, Italy.

An in silico approach was applied to investigate the possible role of microRNAs (miRNA) in the liver transcriptome response to prepartum body condition score (BCS) and feeding management in the weeks before calving. Thirty-two mid-lactation grazing dairy cows of mixed age and breed were randomly allocated to 1 of 4 treatment groups in a 2 × 2 factorial design: 2 prepartum BCS categories [4.0 (thin) and 5.5 (fat)] and 2 feeding management strategies: 2 dietary protein sources: 1) ruminant-processed blood meal (PBM) and 2) USA Lysine (USA-L) - USA Lysine with dried distillers grains w/ solubles (DDGS) replacing most the PBM. Cows were individually fed 1×/d and milked 2×/d. Milk yield and dry matter intake (DMI) were recorded daily and milk components were determined weekly. Data were analyzed with the Mixed procedure of SAS (SAS Institute, Inc. Cary, NC). The model included the fixed effects of treatment, period, week in period (as a repeated measure), sequence, parity, treatment by week in period, treatment by parity and the random effect of cow nested within sequence. Significance was declared at P < 0.05 and tendencies at 0.05 < P < 0.10. There were no differences (P > 0.25) between the CON and USA-L treatment groups in DMI (25.90 vs. 25.63 kg/d) or milk yield (41.71 vs. 41.19 kg/d), respectively. Milk components and component yields, as well as feed efficiency, were nearly identical across treatments. Nitrogen use efficiency in terms of milk urea nitrogen (MUN) was improved with the USA-L diet (9.98 vs. 10.74 mg/dL, P < 0.01) compared with the CON diet. In conclusion, USA Lysine in combination with DDGS effectively replaced PBM as the main source of MP Lys in a diet formulated for high-producing Holstein dairy cows.

Key Words: lysine, blood meal, milk yield
performed on a list of miRNA families and their predicted target genes for *B. taurus* downloaded from the Microcosm targets website (v. 5.0). The dynamic impact approach was used for pathway analysis on the target differentially expressed genes (fold change $<-1.5$ or $>1.5$, $P < 0.05$) of the predicted miRNA at each time point. For BCS 4 there were 9 and 7 miRNA (pre- and postpartum, respectively) predicted to be involved in the response to prepartum nutrition. Their activity was related to cell proliferation and immune signaling. Postpartum, they also involved pyruvate, nitrogen, glutathione, and glycine metabolism. For BCS 5 only 2 (pre-) and 1 (postpartum) miRNA were common to the 3 approaches, with ubiquitin-mediated proteolysis affected prepartum, and mineral absorption, bile secretion, and tryptophan metabolism potentially regulated by miRNA postpartum. Overall, miRNA seem to be involved in the response to prepartum BCS and nutrition, particularly in thinner cows.

**Key Words:** BCS, prepartum nutrition, liver miRNA

M255  **Pre-weaning and post-weaning performance in dairy calves fed an active dry yeast (*Saccharomyces cerevisiae* CNCM I-1077),** A. Faulkner$^2$, A. Clay$^1$, L. Waldron$^2$, A. Aguilar$^*^1$, E. Chevau$^1$, and A. Turney$^1$, $^1$Lallemand Animal Nutrition, Milwaukee, WI, $^2$Lallemand, Auckland, NZ.

A trial was run to examine the influence of feeding a rumen specific live yeast, *Saccharomyces cerevisiae* CNCM I-1077 (SC), on feed intakes and body weights of calves at weaning. Sixty male, Friesian cross calves, aged 4 d old, were randomly allocated to either a control (unsupplemented) creep feed diet or one containing SC at $4 \times 10^9$ cfu/kg. All calves were fed a commercial milk replacer (CMR) and offered straw and creep feed ad libitum from entry, and intakes and weight gains were recorded. At 6 weeks of age, the calves were transferred to grazing with ad libitum access to creep feed and monitored for a further 2 weeks, covering the transition period from milk to grazing. The calves fed SC had higher feed intakes at weaning (1.105 kg/yr versus 1.523 kg/d at 6 wk of age; $P = 0.0434$ and 1.179 kg/d versus 1.965 kg/d at 8 wk of age; $P = 0.0272$ for control and SC respectively). Correspondingly, average daily gain (ADG) was improved. At 7 wk of age, ADG was 0.659 kg versus 0.912 kg ($P = 0.039$), and at 8 wk of age ADG was 0.457 kg versus 0.707 kg ($P = 0.0650$) for control and SC respectively. It was concluded that supplying SC via creep feed prevented the drop off in calf performance at weaning, in terms of both feed intake and weight gain. This is important, as early rumen development and pre-weaning growth rates are related to a reduction in gastric upsets at weaning, future growth performance in calves and future lactation performance.

**Key Words:** calf, live yeast, performance


A standard procedure for measurement of fecal pH in dairy cows does not currently exist. Consequently, sample preparation may influence the precision of this measurement; thus, limiting comparisons across literature reports. The objectives of this study were to determine if differences exist based on preparation method, and to determine variation across methods. Thirty fresh fecal samples were collected from lactating Holstein cows housed in the same pen and consuming the same diet. Five samples were collected at a time and prepared according to the following methods: (1) direct measurement (DIR) in which the pH probe was directly inserted into the fecal sample; (2) strained fecal fluid (STR) obtained by squeezing the fecal sample through 4 layers of cheesecloth. Three dilution rates (distilled water: feces) were also tested: (3) 0.5:1 dilution (D1), (4) 1:1 dilution (D2), and (5) 2:1 dilution (D3). Each sample was prepared using all methods, resulting in a total of 150 pH measurements. The UNIVARIATE and GLM procedures of SAS were used to test normality and homogeneity of variance, respectively. The Shapiro-Wilk test confirmed that data were normally distributed ($P = 0.08$). The Levene’s test showed heterogeneity of variance ($P = 0.02$), thus the SATTERTHWAITE approximation of degrees of freedom for denominator was used for the ANOVA via the GLIMMIX procedure. Sample preparation method affected ($P < 0.01$) pH values, resulting in D3 having the highest pH of 6.91 $\pm$ 0.04, followed by D2 with a value of 6.79 $\pm$ 0.04. Measurements of pH by D1 and DIR were similar, and averaged 6.67 $\pm$ 0.04 ($P = 0.17$); whereas, STR had the lowest value of 6.60 $\pm$ 0.04. Descriptive statistics showed the standard deviation for the STR method was 0.173 and 0.174 for D2, while that of D1, D3 and DIR was 0.224, 0.226 and 0.296, respectively. These results demonstrate that pH measurements in strained fecal fluid or a 1:1 dilution rate have reduced variability when compared with direct measurements and other dilution rates.

**Key Words:** hindgut acidosis, gastrointestinal health, colonic fermentation

M257  **Bioavailability of AjiPro-L 2G and AjiPro-L 3G using the plasma free lysine dose-response technique.** N. Whitehouse$^*^1$, A. Brito$^1$, C. Schwab$^1$, I. Shinzato$^3$, and M. Miura$^1$, $^1$University of New Hampshire, Durham, NH, $^2$Schwab Consulting LLC, Boscobel, WI, $^3$Ajinomoto Heartland Inc., Chicago, IL, $^4$Ajinomoto Co. Inc., Tokyo, Japan.

The objective was to compare the bioavailability of 2 rumen-protected supplements (AjiPro-L 2G vs. AjiPro-L 3G) using the plasma free Lys dose-response technique. Seven lactating multiparous Holstein cows (202 ± 49 DIM) equipped with ruminal cannulas were used in a 7 × 7 Latin square with 7-d periods. The treatments were (1) 0 g/d Lys, (2) 30 g/d of abomasally infused Lys, (3) 60 g/d of abomasally infused Lys, (4) 30 g/d fed Lys from AjiPro-L 2G, (5) 60 g/d fed Lys from AjiPro-L 2G, (6) 30 g/d fed Lys from AjiPro-L 3G, and (7) 60 g/d fed Lys from AjiPro-L 3G. The infusion treatments consisted of Lys-HCI and were infused continuosly into the abomasum via the ruminal cannulas. To ensure complete consumption, the AjiPro-L2G and AjiPro-L 3G were mixed with 1.5 kg of TMR, stored at 4°C for 8 h before feeding and placed in tubs in front of the cows 30 min before each of the 3 daily feedings. Blood samples were obtained from each cow on the last 3 d of each period every 2 h, 4 times daily, from the tail vein, centrifuged, deproteinized, composited into 1 daily sample/cow, and analyzed for AA. Data for plasma AA concentrations (μM basis) were analyzed using the PROC MIXED and PROC REG procedures of SAS. Data from 2 cows was removed from the data set due to very low or negative response to all treatments. The slope for the AjiPro-L 3G (i.e., 0.01407; $P < 0.001$) was greater than the slope for the AjiPro-L 2G (i.e., 0.01257; $P < 0.04$) resulting in a 12% improvement in bioavailability of Lys from the AjiPro-L 3G based on the ratio of the 2 slopes. Although the bioavailabilities of AjiPro-L 2G and AjiPro-L3G were not significantly different, the 12% numerical increase in bioavailability with feeding AjiPro-L3G results in 1.1 g/d more absorbable Lys when 60 g/d of product are fed.

**Key Words:** AjiPro, bioavailability, plasma lysine
M258  In vitro investigation of supplementing microalgal protein precipitate material as a source of dietary protein in a dairy diet using continuous cultures. S. Y. Yang,1 J. M. Yang,1 J. Marriott2, J.-S. Eun4, R. C. Sims2, and R. C. Anderson3, 1Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT, 2Department of Biological Engineering, Utah State University, Logan, UT, 3USDA-ARS, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit, College Station, TX.

The fat-extracted microalgal biomass derived from the biofuel production has been suggested as a promising source of protein supplement in ruminant diets. Consequently, we performed an in vitro experiment to investigate the effects of supplementing microalgal protein precipitate material (APP) obtained via wet lipid extraction procedure. The APP was attained using dairy waste from Caine Dairy Research Center (Wellsville, UT) at Utah State University. The APP used in this study had low bacterial counts on blood agar, approximately $1.8 \times 10^3$ cfu/g. In addition, in a 24-h incubation assay with a suspension of bovine fecal contents, we observed neither an inhibitory nor a stimulatory effect of the APP on growth or survivability of Salmonella enterica serovar Typhimurium ($8.65 \pm 0.18 \log_{10} \text{cfu/mL})$ or Escherichia coli O157:H7 ($8.91 \pm 0.11 \log_{10} \text{cfu/mL}$). The APP contained 30.6% crude protein and 6.60% ether extract on a dry matter (DM) basis. The experiment was performed as a completely randomized design to test 4 dietary treatments in a typical dairy diet (n = 4): 1) no APP (control), 2) 1.2% APP, 3) 2.4% APP, and 4) 3.6% APP in a DM basis. Although increasing APP in the diet tended to linearly decrease culture pH ($P = 0.08$), the culture pH was maintained at least 6.0 throughout culture incubation. Total VFA concentration was similar across dietary treatments. Molar proportion of acetate linearly increased by increasing APP inclusion ($P = 0.03$), while propionate portion did not change, resulting in no effect on acetate-to-propionate ratio. Increasing addition of APP linearly decreased molar proportion of isovalerate ($P < 0.01$). Concentration of ammonia-N averaged 7.32 mg/100 mL, and it was not influenced due to dietary treatments ($P = 0.27$). Additionally, APP supplementation did not affect methane production in the culture regardless of dietary concentration. Overall data in this in vitro study suggest that APP attained from dairy waste did not have any negative impacts when added up to 3.6% DM on ruminal fermentation profiles and show a potential to be a sustainable source of dietary protein in dairy diets.

Key Words: dairy waste, microalgal protein precipitate material, ruminal fermentation

M259  Is a pelleted feed required in an automated milking system (AMS)? K. S. Paddock*, S. B. Menajovsky, and G. B. Penner, University of Saskatchewan, Saskatoon, SK, Canada.

It is recommended that a pelleted concentrate is offered to cows milked in an AMS. The previous recommendation is designed to ensure cattle can consume concentrate rapidly to enable precision feeding strategies; however, it is not clear if pelleted feeds are necessary when low quantities of concentrate are offered. The objective of this study was to evaluate the effect of providing barley grain that was either steam-rolled (SR) or pelleted (PEL) on DMI and milk and milk component yield. Five Holstein cows (98 ± 7.8 DIM) housed in a feed-first guided-traffic flow barn were used in a crossover design with 24-d periods. Cows were fed a common partial mixed ration (PMR) containing a 55:45 forage-to-concentrate ratio and were offered sufficient concentrate in the AMS to achieve either 2.5 kg/d of SR or PEL (DM basis). Milking permissions were granted when predicted milk yield exceeded 9 kg or when the interval from the last milking exceeded 4 h. Dry matter intake (PMR and AMS concentrate), voluntary visits to the AMS, and milk and milk component yield were measured. The form of concentrate offered in the AMS had no effect on total DMI or PMR intake with average values of 29.8 and 27.3 kg/d, respectively ($P \geq 0.79$). Interestingly, cows fed PEL and SR did not differ for concentrate intake in the AMS averaging 2.48 kg/d ($P = 0.16$). The type of concentrate provided in the AMS did not affect variability for AMS or PMR concentrate intake among days ($P \geq 0.59$). Voluntary milking frequency was not affected by form of concentrate offered in the AMS with an average of 3.51 visits/d. Milk yield (41.5 kg/d), and the yield of CP (1.43 kg/d) and fat (1.60 kg/d) did not differ among treatments ($P \geq 0.27$). However, milk fat concentration was reduced for cows fed PEL compared with SR (3.82% vs. 3.92%; $P = 0.03$). Milk urea nitrogen tended to be reduced for cows fed PEL compared with SR (13.8% vs. 15.6 mg/dL; $P = 0.10$). These data indicate that, with a low quantity of concentrate allocated in the AMS, it may be possible to feed steam-rolled barley grain without negatively affecting voluntary visits to the AMS and milk component yield.

Key Words: automatic milking systems, dairy nutrition

M260  Lipidomics reveals phosphatidylcholines as candidate biomarkers for metabolic disease. S. S. Samii*, Y. Zang,1 E. Grilli2, and J. W. McFadden1, 1West Virginia University, Morgantown, WV, 2University of Bologna, Bologna, Italy.

The discovery of novel biomarkers for metabolic disease can refine nutritional interventions aimed at improving dairy cow health and performance. Therefore, our objective was to identify metabolites associated with common markers of metabolic disease. Thirty multiparous Holstein cows were enrolled −28 d prepartum and fed diets formulated to meet or exceed requirements. Blood and liver samples were routinely collected from enrollment through d 14 postpartum (pp). To characterize the plasma lipidome spanning 9 time points, untargeted lipidomics was performed using quadrupole time-of-flight mass spectrometry. Univariate and multivariate analyses of normalized, auto-scaled lipidomic data were performed. Based on pp metabolic health data, cows were separately categorized into low or high free fatty acid area under the curve (FFA_AUC; d 1 – 14 pp; 4,915 ± 1,569 vs. 12,501 ± 2,761 [μmol/L x 14 d]; n = 18), β-hydroxybutyrate area under the curve (BHB_AUC; d 1 – 14 pp; 4,583 ± 459 vs. 7901 ± 1,206 [μmol/L x 14 d]; n = 18), or mean pp liver lipid content (d 5 and 14 pp; 5 ± 1 vs. 12 ± 2% of wet weight; n = 18). Significant variables associated with a specific category were identified based on leverage/squared prediction error plots. Lipidomics revealed 301 plasma lipids including 8 cholesterol esters, 163 phospholipids, and 130 acylglycerols. Independent of category, all cows displayed decreases in plasma triacylglycerols and monoalkyl-diacylglycerols ($P < 0.01$), and the majority of phospholipids reached a nadir at parturition ($P < 0.01$). Analyses revealed that phosphatidylcholine (PC) 32:3, 35:5, 37:5 were specific for high FFA_AUC, PC 31:3, 32:3, 35:5, and 37:5 were specific for high BHB_AUC, and PC 31:2, 31:3, and 32:3 were specific for high liver lipid. Notably, PC 32:3 was specific for high FFA_AUC, BHB_AUC, and liver lipid cows, a metabolite that was lower in abundance relative to the low categories ($P < 0.01$). Other lipids specific for 2 or more categories included phosphatidylglycerol 38:4 and lysophosphatidylcholine 15:0. Nutritional interventions that increase plasma PC 32:3 during the peripartum should be explored.

Key Words: biomarker, lipidomics, metabolic disease
M261  Performance of crossbred Holstein × Zebu cows supplemented with fibrolytic enzyme in diets with different forage levels. A. M. Teixeira1, L. C. Gonçalves2, L. F. Martins1, A. P. D’Abadia Netto1, B. O. Silva1, G. C. Oliveira1, T. T. Santos1, N. Ferreira Junior3, N. D. Walker1, and T. L. Resende1, 1Universidade Federal de Minas Gerais, Belo Horizonte, MG Brazil, 2Universidade Federal de Uberlândia, Uberlândia, MG Brazil, 3Universidade Federal de Minas Gerais, Belo Horizonte, MG Brazil, 4AB Vista Brazil, São Paulo, SP Brazil, 5AB Vista, Marblrough, Wiltshire, UK, 6Auster Nutrição Animal Ltda, Hortolândia, SP Brazil.

The aim of the trial was to evaluate the performance and concentration of ketone bodies in the blood of early lactation cows supplemented with fibrolytic enzymes (750 mL/T DM; Xylanase 350,000 BXU/g, Cellulase 10,000 ECU/g, AB Enzymes, Finland) in diets with different forage levels. Twenty Holstein × Zebu cows (46 ± 31 d in milk), blocked according to yield, DIM and parity, were individually fed for 132 d divided into 6 periods of 8 d adaptation and 14 d measurements in a 2 × 2 factorial randomized complete block design. The treatments consisted of 4 groups: (1) low forage (53.8%) without enzyme (LF); (2) low forage with enzyme (LFE); (3) high forage (62.3%) without enzyme (HF); (4) high forage with enzyme (HFE). The LF (33.07 kg/d) and LFE (31.72 kg/d) groups had higher milk yield than the HF group (27.01 kg/d), with the HFE group being intermediate (30.42 kg/d) (P = 0.02). 4% FCM (P = 0.022) and ECM (P = 0.044) showed the same behavior. There was a tendency for LF (1.27 kg/d) and LFE (1.23 kg/d) to present a higher fat yield than the HF group (1.08 kg/d), with the HFE group being intermediate (1.18 kg/d) (P = 0.068). LF had a tendency (P = 0.058) to present higher solids yield in relation to the HF and HFE groups. The LFE and LF groups had higher lactose content than the HF group, with the HFE group being intermediate (P = 0.001). Cows fed the LF diet had higher (P < 0.001) protein content (3.30 vs 3.14%) and higher (P = 0.001) lactose yield in relation to the cows that received the HF diet, regardless of enzyme addition. The protein yield of the LF group was similar to the LFE group and higher than the others (P = 0.005). The HFE group presented higher urea nitrogen content (P = 0.001) and tendency of higher blood ketone concentration (P = 0.059) compared with the others. To conclude, addition of enzyme improved the performance of cows fed diets containing a higher proportion of forage, approaching a similar level of performance as animals receiving a lower proportion of forage and higher proportion of concentrate.

Key Words: digestibility, enzyme, fiber

M262  Kinetics of trans-10,cis-12 and cis-9,trans-11 conjugated linoleic acid (CLA) transfer to plasma and milk following an abomasal bolus in lactating dairy cows. N. L. Urrutia1,2, R. Bomberger1, M. Baldini1, M. Toledo1, and K. J. Harvatin1, 1The Pennsylvania State University, University Park, PA, 2Instituto Nacional de Investigaciones Agropecuarias-Remehue, Osorno, X Region de Los Lagos, Chile.

Dietary fatty acids (FA) are directly transferred to milk through chylomicrons and indirectly through tissue recycling. The objective of this study was to characterize the direct and indirect transfer rates of the cis-9,trans-11 (c9t11) and trans-10,cis-12 (t10c12) CLA through plasma to milk following a single abomasally infused bolus. Five ruminally cannulated multiparous mid-lactation cows (148 ± 86 DM; Milk 44.1 ± 11.2 kg/d) received a single abomasal bolus infusion of an enriched CLA mixture providing 15 g of each CLA isomer (c9t11, t10c12) over a 30 min period. Total transfer of CLA was analyzed in a model that included cow as a random effect and CLA isomer as a fixed effect (JMP Pro). Time course data were analyzed as repeated measures in SAS and least squares means were fit to a double exponential decay function by nonlinear curve fitting (JMP Pro) to characterize direct (fast pool) and indirect (slow pool) transfer of CLA isomers to milk. Plasma CLA concentration peaked at 2 h, reaching 0.32 and 0.31% of plasma FA for c9t11 and t10c12, respectively, and returned to baseline at 72 h. Milk t10c12 concentration peaked at 14 h (0.5% of FA) and returned to baseline at 86 h post infusion. Milk c9t11 concentration initially peaked at 14 h (0.98% of FA), returned to baseline at 86 h post infusion, and then had a second peak between 146 and 158 h (0.56% of FA) post infusion. Total transfer of CLA to milk differed between isomers and was 79.3 and 40.8% of the bolus for c9t11 and t10c12, respectively (P < 0.001). Time course of CLA isomers transferred to milk fit a biexponential model (R² = 0.99). The area (% of total) under the first exponential representing direct transfer was 17 and 73% and the second exponential representing indirect transfer was 83 and 27% of the total CLA isomers transferred for c9t11 and t10c12, respectively. In conclusion, although plasma kinetics of c9t11 and t10c12 were similar, transfer of CLA isomers to milk differed greatly in their transfer efficiency and major pool of transfer.

Key Words: conjugated linoleic acid, plasma, milk

M263  Palmitic acid feeding increases plasma ceramide concentrations in Holstein dairy cows during early lactation. A. N. Davis1, Z. C. Phipps1, Z. Q. Zeng2, J. de Souza3, J. E. Rico1, A. L. Lock2, and J. W. McFadden1, 1West Virginia University, Morgantown, WV, 2Michigan State University, East Lansing, MI.

Reduced insulin action is an adaptation that develops during early lactation to enhance lipolysis and promote glucose partitioning for the mammary synthesis of milk. The onset of lactation is accompanied by the accumulation of ceramides, antagonists of insulin signaling, which have been reported to be enhanced by palmitic acid (C16:0) feeding in monogastrics. To determine whether C16:0 feeding increases plasma ceramides during early lactation, 37 multiparous cows were assigned to 1 of 3 treatments: CON-CON (control diet with no supplemental fat; 1–67 DIM); CON-PA (control diet fed from 1 to 24 DIM, and a C16:0-supplemented diet from 25 to 67 DIM); and PA-PA (C16:0 supplemented diet; 1–67 DIM). Diets were formulated to contain a minimum of 17% CP, 29% NDF, and 24% starch. The C16:0 supplement (85% C16:0), added at 1.5% of diet DM, replaced soyhulls in the CON diets. Blood was collected at 11, 32, and 60 DIM. Plasma ceramide and monohexosylceramide (GlcCer) concentrations were determined using mass spectrometry. Data were analyzed as repeated measures using a mixed model, and Pearson correlations were analyzed. Circulating ceramides increased with the progression of lactation (P < 0.01), albeit the magnitude was greater with PA feeding (day by treatment interaction; P < 0.05). PA increased total ceramide levels by 39% (P < 0.01). The addition of C16:0 in CON-PA increased total ceramides by 60% at d 32, relative to d 11 (P < 0.01). Relative to CON-CON, PA-PA increased C24:0-ceramide levels by 26, 48, and 58% at d 11, 32, and 60, respectively (P < 0.05). The ratio of C24:0- to C16:0-ceramide decreased over time and was elevated in PA-PA (P < 0.05). The majority of GlcCer increased over time, and were elevated in PA-PA, relative to CON-CON (P < 0.01). For example, total GlcCer increased 25% from d 11 to 60, and was 25% higher in PA-PA, relative to CON-CON (P < 0.01). Plasma total ceramide and C24:0-ceramide levels were positively correlated with milk yield and energy-corrected milk (r = 0.27 to 0.52; P < 0.01). The ability of palmitic acid-induced ceramide to suppress insulin action during early lactation requires further investigation.

Key Words: ceramide, insulin resistance, palmitic acid

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M264 Effect of rumen fluid inoculum and substrate on in vitro volatile fatty acid production and fiber digestibility. R. A. Judd1, C. Stoffel2, and E. Evans3, 1University of Maryland, College Park, MD, 2Papillon Agricultural Company, Easton, MD, 3Essi Evans Technical Advisory Services, Bowmanville, Canada.

This in vitro experiment used a 4 × 2 × 2 factorial design with 6 replicates (3 per run) to determine effects of rumen fluid treatment, substrate and probiotics. There were 4 treatments of rumen fluid inoculant, 2 preweighed substrates (0.5 g timothy hay or 0.5 g timothy hay with 0.5 g corn grain), each with or without a probiotic supplement containing yeast, lactic acid bacteria, and digestive enzymes. Control (C) rumen fluid (10 mL/flask) was collected 4 h after feeding, and was blended and strained. Other rumen fluid treatments used 0.5 mL rumen fluid: otherwise as control (A), collected before feeding (B), or not blended (D). Samples in 40 mL of medium plus inoculant were incubated at 39°C for 24 h. Results were analyzed by the model: Y = μ + T + S + P(D). Samples in 40 mL of medium plus inoculant were incubated at 39°C for 24 h. Results were analyzed by the model: Y = μ + T + S + P(D). Samples in 40 mL of medium plus inoculant were incubated at 39°C for 24 h. Results were analyzed by the model: Y = μ + T + S + P(D).


Twenty-four cows were used in a 3 × 3 Latin square design experiment with 21-d periods to evaluate production responses to 2 highly saturated fatty acid (FA) supplements enriched in either stearic or palmitic acid. Cows (122 ± 39 DIM; 43.5 ± 9 kg/d milk yield) were randomly assigned to squares and treatment sequences within square. Treatments were: control (CTR; base diet with no supplemental FA; 53% forage, 1:1 corn silage to alfalfa ratio), an enriched palmitic acid supplement (PALM; > 80% C16:0), and an enriched stearic acid supplement (STEAR; 65–75% C18:0, 20–25% C16:0). Both free FA supplements were added to the base diet at 2.0% of the diet DM. Milk yield and DMI from d 14 to 20 and milk composition from d 20 of each period were used for the analysis. Contrasts compared CTR vs. PALM and STEAR, and PALM vs. STEAR. FA supplementation decreased dry matter intake (DMI) by 1.3 kg/d (22.0 vs. 23.3 kg/d; P = 0.03), but did not affect net energy intake compared with CTR. Treatments did not affect fat or protein concentrations in milk or yields of milk, 3.5% fat-corrected milk, and milk components. FA supplementation increased components efficiency (yields of milk fat and protein/DMI; 11.3 vs. 10.7%; P = 0.04) and feed efficiency (milk yield/DMI) compared with CTR (1.77 vs. 1.66, P = 0.01). Treatments did not affect body weight or body condition score. Production responses to PALM were not different from those of STEAR. Overall, FA supplementation decreased concentration of FA from de novo synthesis in milk compared with CTR (24.6 vs. 26.9%; P < 0.01), and the effect tended to be more pronounced for PALM than STEAR (24.1 vs. 25.1%; P = 0.07). FA supplementation increased mixed-source (P < 0.01) but not pre-formed FA in milk compared with CTR. PALM increased concentration of mixed-source FA in milk compared with STEAR (35.3 vs. 30.6%; P < 0.01), while STEAR increased that of pre-formed FA compared with PALM (41.2 vs. 37.1%; P < 0.01). In conclusion, FA supplements decreased DMI, did not affect milk yield or concentrations of milk fat or protein, and increased feed efficiency compared with a control diet with no supplemental FA.

Key Words: fatty acids, palmitic acid, stearic acid

M266 Estimation of daily dry matter intake of individual cows fed in a group setting using common on-farm measurements. M. E. Ivaniuk*,1, E. E. Connor2, and R. A. Erdman1, 1University of Maryland, College Park, MD, 2USDA-ARS, Beltsville, MD.

Feed is the single largest expense for producing milk on dairy farms so producers are interested in exploring methods to improve dairy feed efficiency (FE). To calculate FE and to allow for genetic selection of the most feed-efficient cows within a herd, knowing the dry matter intake (DMI; kg/d) of individual cows is required. The vast majority of dairy cows are fed in large groups such that the DMI of an individual cow within a group is unknown. The objective of this study was to develop and validate a system to estimate DMI of individual cows using measurements that are already commonly recorded on dairy farms. The proposed approach is a modification of an original model developed by Jonker et al. (1998; J. Dairy Sci. 81:2681–2692). For this study, the data set included 2-wk averages for dietary nitrogen (DietN) concentrations (g/kg DM) as well as DMI and milk production (kg/d), milk protein (g/d), and milk urea N concentrations (MUN; mg/dL) from 167 individual cows (2,157 cow observations) across 52 2-week periods. The data were used to predict N losses in milk, urine, and feces to estimate DMI of individual cows fed the same diet by 2-wk periods using the following equation: DMI (kg/d) = [(MilkN + A + (B × MUN))/0.8 × DietN + 5 – C] where: MilkN = milk N output (g/d), A = intercept for predicted urinary N losses (g/d), B = coefficient used to predict urinary N output based on changes in MUN, 0.8 = availability of DietN digested, DietN = dietary N concentration (g/kg diet DM), 5 = mean metabolic fecal N (MFN; g/kg diet DM), and C = adjustment in differences in diet N availability and MFN. Predicted DMI explained 59% of the total variation in measured DMI (R2 = 0.59; RMSE = 2.15; P < 0.0001). Residual plots of the deviations in actual vs. predicted DMI indicated no mean or linear biases. Therefore, it was concluded that DMI can be estimated on an individual cow basis using common on-farm measurements even if the cows are fed in a group setting. These results will enable producers to calculate individual cow DMI as well as FE to allow for the genetic selection of more feed-efficient cows.

Key Words: DMI, dairy cow, prediction model

M267 Effects of a starch binding agent on in vitro rumen degradability of corn and sorghum starch. M. N. T. Shipan-del1,2, E. Raffrenato1, and C. W. Cruywagen1, 1Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa,
The objective of the trial was to quantify the potential of a starch binding agent (BioProtect) to reduce in vitro rumen starch degradation of corn and sorghum particles varying in size. Corn and sorghum grain samples were ground through 1 and 2 mm sieves using a Wiley mill and subsequently sieved to obtain the following sizes: <250, 250–500, 500–1180 and 1180–2000 µm. All fractions were separately analyzed for starch. Samples were treated 24 h before fermentation by spraying with BioProtect according to product guidelines. Both, treated and untreated corn and sorghum samples were fermented in vitro for 0, 3, 6, 9, 12, 24 and 48 h to quantify starch digestibility (Sd). Rates of digestion (kd) were calculated using a first order decay model and 48 h fermentation residuals were used to estimate indigestible starch. Data were analyzed according to a randomized complete block design with a factorial arrangement of treatments. The main effects tested were grain, particle size, product, time (for Sd only) and their interactions. Fermentation run (n = 3) was considered a random effect. As particle size decreased, starch increased from 701 to 821 g/kg and from 730 to 810 g/kg for corn and sorghum, respectively. For both grain types, Sd and kd increased linearly with decreased particle size (P < 0.01). Sd (kd) increased from 41 (0.10) to 58% (0.26 h⁻¹) and 30 (0.11) to 53% (0.24 h⁻¹) for corn and sorghum, respectively. BioProtect was effective (P < 0.001) in decreasing starch digestibility for both grains. The product was more effective with smaller particle size, by reducing Sd 17%-units for the smallest particles vs. 7%-units for the largest particles. A time interaction was observed (P < 0.0001), showing that the highest impact of BioProtect occurred after 12 h of fermentation for both grains. The starch binding agent resulted in an effective decrease of in vitro starch digestion, but results would be affected by particle size and fermentation time.

Key Words: BioProtect, starch, in vitro

M268 Effects of oilseed supplementation on performance, methane emission and nitrogen utilization efficiency of lactating dairy cows. C. Muñoz*,†, R. C. Sánchez‡, A. M. T. Peralta§, S. Espindola##, T. Yan¶, R. Morales**, and E. M. Ungerfeld†*, 1Facultad de Ciencias Veterinarias, Universidad de Concepción, Concepción, Chile, 2Instituto de Investigaciones Agropecuarias, INIA Remue, Ovalle, Chile, 3Departamento de Animal Science, University of Namibia, Windhoek, Namibia.

Oilseeds can decrease enteric methane emission and affect nutrient utilization in dairy cows. The objective of this study was to evaluate the effects of oilseed supplementation on milk production and composition, methane emission and efficiency of nitrogen (N) utilization of dairy cows. Eight multiparous Holstein Friesian cows (75.4 ± 15.9 d in milk) were randomly allocated to treatments in a double 4 × 4 Latin square with 4 periods (22 d for adaptation and 6 d for measurements in digestibility units). Diets fed as TMR consisted of: maize silage, a concentrate based on ground corn and soybean meal, and 1 of 4 treatments: control (CON); commercial fractionated palm oil protected fat; 3.5% DM basis), whole linseed (LNS; 7.5% DM basis), whole rapeseed (RPS; 6.9% DM basis) and whole cottonseed (CTS; 18.4% DM basis). Measurements of methane emissions were conducted using the SF₆ technique. Data were analyzed using a mixed model including the fixed effects of treatment and period and the random effect of cow. The RPS diet increased (P < 0.05) DM intake compared with the other treatments. Treatments had no effect on milk yield. Milk fat content was decreased (P < 0.001) by LNS and RPS compared with CON. CTS tended to increase (P = 0.07) milk protein and increased (P < 0.001) milk lactose compared with CON, but was not different (P > 0.05) from LNS or RPS. Methane emission per cow (P < 0.001) and per kg of DM intake (P < 0.01), were decreased by CTS compared with RPS and LNS. Methane emission per kg of energy corrected milk (P = 0.03) was decreased by CTS compared with RPS, but was not different (P > 0.05) than CON and LNS diets. Total N intake was increased (P < 0.05) by RPS compared with CON. All oilseeds increased (P < 0.001) fecal N output (g/d) compared with CON. Feeding CTS increased (P < 0.001) urine N output compared with LNS, RPS and CON, and tended (P = 0.05) to decrease retained N compared with RPS. In conclusion, the inclusion of CTS in the diet of lactating dairy tended to increase milk protein and lowered methane emissions compared with LNS and RPS, but increased N excretion in urine.

Key Words: oilseeds, methane, cottonseed

M269 Effect of different physiological stages on plasma adropin, insulin, nonesterified fatty acids, and glucose concentration in lactating dairy cows. H. M. Edvardsson* and A. E. Relling, Department of Animal Sciences, The Ohio State University, Wooster, OH.

The objective of this research was to investigate the effect of physiological stage on plasma concentrations of adropin, nonesterified fatty acids (NEFA), glucose, and insulin in lactating dairy cows. Twenty-three lactating dairy cows were selected based on days in milk (DIM) and daily average milk yield. There were 7 early lactation cows (EL, < 50 DIM), 8 in mid-lactation high producing (HPML) and 8 in mid-lactation low producing (LPML). High and low production mid-lactation (100–200 DIM) were determined by taking an average of all DIM appropriate cows, and using plus or minus a standard deviation to create the minimum milk yield for the HPML cows and the maximum milk yield for the LPML cows. Blood samples from the cows were drawn once via the coccygeal vein before feeding time and the plasma was used for glucose, NEFA, insulin, and adropin assays. Data were analyzed as a complete randomize design with a mixed model (SAS 9.4) considering each treatment as a fixed variable and the cow as a random variable. The option PDIFF of SAS was used for mean separation if overall treatment effect has a P value < 0.05. We were able to validate a human adropin assay as a valid method to measure bovine adropin, using parallel displacement and recovery points. Plasma glucose (EL: 72.15 mg/dL, HPML: 73.88 mg/dL) and insulin (EL: 0.25 ng/mL, HPML: 0.33 ng/mL) concentrations of EL and HPML cows were similar (P > 0.1) while LPML had greater (P < 0.05) concentrations (79.82 mg/dL and 0.5382 ng/mL for glucose and insulin respectively). Average NEFA concentrations of HPML (218 µEq/mL) and LPML (254 µEq/mL) were similar (P > 0.1) while EL had much greater concentrations (537 µEq/mL, P < 0.05). There was a trend (P < 0.1) for adropin to have a lower concentration in EL (0.48 pg/mL) than HPML (0.78 pg/mL), while LPML had similar concentrations to both (0.63 pg/mL, P > 0.1). Our results show that different stages of lactation tend to have different concentrations of adropin, insulin NEFA and glucose and the concentration is not dependent of physiological stage or milk yield but the interaction between them.

Key Words: metabolism, endocrinology, milk yield

M270 Effect of different heat processing methods on both morphological changes of starch granules and degradability of barley grain. S. Shirmohammadi*, A. Taghizadeh, G. A. Moghaddam, and A. H. Khani, University of Tabriz, Tabriz, East Azerbaijan, Iran

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The aim of this study was to evaluate the effect of different heat-processing methods on both morphological changes of starch granules and degradability of barley grain. Treatments included 1) control: whole barley grain with no processing(WBG), 2) roasted: roasting for 5 min at 130°C (RBG), 3) microwave irradiated: microwaved for 2 min at 1200W(MBG), and 4) steam flaked: moisturized for 30 min on direct steam flow of boiling water and flaked just after moisturizing(SBG). To evaluate degradability by treatments, a gas production technique and an in situ method were utilized. For estimating post-ruminal digestibility, a modified 3-step method was adopted. Scanning electron microscopy (SEM) was used to identify morphological changes and also as a new method for explaining digestion kinetics. Cumulatively produced gas was recorded at 2,4,6,8,12,16,24,36,48,72, and 96 h of incubation. Kinetics of digestion were estimated using the model of Gp = A(1 − e−ct). The in situ method used for determination of ruminal digestion kinetics. Two bags were incubated in the rumen of 3 wethers fitted with a rumen canula. Incubation times were 0,2,4,8,12,16,24,36, and 48 h. Degradation kinetics of DM were calculated using the model of y = a + b(1 − e−ct).

In vitro intestinal disappearance of ruminal DM residue after 12 h of incubation was estimated using a modified 3-step procedure. Different heat-processing methods increased \( P < 0.05 \) cumulative gas production, being 179.9, 190.2, 200.6, and 211.8 mL/g of DM for WBG, RBG, MBG, and SBG respectively. Extent of intestinal disappearance of DM was greater for MUL than PRIM while RT and IT did not differ \( P > 0.10 \) between parities during access time to pasture. When INT were evaluated, differences were detected only in INT 3: MUL cows performed 591 more NB \( P = 0.013 \) and grazed 14 min more \( P = 0.012 \) than PRIM, while PRIM ruminated 7 min more \( P = 0.048 \) and idled 7 min more \( P = 0.0543 \) than MUL. Results suggest that the different grazing behavior performed by MUL and PRIM could be associated with different pasture intake.

**Key Words:** ruminant behavior, dairy cow, oat pasture

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Information concerning to grazing pattern of multiparous (MUL) and primiparous (PRIM) cows is especially valuable to understand mechanisms involved in feed intake as well as to improve dairy grazing management. The study was conducted in a randomized block design to assess the effect of parity (3 block; n = 9 cows per treatment) of Holstein dairy cows (days in milk = 73 ± 7; BW = 521 ± 32 kg; milk yield = 26 ± 3 kg) grazing a vegetative oat pasture (8 h of access to pasture from 8:30 to 16:30 h; pasture allowance = 30 kgDM/cow/day; DM = 14%, CP = 23%, NDF = 46%, dry basis) on grazing behavior (grazing, ruminating and idling times; GT, RT and IT, respectively) and number of prehension bites (NB). Cows were milked twice daily and fed, after the afternoon milking, 6 kg DM/day of a mixture (70:30 forage to concentrate ratio as-fed basis). Individual cows were observed every 5 min on 3 consecutive days and grazing, ruminating or idling and other activities were recorded. The GT, RT and IT were estimated assuming that the recorded activity was maintained between 2 consecutive records. The number of bites was estimated by counting prehension bites/min every 5 min during the grazing activity. All variables were calculated for the 8 h of access to pasture and for 4 intervals of 2 h each during the access time to pasture (INT1 to INT4). Data were analyzed as a repeated measures using a mixed model with treatment (MUL and PRIM), day and INT (when corresponded) as fixed effects and block as a random effect. The NB (14,990 vs 13,670; \( P = 0.033 \)) and GT (320 vs 300 min; \( P = 0.075 \)) were greater for MUL than PRIM while RT and IT did not differ \( P > 0.10 \) between parities during access time to pasture. When INT were evaluated, differences were detected only in INT 3: MUL cows performed 591 more NB \( P = 0.013 \) and grazed 14 min more \( P = 0.012 \) than PRIM, while PRIM ruminated 7 min more \( P = 0.048 \) and idled 7 min more \( P = 0.0543 \) than MUL. Results suggest that the different grazing behavior performed by MUL and PRIM could be associated with different pasture intake.

**Key Words:** barley grain, scanning electron microscope, gas production technique

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**M272** **Changes in rumen bacteria communities in continuous cultures fed high and low levels of unsaturated fatty acids with increasing rates of starch degradability.** V. Richards, T. Jenkins, L. Koch, and G. Lascano*, *Clemson University, Clemson, SC.*

Dietary changes can alter the rumen environment and provoke shifts in microbial communities leading to incomplete biohydrogenation (BH). The objective of this study was to compare bacterial diversity in diets previously shown to cause shifts in BH intermediates. Diets containing low (LF) or high (HF) concentrations of unsaturated fatty acids (0 or 3.3% soybean oil added) were modified using corn sources with low (L), medium (M) or high (H) starch degradability (SKd; 48.4 L, 66.2 M, or 84.0% h-1 in 7 h in vitro test) and arranged in a 2 × 3 factorial design. Diets were fed for 4 10 d periods. Bacterial community composition from overflow samples was determined using Illumina MiSeq16S rRNA gene V4 variable region amplicon sequencing. Significant differences in β diversity among sample groupings were determined using a Python script within QIIME to perform a PERMANOVA. For individual relative abundance (rA) of interest, the MIXED procedure of SAS was used. Significance for main effect of fat and linear and quadratic contrasts for SKd were set at \( P \leq 0.10 \). Results showed 519 species belonging to 248 genera across treatments. Beta diversity was different between LF and HF \( P = 0.10 \); and significantly increased with SKd \( P = 0.02 \) with a pronounced separation between L and H SKd \( P < 0.01 \). Among the 60 species of most frequently detected taxa, 29 showed a progressive decrease \( n = 20 \) or increase \( n = 9 \) in frequency moving from L to H SKd respectively. The rA of fibrolytic bacteria *Prevotella ruminicola* was increased while *Ruminococcus flavefaciens* was reduced \( P < 0.01 \) as fat was added. There was a linear decrease in rA of *Buty vibrio fibrisolvens* and *Buty vibrio hungatei* (BH enabling; \( P \leq 0.10 \)) as SKd increased. Whereas *Streptococcus bovis* and *Prevotella bryantii* (amylolytic) showed a linear increase as SKd increased \( P \leq 0.05 \). Taxa responsible for lipolysis (*Anaerovibrio lipolyticus*) or utilizing lactic acid (*Megasphaera elsdenii*) were not different. These results suggest that fat level affect bacteria diversity and that increasing the SKd with constant starch level causes significant changes in microbial communities.

**Key Words:** lipid, starch, bacteria diversity

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**M273** **In vitro fermentation of Moringa oleifera leaves supplemented in a ruminant diet.** S. Chizonda*, J. Allen, and V. Fellner, *North Carolina State University, Raleigh, NC.*

Global population growth continues to drive the need for dairy farm sustainability and improvement of system efficiencies. This has led to the exploration of alternative feed sources. *Moringa oleifera* is a multipurpose tree whose leaves are used as animal feed in many parts of the world and is a potential dairy animal feed. The objective of this study was to explore the potential use of *Moringa* as a dairy feed through analysis of in vitro fermentation properties. A batch culture in vitro digestibility study was carried out to analyze the effect of *Moringa* on rumen fermentation of a corn-based diet. Three levels of *Moringa*
Impact of feed restriction-induced negative energy balance on the fatty acid profile of liver lipid fractions of dairy cows. C. M. Prom*, L. C. Worden, S. E. Schmidt, G. A. Contreras, and A. L. Lock, Michigan State University, East Lansing, MI.

Our objective was to determine the impact of feed restriction-induced negative energy balance on the fatty acid (FA) profile of lipid fractions in liver. Ten multiparous Holstein cows were randomly assigned to treatments of ad libitum feed intake (CON; n = 5) or feed restriction (RES; n = 5), with RES resulting in a mean energy balance of -13.1 ± 1.46 Mcal/d following 4 d of treatment. Cows were euthanized on d 4 after 48 h of treatment with RES (n = 5), with RES resulting in a mean energy balance of -13.1 ± 1.46 Mcal/d. The FA profiles of liver lipid fractions were analyzed from control (CON) and RES treatments. Liver lipids were extracted, fractionated, and analyzed for short-chain fatty acids (SCFA), pH, neutral detergent fiber (NDF), and methane from rumen samples were analyzed and dry matter disappearance (DMD) was calculated. Analysis indicated that feed restriction had a crude protein (CP) content of 22% CP for Moringa, 15% CP alfalfa, and 10% CP for corn. The results indicated that methane production was significantly lower (P < 0.0001) with Moringa inclusion (14.56 ± 1.72 mM at 100%) than alfalfa (891.88 ± 351.89 mM at 100%). The total SCFA was not significantly different across all treatments at 48 h, but 128.53 ± 4.7 mM for Moringa inclusion and 129.64 ± 3.6 mM for the control. Moringa inclusion increased digestibility of DM and NDF. Inclusion of Moringa increased NDF from 9.1 ± 0.95% at 50% to 19.4 ± 2.26% at 100%. DMD increased only up to 75% Moringa inclusion (42.3 ± 0.85%) then dropped to 35.2 ± 1.34% at 100% inclusion and control was at 29.2 ± 0.31%. There was more butyrate produced from the control diet (13.1 ± 0.46 mM versus 7.11 ± 0.61 mM for Moringa) but Moringa increased propionate levels (47.23 ± 0.97 mM versus 31.28 ± 1.58 mM for control). The pH increased across all treatments, with time. Suppression of methane saves energy that might be wasted as gas production in the ruminant animal. The results suggest Moringa performs similarly to alfalfa as a high protein feed ingredient.

Key Words: efficiency, dairy, nutrition

M274 Impact of feed restriction-induced negative energy balance on the fatty acid profile of liver lipid fractions of dairy cows. C. M. Prom*, L. C. Worden, S. E. Schmidt, G. A. Contreras, and A. L. Lock, Michigan State University, East Lansing, MI.

Our objective was to determine the impact of feed restriction-induced negative energy balance on the fatty acid (FA) profile of lipid fractions in liver. Ten multiparous Holstein cows were randomly assigned to treatments of ad libitum feed intake (CON; n = 5) or feed restriction (RES; n = 5), with RES resulting in a mean energy balance of -13.1 ± 2.0 Mcal/d following 4 d of treatment. Cows were euthanized on d 4 and liver samples collected. Liver lipids were extracted, fractionated, and FA concentrations determined for triglycerides (TG), phospholipids (PL), cholesterol esters (CE), and free FA (FFA). The statistical model included the fixed effects of treatment, block, fraction, and interactions, as well as the random effect of cow nested within block. Compared with CON, RES altered the distribution of FA in liver lipid fractions. The proportion of FA in each fraction for CON and RES was 5.82% and 35.2% for TG (P < 0.01), 3.18% and 3.45% for FFA (P = 0.96), 88.1% and 59.0% for PL (P < 0.01), and 2.89% and 2.34% for CE (P = 0.91), respectively. Compared with CON, RES increased the concentration of C16:0 (P < 0.01 for all) and cis-9 18:1 (P < 0.01 for all) and decreased the concentration of C18:0 (P < 0.05 for all) in all fractions. The largest fold increase for C16:0 and cis-9 18:1 was in TG and FFA, respectively; the greatest fold decrease for C18:0 was in TG. The concentration (g/100 g FA) of C16:0, C18:0, and cis-9 18:1 for CON vs. RES was 27.2 vs. 40.7, 12.4 vs. 5.93, and 11.1 vs. 25.6 in TG, respectively; 16.1 vs. 21.8, 33.2 vs. 22.8, and 5.24 vs. 17.6 in FFA; 9.17 vs. 13.2, 31.6 vs. 28.2, and 7.09 vs. 14.8 in PL; and 15.6 vs. 21.8, 12.7 vs. 10.6, and 5.76 vs. 13.8 in CE. Compared with CON, RES decreased cis-9, cis-12 C18:2 (g/100 g FA) in TG (8.27 vs. 4.75; P < 0.01) but did not alter it in FFA (8.80 vs. 8.07; P = 0.36), PL (10.2 vs. 11.7; P = 0.35), or CE (25.7 vs. 22.6; P = 0.16). RES decreased cis-5, cis-8, cis-11, cis-14 C20:4 in TG (P < 0.01), FFA (P < 0.01), and CE (P = 0.08), but not in PL (P = 0.74). In conclusion, feed restriction induced-negative energy balance increased the proportion of FA in TG and increased C16:0 and cis-9 18:1 in all liver lipid fractions.

Key Words: fatty acids, liver, negative energy balance


Twenty-four Holstein cows were used in a replicated 4×4 Latin square design experiment with 28-d periods to evaluate the effects of different sources and levels of total undigested neutral-detergent fiber (uNDF; determined after 240 h of in vitro fermentation) on animal performance. Treatments, expressed as % of body weight, were: 1) 0.40% uNDF with 33% forage (high uNDF, low forage: HULF), 2) 0.40% uNDF with 38% forage (high uNDF, medium forage: HUMF), 3) 0.40% uNDF with 48% forage (high uNDF, high forage: HUFM), and 4) 0.30% uNDF with 38% forage (low uNDF, medium forage: LUMF). Linear and quadratic contrasts were used to evaluate the effect of increasing levels of dietary forage at a constant level of uNDF. In addition, a simple contrast was run between treatments HUMF and LUMF to evaluate the effect of 2 levels of uNDF at a similar forage level. There was a quadratic negative effect of increasing forage level on dry matter intake (DMI) and yields of milk, milk fat, and milk protein (Table 1). Feed efficiency (milk yield/DMI) increased as forage in the diet increased at the same level of uNDF. Forage level affected total-tract NDF digestibility (TTNDFD) quadratically: HUMF increased TTNDFD the most, followed by LHLF, and then HFL. LUMF decreased DMI and increased TTNDFD compared with the HUMF, but did not affect milk yield, resulting in increased feed efficiency. Under the conditions of this study, level of forage was better related to changes in DMI and performance than total uNDF intake.

Key Words: indigestible NDF, control of intake, undigested NDF

Table 1 (abstract M275). Effect of source of undigested uNDF on performance of dairy cows

<table>
<thead>
<tr>
<th>Item</th>
<th>HULF</th>
<th>HUMF</th>
<th>HUHF</th>
<th>LUMF</th>
<th>SEM</th>
<th>P-value</th>
<th>Linear</th>
<th>Quadratic</th>
<th>HUMF vs. LUMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>29.3</td>
<td>25.4</td>
<td>24.3</td>
<td>24.3</td>
<td>0.79</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>35.2</td>
<td>31.9</td>
<td>32.4</td>
<td>32.4</td>
<td>2.06</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.31</td>
</tr>
<tr>
<td>Milk fat yield, kg/d</td>
<td>1.34</td>
<td>1.21</td>
<td>1.2</td>
<td>1.22</td>
<td>0.07</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.04</td>
<td>0.71</td>
</tr>
<tr>
<td>Milk protein yield, kg/d</td>
<td>1.13</td>
<td>1.01</td>
<td>1.0</td>
<td>1.0</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.98</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>1.26</td>
<td>1.32</td>
<td>1.38</td>
<td>1.40</td>
<td>0.06</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.84</td>
<td>0.04</td>
</tr>
<tr>
<td>TTNDFD, %</td>
<td>40.4</td>
<td>43.3</td>
<td>42.5</td>
<td>51.1</td>
<td>0.76</td>
<td>&lt;0.01</td>
<td>0.03</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
The objective of this study was to investigate the curve-linear relationship between altered carbohydrate (CHO) traits (different levels of amylose, amylopectin and β-glucan) and rumen and intestinal digestion in dairy cattle in CDC developed hull-less barley cultivars. Four hull-less barley cultivars (zero-amylose waxy, Fibar; 5%-amylose waxy, Rattan; normal amylose, McGwire and high-amylose, HB08302) were developed at the Crop Development Centre, University of Saskatchewan, with differences in carbohydrates traits on the basis of amylose (1–40% DM) and b-glucan (5–10% DM) content. All cultivars were planted and grown in Saskatoon (Canada) and then harvested in 3 consecutive years for experimental purposes. The digestion in the rumen and intestine was determined using situ dairy cows with RCBD design with samples year as a random effect. The rumen degradation was carried out with in situ nylon bag technique. The intestinal digestibility was carried out with 3-step in vitro technique with 16 h pre-incubation. The data were analyzed with Proc mixed model. Results showed that: 1) intestinal (IP: 38 to 69 g/kg DM) and total digestible protein (TDP: 106 to 154 g/kg DM) had cubical (P < 0.05) relation and truly digestible neutral detergent fiber (TDNDF: 53 to 105 g/kg DM) had quadratic (P < 0.05) relation with the β-glucan level. TDP and TDNDF showed a cubical effect and IDP showed a quadratic effect (P < 0.05) with the ratio of amylose to amylopectin (A:AP). There were cubical (P < 0.05) relation between intestinal digestible rumen undegraded starch (IDBST: 78–184 g/kg DM) and A:AP, quadratic (P < 0.05) relationship between IDBST and β-glucan level. Total digestible starch (TDSST) had a quadratic P < 0.05 relation with the ratio of amylose to amyllopectin (A:AP). Intestinal and rumen undegraded CHO (IDBCCHO: 15 to 33 g/kg DM) was cubically related to the A:AP (P < 0.05). In conclusion, the alternation of carbohydrate traits in hull-less barley through modern breeding tool results in curve-linear relationship with rumen and intestinal digestion in dairy cows.

Key Words: alteration of carbohydrate traits, ratio of amylose to amylopectin and β-glucan, rumen and intestinal digestion

M277 Interactions between levels of flaxseed oil and corn grain particle size on milk yield and nutrient digestibility in Jersey cows. V. Brossillon1, A. F. Brito2, S. F. Reis2, D. C. Moura3, J. G. B. Galvão Jr4, C. Córtes5, and A. S. Oliveira5, 1Ecole Supérieure d’Agricultures, Angers, France, 2University of New Hampshire, 3Department of Biological Sciences, Durham, NH, 4Programa de Pós Graduação em Ciência Animal, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil, 5Instituto Federal de Educação do Rio Grande do Norte, Ipanguaçu, RN, Brazil, Ipanguaçu, RN, Brazil, 5Instituto de Ciências Agrárias e Ambientais, Universidade Federal de Mato Grosso – Campus Sinop, Sinop, MT, Brazil.

Organically certified cows in the United States must have year-round access to the outdoors to comply with the National Organic Program rules. Thus, increasing dietary energy via flaxseed oil (FO) may be an attractive strategy to increase milk yield during the winter season. In addition, FO may interact with corn grain of 2 particle sizes [cracked corn (CC) vs. ground corn (GC)] to modulate nutrient digestibility ultimately affecting DMI and milk yield. Sixteen mid-lactation organically certified Jersey cows were randomly assigned to treatments in a replicated factorial 4 × 4 Latin square design with 24-d periods. Treatments were fed as TMR (55:45 forage-to-concentrate ratio) and consisted (DM basis) of (1) 0% FO + 28.3% CC, (2) 0% FO + 27.1% GC, (3) 3% FO + 28.3% CC, and (4) 3% FO + 27.1% GC. Diets averaged 19.5% starch, and 4.9 and 7.6% ether extract for 0 and 3% FO, respectively. Corn grain mean particle size averaged 2,047 µm (CC) and 580 µm (GC). Contrasts were used to compare: FO level, corn particle size, and oil × corn interaction. Data are presented in Table 1. DMI and yields of milk fat and protein were not affected by treatments. Cows fed 3% FO produced more milk than those fed no FO. Conversely, concentrations of milk fat and protein, and total-tract aNDFom digestibility decreased with feeding 3% FO vs. 0% FO. Total-tract digestibilities of OM and starch were greater in cows fed GC than CC. Overall, FO appears to be viable strategy to increase milk yield during the winter season when cows are housed outdoors.

Key Words: corn grain, dairy cow, flaxseed oil


Adipose tissue mobilization increases circulating fatty acid (FA) concentration, hepatic FA uptake, and influences hepatic metabolism. The objective of this experiment was to examine the effect of FA challenge on complete and incomplete oxidation, glucose output, and oxidative stress in bovine primary hepatocytes. Primary hepatocytes isolated from 3 neonatal Holstein calves were maintained as monolayer cultures for 24 h. At 24 h, media was refreshed with a glucose-free media containing only pyruvate as a gluconeogenic precursor, and cells were exposed to 0 or a 1 mM FA cocktail that reflected the circulating FA profile at calving.
After 21 h of treatment, $^{14}$C-labeled palmitate or pyruvate was added to the media and both CO$_2$ and acid soluble products (ASP) were collected after a 3-h incubation. Media was harvested to quantify glucose and reactive oxygen species (ROS). Cell lysates were collected and DNA quantified to normalize all data. Data were analyzed by PROC MIXED (SAS 9.4) in a model accounting for fixed effect of FA treatment and random effect of calf. Data are reported as least squares means ± SE and differences declared at $P < 0.10$ and tendencies at $P < 0.15$. Fatty acid treatment decreased ($P = 0.01$) the relative recovery of pyruvate label as CO$_2$ (3.8 vs. 1.94 ± 0.31 arbitrary units) and increased ($P = 0.02$) label recovery as ASP (20.0 vs. 27.3 ± 1.4 arbitrary units). Recovery of palmitate label as CO$_2$ tended to be greater ($P = 0.14$) in FA treated cells (0.87 vs. 1.05 ± 0.15 arbitrary units) but ASP from palmitate was not affected ($P > 0.18$) by FA treatment. Glucose output from cells exposed to FA was increased ($P < 0.007$) by 35%. Treatment with FA increased ($P = 0.07$) ROS in cell culture media. Fatty acid challenge appears to shift oxidative and gluconeogenic capacity in a substrate-specific manner. Decreased complete and increased incomplete oxidation of pyruvate indicates a shift of pyruvate conversion toward ASP or glucose production. Conversely, fatty acid challenge increased complete oxidation of palmitate, consistent with previous indications that FA can upregulate key oxidative enzymes at the time of calving.

**Key Words:** gluconeogenesis, ketones, flux

### M279 Nutritive value of common feedstuffs fed to dairy cows measured using the in vitro gas production technique. K. Mjoun*, L. Shearer, and B. Kubat, Altech, Brookings, SD.

The fermentation parameters of feedstuffs commonly fed to dairy cows were evaluated using the in vitro gas production technique. Feeds were grouped into different categories; corn silage (n = 15), small grains silages (n = 15), alfalfa hay (n = 11), alfalfa haylage (n = 4), byproducts (n = 20); protein meals (n = 7), energy feeds (n = 17), and lactation TMR (n = 12). Incubations (48h) were completed using rumen fluid from a lactating cow fed a 50:50 forage to concentrate diet. The fermentation kinetics were estimated using a logistic model separating gas production per 1 g of DM truly digested into fast pool (FP), slow pool (SP) and their respective rates of fermentation (FR) and (SR) (Table 1). Grain silages produced the most gas (GP) mainly from the SP. Protein meals had the highest FP:SP and haylage had the highest fermentation rates.

The partitioning of digested DM into gases, VFA, and microbial protein (MPS) suggests that the fermentation efficiency ($Y_{MPS}$) is highest for protein feeds, alfalfa forages and TMR, intermediate for energy feeds, byproducts and small grains silages, and lowest for corn silages.

**Key Words:** in vitro, rumen, feedstuffs

### M280 Effect of RDP:RUP ratio and corn processing on lactation performance, milk quality and efficiency of nutrients utilization in lactating dairy cows. C. M. R. Martins*, D. C. M. Fonseca1, M. A. Arcari1, B. G. Alves1, K. C. Welter2, F. P. Rennó1, and M. V. Santos1, 1Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, São Paulo, Brazil, 2Department of Animal Science, School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil.

The study aimed to evaluate the effect of RDP:RUP ratio and corn processing on lactation performance and milk heat coagulation time (HCT) at 140°C. Twenty Holstein cows (8 fitted with ruminal cannulas) averaged 162 ± 70 DIM, 666 ± 68 kg of body weight and 36 ± 7.8 kg/d of milk yield, were distributed in a Latin Square design with 5 contemporary squares, 4 periods of 21 d and 4 treatments (factorial arrangement 2 × 2). Factor 1 was corn processing (ground through 2-mm screen [GC] or steam-flaked [SFC]), and factor 2 was RDP:RUP ratio (High: 11% of RDP and 5.3% of RUP or Low: 9.8% of RDP and 6.5% of RUP; DM basis; NRC, 2001). For diets with High RDP:RUP ratio, 103.4 g/kg of conventional soybean meal (SM) and 7.8 kg of urea were included. For diets with Low RDP:RUP ratio, 91.9 of SM, 2.8 of urea and 44.7 g/kg of heated SM (SOYPASS, Brazil) were included. There was a tendency of interaction ($P = 0.06$) between RDP:RUP ratio and corn processing on DMI. Cows fed GC had lower DMI than cows fed GC with high RDP:RUP ratio. No effect of RDP:RUP ratio was found when cows were fed SFC. Cows fed SFC had lower DMI ($P = 0.007$), and higher DM ($P = 0.03$) and starch total apparent digestibility ($P = 0.02$), and higher productive efficiency ($P = 0.002$) than cows fed GC. Rumen pH was not affected by diets. It was observed an interaction between treatments on MY ($P = 0.04$), HCT ($P = 0.029$) and milk lactose content ($P = 0.04$). Cows fed GC had reduced MY by 2.3 kg/d when the diet had high RDP:RUP ratio, than cows fed low RDP:RUP ratio. However, when cows were fed SFC, MY did not

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**Table 1 (abstract M279)**

<table>
<thead>
<tr>
<th></th>
<th>Corn silage</th>
<th>Small grains silage</th>
<th>Alfalfa haylage</th>
<th>Alfalfa hay</th>
<th>By-products</th>
<th>Protein meals</th>
<th>Energy feeds</th>
<th>Lactation TMR</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP, mL</td>
<td>78.4$^a$</td>
<td>115.2$^a$</td>
<td>79.9$^a$</td>
<td>85.2$^a$</td>
<td>100.8$^b$</td>
<td>104.3$^b$</td>
<td>73.5$^c$</td>
<td>74.5$^c$</td>
<td>8.13</td>
</tr>
<tr>
<td>FR, %h</td>
<td>23.6$^a$</td>
<td>18.5$^a$</td>
<td>27.4$^a$</td>
<td>18.7$^b$</td>
<td>18.1$^c$</td>
<td>17.4$^c$</td>
<td>14.9$^c$</td>
<td>22.8$^c$</td>
<td>2.65</td>
</tr>
<tr>
<td>SP, mL</td>
<td>236.7$^a$</td>
<td>230.9$^a$</td>
<td>158.4$^b$</td>
<td>151.1$^a$</td>
<td>169.2$^b$</td>
<td>109.4$^b$</td>
<td>174.0$^b$</td>
<td>176.5$^b$</td>
<td>10.9</td>
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<tr>
<td>SR, %h</td>
<td>4.65$^bc$</td>
<td>4.17$^d$</td>
<td>4.57$^d$</td>
<td>4.87$^d$</td>
<td>4.84$^d$</td>
<td>4.41$^d$</td>
<td>3.43$^c$</td>
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<td>251.6$^bc$</td>
<td>260.5$^a$</td>
<td>209.1$^bc$</td>
<td>202.8$^b$</td>
<td>239.2$^bc$</td>
<td>203.1$^c$</td>
<td>232.4$^d$</td>
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<td>FP:SP</td>
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<td>0.51$^c$</td>
<td>0.51$^c$</td>
<td>0.57$^b$</td>
<td>0.62$^b$</td>
<td>0.95$^a$</td>
<td>0.43$^c$</td>
<td>0.43$^c$</td>
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</tr>
<tr>
<td>TDMD, %</td>
<td>73.4$^d$</td>
<td>63.7$^e$</td>
<td>81.9$^c$</td>
<td>78.0$^a$</td>
<td>82.5$^b$</td>
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<td>91.5$^c$</td>
<td>79.4$^a$</td>
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</tr>
<tr>
<td>VFA, mM</td>
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<td>21.4$^d$</td>
<td>25.8$^b$</td>
<td>24.7$^c$</td>
<td>28.3$^b$</td>
<td>29.1$^b$</td>
<td>31.2$^c$</td>
<td>24.1$^d$</td>
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<td>Acetate, %</td>
<td>38.6$^d$</td>
<td>48.4$^a$</td>
<td>56.6$^a$</td>
<td>55.7$^a$</td>
<td>45.4$^b$</td>
<td>43.9$^b$</td>
<td>28.4$^a$</td>
<td>40.2$^d$</td>
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<td>Propionate, %</td>
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<td>30.6$^d$</td>
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<td>25.3$^e$</td>
<td>33.3$^c$</td>
<td>31.3$^c$</td>
<td>41.1$^a$</td>
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<td>Butyrate, %</td>
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<td>15.1$^d$</td>
<td>9.9$^e$</td>
<td>11.3$^e$</td>
<td>16.1$^c$</td>
<td>13.7$^c$</td>
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<td>1.11</td>
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<tr>
<td>MPS, mg</td>
<td>138$^d$</td>
<td>162$^a$</td>
<td>273$^bc$</td>
<td>275$^b$</td>
<td>212$^c$</td>
<td>320$^b$</td>
<td>249$^bc$</td>
<td>270$^bc$</td>
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<td>YATP, mL/g of DM</td>
<td>41.1$^ab$</td>
<td>42.6$^a$</td>
<td>47.5$^a$</td>
<td>43.6$^a$</td>
<td>36.2$^bc$</td>
<td>37.1$^{abc}$</td>
<td>26.8$^a$</td>
<td>31.5$^d$</td>
<td>4.21</td>
</tr>
</tbody>
</table>

$^a$–$^c$Values with different superscripts within the same row differ at $P < 0.05$.
change according to the RDP:RUP ratio. Similar interaction results were found for HCT and lactose content. Cows fed low RDP:RUP ratio had higher fat-corrected milk, milk protein content, and efficiency of N utilization for milk protein yield. Thus, diets with GC and high RDP:RUP ratio reduces MY and milk quality. For SFC corn diets the RDP:RUP ratio does not affect the lactation performance. Overall, feeding cows with low RDP:RUP ratio results in higher efficiency of N utilization to milk protein yield.

**Key Words:** casein, dairy industry, milk stability

**M281 Impact of dietary starch concentration formulated with two types of corn silage on methane and ammonia emissions in dairy cows.** J. I. Sanchez-Duarte*1, K. F. Kalscheur2, and J. M. Powell2, 1South Dakota State University, Brookings, SD, 2US Dairy Forage Research Center, USDA, ARS, Madison, WI.

The objective of this study was to evaluate methane (CH4) and ammonia (NH3) emissions of lactating dairy cows fed different starch level and corn silage type. After the completion of an 8-wk production study, 48 Holstein cows were allocated to 1 of 4 air-flow controlled chambers (2 cows/chamber) for 6 d in a randomized complete block design. Chamber was the experimental unit. Cows were fed 1 of 4 diets arranged as a 2 × 2 factorial with 2 corn silage hybrids (conventional (CS) and brown midrib (BMR) corn silage) and 2 dietary starch concentrations (19 and 25% of DM). Performance data from the last 6 d and emission measurements last 3 d were recorded and used for analysis. Soyhulls and beet pulp replaced corn grain in the diet to decrease starch concentration. There was no effect of dietary starch concentration and corn silage on DMI, ECM, ECM/DMI, and milk protein percentage, however milk fat percentage was greater (P < 0.03) for cows fed diets formulated at 25% starch rather than diets with 19% starch. An interaction of silage × starch (P < 0.03) was observed for CH4 expressed as per unit of DMI and for MUN. Cows fed CS-25% starch had the lowest MUN. Cows fed BMR-25% starch produced 1.3 g CH4 less per unit of DMI than cows and for MUN. Cows fed CS-25% starch, but were similar to cows fed 21% starch for any silage type. Emissions of CH4 and NH3 (g/d), and CH4/ECM did not differ among treatments. It was concluded that cows fed the BMR-25% starch have the potential to reduce CH4 emissions per unit of DMI even though productive performance was not improved.

**Key Words:** nitrogen use efficiency, iNDF, crude protein

**M282 Forage fiber quality interacts with dietary protein level to determine nitrogen use efficiency.** C. S. Malherbe* and E. Raffrenato, Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa.

The aim of this study was to improve nitrogen use efficiency (NUE) by optimizing the use of dietary protein and higher quality fiber from forage. Four lactating Holstein cows were used in a 4 × 4 Latin square design balanced for carryover effects with a 2 × 2 factorial arrangement of treatments. Treatments were low CP concentration with high NDF digestibility (LpHd), high CP concentration with high NDF digestibility (HpHd), low CP concentration with low NDF digestibility (LpLd) and high CP concentration with low NDF digestibility (HpLd). Crude protein concentrations for the rations were formulated to be 18 and 15% for the Hp and Lp diets, respectively. Diets were formulated around the LpLd diet to satisfy 105% of metabolizable protein requirements. Data were evaluated using the Mixed procedure in SAS with cow and period as random effects and diet as fixed effect. The indigestible NDF, as % of the NDF, of the 2 out hays used were 40.80% for Ld hay and 31.54% for the Hd hay and wheat straw was included in the Ld diets to obtain iso-NDF diets. Cows were fed ad libitum for 14 d with data collection over the last 4 d of each period. Dry matter intake (DMI) for 3 of the diets were found to be similar, with the exception of the LpLd diet having lower DMI (P < 0.001) than the other 3 diets, showing how protein can counteract the lower forage quality by stimulating fibrolytic bacteria. Energy corrected milk yield decreased (P < 0.01) when feeding less CP, specifically of 2.46 kg/d for the Hd forage diet and 3.00 kg/d for Ld forage diet. However, nitrogen use efficiency was the highest (P < 0.05) for the lower protein diet in combination with Hd forage (32.3%). We conclude that lowering protein improved NUE significantly, probably due to higher N recycling by the animals, with forage digestibility and iNDF contributing to the level of improvement.

**Key Words:** nitrogen use efficiency, iNDF, crude protein

**M283 Predicting rumen passage rate of NDF fractions in lactating dairy cows.** J. R. R. Dórea*1, E.B. Alves2, and D. K. Combs3, 1University of Wisconsin, Madison, WI, 2Federal University of Lavras, Lavras, MG, Brazil.

Measures of passage rates (kp) of potentially digestible NDF (pdNDF) and indigestible NDF (iNDF) are needed to model the process of rumen fiber digestion. Our objective was to develop and validate models to predict kp of iNDF and pdNDF. Nineteen flow influx studies with lactating dairy cows were compiled (n = 73, treatment means). Three empirical models to predict kp iNDF were developed. The following parameters were included in all 3 models: milk yield (MY, kg/d), DIM, iNDF incubation time (h), diet NDF (%). The 3 models differed by one parameter: Model 1 (M1) also included diet iNDF:NDF ratio, Model 2 (M2) included diet iNDF(%), and Model 3 (M3) included ratio of diet concentrate proportion to diet NDF. Models were validated with an independent data set from 2 experiments (n = 64, individual animals). M1, M2 and M3 were used to predict kp iNDF of individual animals in the independent data set. The relationship between kp iNDF and kp pdNDF was best described with a segmented regression with kp iNDF as an independent variable and kp pdNDF the dependent variable. When kp iNDF <2.45%/h, kp of pdNDF was constant, but when kp iNDF >2.45%/h, a regression: kp pdNDF = −0.011 + 0.942 x kp iNDF (%/h) described the passage rates of iNDF and pdNDF. Concordance correlation coefficient (CCC) of kp iNDF predictions and observed kp iNDF was 0.40, 0.44, and 0.35, for M1, M2, and M3, respectively. When kp pdNDF calculated from M1, M2, and M3 was compared with observed, CCC was 0.33, 0.36, and 0.26, respectively. Rumen NDF digestibility

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**Table 1 (abstract M281).**

<table>
<thead>
<tr>
<th>Item</th>
<th>CS 19%</th>
<th>25%</th>
<th>SEM</th>
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</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>25.4</td>
<td>25.1</td>
<td>24.9</td>
<td>25.9</td>
</tr>
<tr>
<td>ECM, kg/d</td>
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<td>44.7</td>
<td>40.8</td>
<td>44.6</td>
</tr>
<tr>
<td>ECM/DMI</td>
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<td>1.79</td>
<td>1.65</td>
<td>1.74</td>
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<tr>
<td>Fat, %</td>
<td>3.91</td>
<td>4.07</td>
<td>3.68</td>
<td>4.02</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.04</td>
<td>3.10</td>
<td>3.05</td>
<td>3.08</td>
</tr>
<tr>
<td>MUN, mg/dL</td>
<td>11.9</td>
<td>10.8</td>
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<td>11.8</td>
</tr>
<tr>
<td>CH4, g/d</td>
<td>503.0</td>
<td>515.9</td>
<td>494.0</td>
<td>497.4</td>
</tr>
<tr>
<td>NH3, g/d</td>
<td>22.7</td>
<td>27.6</td>
<td>23.4</td>
<td>23.1</td>
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<tr>
<td>CH4/DMI</td>
<td>19.8</td>
<td>20.5</td>
<td>19.9</td>
<td>19.2</td>
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<tr>
<td>CH4/ECM</td>
<td>11.5</td>
<td>11.6</td>
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**Table 2 (abstract M281).**

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<td>25.4</td>
<td>25.1</td>
<td>24.9</td>
<td>25.9</td>
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<tr>
<td>ECM, kg/d</td>
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<td>44.7</td>
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<td>Protein, %</td>
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<tr>
<td>CH4, g/d</td>
<td>503.0</td>
<td>515.9</td>
<td>494.0</td>
<td>497.4</td>
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<tr>
<td>NH3, g/d</td>
<td>22.7</td>
<td>27.6</td>
<td>23.4</td>
<td>23.1</td>
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<td>CH4/ECM</td>
<td>11.5</td>
<td>11.6</td>
<td>12.1</td>
<td>11.2</td>
</tr>
</tbody>
</table>

Mean differences (P < 0.05).

1S = starch effect; I = silage by starch effect; NS = not significant.

**Key Words:** BMR corn silage, milk, gas emissions
was calculated as \( \text{kd}/(\text{kd} + \text{kp}) \times \text{pdNDF} \), where \( \text{kp} \) was predicted by M2 and adjusted by the segmented regression, \( \text{kd} \) (degradation rate) was estimated by in vitro incubation (24, 48, and 72 h), and \( \text{pdNDF} \) was estimated using 240 h in situ incubation. When M2-predicted NDF digestibility was compared with observed, CCC and root mean square error of prediction (RMSEP) was 0.60 and 7.6%, respectively. When an equation using kp \( \times \text{NDF} \) was used (Kriszan et al., 2010), CCC and RMSEP were 0.51 and 8.0%, respectively. These data suggest that kp \( \times \text{NDF} \) can increase the accuracy and precision of rumen NDF digestibility predictions.

**Key Words:** fiber digestion, fiber passage, iNDF

**M284** Effect of contrasting predicted residual feed intake on performance and \( \text{CH}_4 \) emission of dairy cows fed 2 levels of forage neutral detergent fiber. M. Aguerre*1, F. Sun2, J. M. Powell1, K. Weigel3, A. Pelletier3, P. Crump3, and M. Wattiaux2, 1Animal and Veterinary Science Department, Clemson University, Clemson, SC, 2Dairy Science Department, University of Wisconsin-Madison, Madison, WI, 3U.S. Dairy Forage Research Center, Madison, WI, 4Soils Science Department, University of Wisconsin-Madison, Madison, WI, 5Department of Computing and Biometry, University of Wisconsin-Madison, Madison, WI.

The objective of this study was to determine the effects of selecting cows with contrasting predicted residual feed intake (RFI) on animal performance and \( \text{CH}_4 \) emission and whether cow responses were affected by dietary forage neutral detergent fiber (NDF) level. Mid-lactation multiparous Holstein cows (n = 24) with contrasting predicted RFI were selected from a cohort of 47 cows using genotypic and phenotypic data from previous studies and grouped as low RFI (mean ± SD: 0.42 ± 0.13 kg/d) and high RFI (0.34 ± 0.11 kg/d), respectively. Half of the cows in each RFI group were randomly assigned to a dietary treatment with either 22% or 30% forage NDF in a randomized complete block design. Following a 2-wk covariate period, cows were fed their assigned treatment diets for 4 weeks. Gas emission measurements were conducted in 4 tie-stall emission chambers during 3 consecutive days the last week of the covariate and experimental periods. Results are covariate-adjusted least squares means (±SEM). There was no RFI by forage NDF level interaction (\( P > 0.05 \)) for any of the measured variables. A tendency (0.05 < \( P < 0.10 \)) was observed for higher MUN and \( \text{CH}_4/\text{DMI} \) for high RFI-high forage NDF. Except for fat yield (1.74 vs. 1.50 ± 0.06 kg/d for low and high RFI, respectively), predicted RFI did not affect any measured responses. Increasing forage NDF reduced (\( P < 0.05 \)) milk yield (44.3 vs. 40.6 ± 0.74 kg/d), fat-and-protein corrected milk (FPCM; 43.5 vs. 38.0 ± 1.24 kg/d), fat (1.77 vs. 1.51 ± 0.06 kg/d) and milk true protein yield (1.28 vs. 1.10 ± 0.04 kg/d). Greater forage NDF tended (\( P = 0.08 \)) to decrease FPCM/DMI (1.56 vs. 1.43 ± 0.05), but increased MUN (11.2 vs. 12.7 ± 0.54 mg/dl) and \( \text{CH}_4 \) emission (533 vs. 564 ± 19.7 g/d). Forage NDF level did not affect DMI (27.0 ± 0.74 kg/d), milk/DMI (1.58 ± 0.36), \( \text{CH}_4/\text{DMI} \) (23.5 ± 0.77 g/kg), \( \text{CH}_2/\text{milk} \) (15.4 ± 0.75 g/kg), and \( \text{CH}_4/\text{FPCM} \) (14.9 ± 0.72 g/kg). Under the conditions of this study, selecting cow with lower RFI (presumably higher efficiency) had negligible effects on animal performance and \( \text{CH}_4 \) emission. Increasing forage NDF level has negative impacts on animal performance and \( \text{CH}_4 \) emission.

**Key Words:** greenhouse, forage, residual feed intake

**M285** Effects of supplementing active dry yeast, a blend of probiotic bacteria, or a combination of both on rumen fermentation profiles and nutrient digestion in continuous rumin

fermentors. Y. Liang*, E. Davis, and M. A. Ballou, Texas Tech University, Department of Animal and Food Sciences, Lubbock, TX.

This study evaluated the effects of supplementing yeast, probiotic bacteria, or a combination on rumen fermentation and nutrient digestion in continuous rumen fermentors. Rumen fluid (10 L) from a mid-lactation fistulated Holstein cow was collected and used to inoculate eight 1-L glass continuous culture fermenters. Fermentors were randomly assigned to 1 of 4 dietary treatments in a 2 × 2 factorial arrangement, including: Control, no supplement; Yeast, supplemented with 3.6 × 10^11 cells/ml Saccharomyces cerevisiae; Probiotic, supplemented 5.4 × 10^11 cells/ml of a blend of Enterococcus faecium and Lactobacillus casei; or Combination, supplemented with both the Yeast and Probiotic treatments. The study was conducted in 2 blocks with 8 fermentors / block (n = 4). Fermentors were fed 20 g twice daily. Artificial saliva was added for a 6% dilution/h. Samples were collected from each fermentor on d 5 and 7 at 0, 2, 4, 6, 8, and 12 h relative to the AM feeding and pH recorded and samples collected for volatile fatty acids. On d 5, 6, and 7 total collection of the 24-h effluent was used to determine digestibility of DM and NDF. Data were analyzed as a repeated measures for a 2 × 2 factorial design using the Mixed procedure of SAS with period included as a random effect. Data are reported as Control, Yeast, Probiotic, and Combination throughout. There were no treatment × time or treatment effects (\( P ≥ 0.582 \)) on pH (6.24, 6.23, 6.19, and 6.21 ± 0.081). Additionally, there were no treatment × time or treatment effects on concentrations of acetate (\( P ≥ 0.237 \); 62.2, 63.7, 64.6, and 63.8 ± 0.99 mM), propionate (\( P ≥ 0.263 \); 24.6, 25.8, 24.9, and 26.0 ± 1.48 mM), or total volatile fatty acids (\( P ≥ 0.450 \); 109.7, 113.4, 113.1, and 114.6 ± 4.78 mM). There was a tendency for yeast supplementation (\( P = 0.067 \); 51.5, 56.8, 53.1, and 54.1 ± 1.54%) to increase DM digestion. There was no treatment effects on digestion of NDF (\( P ≥ 0.715 \); 56.9, 59.6, 59.0, and 59.0 ± 4.07%). These data indicate that supplementing active dry yeast may improve DM digestion.

**Key Words:** probiotic, rumen, yeast

**M286** Performance of dairy cows fed conventional sorghum or corn silages compared to brown midrib sorghum silage: A meta-analysis. J. I. Sanchez-Duarte*1, K. F. Kalscheur2, A. D. Garcia1, and F. E. Contreras-Govea3, 1South Dakota State University, Brookings, SD., 2US Dairy Forage Research Center, USDA, ARS, Madison, WI, 3University of Wisconsin, Madison, WI.

A meta-analysis was conducted to compare the effects of feeding dairy cows conventional sorghum (CSS) or corn silages (CCS) vs. brown midrib sorghum silage (BMRSS) on dry matter intake (DMI), milk yield, and milk composition. Data from 9 published articles (1984–2015) were used to contrast CSS (7 comparisons; 104 cows) or CCS (13 comparisons; 204 cows) vs. BMRSS. Statistical analysis was performed using fixed or random effects models in R. The degree of heterogeneity was measured with \( I^2 \) statistic and publication bias was determined with funnel plots and Egger’s regression test. Other sources of heterogeneity of response were analyzed through meta-regression. Estimated effect size was calculated for DMI, milk yield, and milk composition. No evidence of publication bias was observed for all variables tested. DMI and milk yield had the highest (\( I^2 = 41.5 \) [CSS vs. BMRSS]; \( I^2 = 72.6\% \) [CCS vs. BMRSS]) and lowest (\( I^2 = 0\% \)) degree of heterogeneity, respectively. With CSS, cows fed BMRSS tended to increase DMI (0.83 kg/d; \( P = 0.09 \)), and significantly increased milk yield (1.64 kg/d; \( P < 0.001 \)), milk fat (0.09%; \( P = 0.03 \)), and yields of milk fat (0.08 kg/d; \( P < 0.001 \)), protein (0.04 kg/d; \( P < 0.001 \)), and lactose (0.16 kg/d; \( P = 0.02 \)). No differences were observed for milk protein and lactose percentage (\( P > 0.05 \)). Compared with CCS, cows...
fed BMRSS increased milk fat (0.10%; $P = 0.009$), but decreased milk protein (0.06%; $P = 0.03$). There were no effects on DMI, milk yield, yields of milk fat, protein, and lactose, and lactose percentage between CCS and BMRSS. Meta-regression indicated that days in milk affected DMI and milk production when CSS was compared with BMRSS and DMI when CCS was compared with BMRSS. Overall, lactation performance improved when cows were fed diets formulated with BMRSS compared with cows fed diets formulated with CSS; however, performance was not different between cows fed BMRSS and CCS. Future research comparing BMRSS with CSS or CCS needs to consider days in milk because cows respond differently throughout their lactation according to meta-regression analysis.

Key Words: sorghum silage, corn silage, meta-analysis

M287 Effects of experimental design and protein substitution strategy on production responses to feeding different levels of protein to primiparous dairy cows. G. I. Zanaton*, USDA-Agricultural Research Service; Dairy Forage Research Center, Madison, WI.

The objective of this study was to evaluate the effects of reducing crude protein (CP) and CP substitution strategy on performance when primiparous dairy cows were fed diets continuously or according to a change-over experimental design. Fifty-four primiparous, Holstein cows were randomly assigned to either a randomized complete block design (CONT; $n = 36$, initial mean ± SD: $129 ± 36$ DIM, $580 ± 40$ kg BW, $44.1 ± 3.2$ kg milk) or to a replicated, $3 	imes 3$ Latin square design balanced for the effects of previous treatment (CHANGE; $n = 18$, initial mean ± SD: $129 ± 35$ DIM, $583 ± 39$ kg BW, $44.4 ± 3.7$ kg milk). Experimental designs were run concurrently with three 28-d periods and sampling on d 22–28 of each period. Cows were milked 3× daily and were individually fed once daily a diet that was predicted to be either adequate (ADMP; 16.7%CP, 28.3%NDF, 25.5% starch) or deficient (LOMP) in metabolizable protein (MP). ADMP contained expellers soybean meal, whereas this was removed in LOMP diets and replaced with either dry ground corn (STARCH; 14.9%CP, 28.1%NDF, 28.6% starch) or soyhulls (FIBER; 15.1%CP, 30.7%NDF, 25.4% starch) for a total of 3 diets. Contrasts for the effects of MP (ADMP vs LOMP) and carbohydrate source (CHO: STARCH vs FIBER) were evaluated for both experimental designs, with $P < 0.05$ significant and $P < 0.10$ trends. DMI was greater for ADMP than LOMP in CONT, whereas DMI only tended to be greater in CHANGE. Yield of milk and protein were greater for ADMP in both designs whereas yield of fat was greater for ADMP in CHANGE, but only tended to be greater for ADMP in CONT. Within CONT, CHO did not affect DMI, milk, or component yield; however within CHANGE, cows fed STARCH produced more milk and tended to produce more protein. This discrepancy was not due to statistical power of the designs because the results were directionally opposite from CONT where cows fed FIBER had yields that were numerically, though not significantly, greater. Inferences about MP status were generally similar for production measures in both designs whereas inferences about CHO were affected by experimental design.

Key Words: experimental design, protein, carbohydrates

M288 Direct and indirect causal effects of dietary starch on fiber digestibility. J. R. Dórea*, G. J. M. Rosa, and D. K. Combs, University of Wisconsin, Madison, WI.

Depression in fiber digestibility in lactating dairy cows has been widely associated with increases in dietary starch (DS) due to reductions in rumen pH. However, greater starch intake (SI) can increase fiber passage rate (kp) that may also depress NDF digestibility. The objective of this study was to infer if the depression in NDF digestibility associated with DS is due to pH or fiber kp. Two structural equation models were built using 2 research trials ($n = 64$, Latin square design) in which DS, SI, total-tract NDF digestibility (TTNDFD), pH, and kp of potentially digestible NDF (pdNDF) were measured. All variables were adjusted for the fixed effect of diet and random effects of period within square, cow within square, and trial. The first model (M1) tested the direct effects of DS on TTNDFD and SI on TTNDFD, and the indirect effect of DS on TTNDFD. The indirect effect of DS on TTNDFD was composed by the effects of DS on SI, SI on pH, and then pH on TTNDFD. The same approach was used in the second model (M2), but pH was replaced with kp pdNDF. Results (Table 1) suggest that there is an indirect effect of DS on TTNDFD through the increase in kp pdNDF caused by greater SI ($P < 0.05$). However, there is no evidence of causal effect of DS on TTNDFD due to changes in rumen pH ($P > 0.05$). The chi-squared test ($\chi^2$), comparative fit index (CFI), Akaike information criteria (AIC), and root mean square of the approximation (RMSEA) for M1 were: $\chi^2 (P = 0.07)$, CFI = 0.89, AIC = 685, and RMSEA = 0.31, while for M2 were: $\chi^2 (P = 0.95)$, CFI = 1.0, AIC = 660, and RMSEA < 0.001. Increase in passage rate is the main factor that depresses TTNDFD when SI increases in diets with DS ranging from 16 to 27%.

Table 1 (abstract M288). Direct and indirect effects of dietary starch (DS, % of DM) on total tract NDF digestibility (TTNDFD, %)

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<tr>
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<td>0.11</td>
<td>&lt;0.001</td>
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</table>

SI = starch intake (kg/d), pH = rumen pH, kp pdNDF = passage rate of potentially digestible NDF (%/h).

Key Words: pH, starch, fiber

M289 Physical characterization of fat supplements highly enriched in palmitic and stearic acid. R. P. Shepardson*, E. Bazileyskaya, and K. J. Harvatine, Penn State University, University Park, PA.

Fatty acid (FA) supplements are widely used in lactating cow diets to increase energy intake. Previous published research has reported that supplements with moderate enrichment (~85%) of palmitic acid have expected digestibility, while very high enrichments (~98%) have lower digestibility. Saturated FA have the potential to form organized secondary structures at high purity. Differential scanning calorimetry (DSC) is a thermal technique commonly used in material science to measure the change in heat flow as energy is absorbed or released from a sample during heating. Our hypothesis was that a supplement with a very high enrichment would differ in physical characteristics due to the formation of a secondary structure, which may contribute to a decreased digestibility. A 98% stearic acid (SA; 98.5% C18:0, 0.4% C16:0), 98% palmitic acid (PA; 98.5% C16:0, 0.7% C18:0), and a mixture of palmitic and stearic (PA/SA; 54.5% C16:0, 44.5% C18:0) sample were used for this

Key Words: experimental design, protein, carbohydrates
M290 Circulating blood metabolites in early lactation dairy cows fed canola or soybean meals. S. A. E. Moore*1 and K. F. Kalscheuer2, 1University of Wisconsin, Madison, WI, 2US Dairy Forage Research Center, USDA-ARS, Madison, WI.

A successful transition from pregnancy to lactation is imperative for dairy cows to maximize milk production potential. Altering the dietary protein source can change the availability of energy and protein to the cow. The objective of this experiment was to evaluate the effect of CP source [canola meal (CM) or soybean meal (SBM)] and CP concentration [HI (18.1%) or LO (16.2%) DM basis] on circulating blood metabolites. At calving, multiparous Holstein cows (n = 79) were enrolled into a 2 × 2 factorial arrangement of treatments in a randomized complete block design. Cows were blocked by calving date and individual cow was the experimental unit. Enzymatic colorimetry was used to evaluate circulating concentrations of glucose, nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHB), triglycerides (TG), and plasma urea nitrogen (PUN). Serum and plasma coccygeal vein samples were collected 2 × during wk 1, 2, 3, 4, 6, and 8 postpartum. Samples were pooled by wk for each cow. Data were analyzed using the MIXED procedure of SAS. BCS and BW at calving were used as covariates when appropriate. Total milk yield was greater for CM-fed than SBM-fed cows during wk 1–8 of lactation (mean ± SEM; 53.2 vs 49.2 ± 0.98 kg/d; P = 0.001), while there was no difference in DMI (P = 0.11) to support additional production. No treatment effect was observed for glucose or BHB. Circulating TG concentration was greater for cows fed CM compared with SBM-fed cows (0.125 vs 0.118 ± 0.002 mM; P = 0.02). There was an interaction of source and wk for NEFA concentration (P = 0.04). Efficiency of nitrogen utilization favored CM vs SBM-fed cows for both circulating PUN (0.37 vs 0.40 ± 0.11 mM; P = 0.02) and concentration of milk urea N (MUN; 10.7 vs 11.4 ± 0.24 mg/dL; P = 0.04). HI-fed cows were greater in PUN (0.44 vs 0.33 ± 0.01 mM; P < 0.001) and MUN concentration (9.64 vs 12.5 ± 0.24 mg/dL; P < 0.001) compared with LO-fed cows. The increase in milk yield can be attributed in part, to an increase in circulating TG and nitrogen utilization. However, further investigation into the CM vs SBM milk disparity in early lactation is needed.

Key Words: canola meal, early lactation, energy

M291 Effect of supplementing rumen-protected methionine pre- and postpartum on milk yield and components of dairy cows during early lactation. M. L. Stangaferro*1, M. M. Perez1, M. Masello1, R. Wijma1, M. E. Van Amburgh1, T. R. Overton1, D. Luchini1, M. C. Wiltbank2, R. D. Shaver2, and J. O. Giordano1, 1Cornell University, Ithaca, NY, 2University of Wisconsin-Madison, Madison, WI, 3Adisseo USA Inc., Alpharetta, GA.

The objective of this study was to assess the effect of feeding rumen-protected methionine (RPM) pre- and postpartum on milk yield (MY) and composition up to 14 wk of lactation. Three weeks before calving, multiparous Holstein cows (n = 211) were randomly assigned to a control (CON) or RPM group, whereby the only difference in the diets was the inclusion rate of Smartamine M pre- (PreP) and postpartum (PostP). Methionine feeding (MP Met as % of MP) in the diet were 2.76 vs 3.35 PreP, and 2.10 vs 2.68 PostP, for CON and RPM, respectively. Diets were formulated using CNCPS v7 at DMI of 12.7 and 24.4 kg of DM/cow/d PreP and PostP, respectively. Diets were isoenergetic, and provided the same lysine supply (9.73 PreP and 7.10% of MP PostP, respectively), with a Lys:Met ratio of 3.5 vs 2.9 for CON and RPM PreP, and 3.4 vs 2.7 for CON and RPM PostP. Cows were allocated to pens (1 pen per group PreP, and 6 pens of 16 cows per group PostP), and the diets were fed to pens. Milk samples were collected at wk 1 and 2, and then biweekly until 14 weeks PostP. Data were analyzed by ANOVA with repeated measurements using PROC MIXED of SAS, with treatment (trt), pen(trt), week and trt by week as fixed effects and cow(pen trt) as a random effect. Supplementing RPM PreP and PostP increased milk true protein percentage from wk 2 to 14 (2.9 vs. 3.0%; P < 0.01), milk fat percentage from wk 8 to 14 (3.5 vs. 3.7%; P < 0.01), total solids percentage from wk 6 to 14 (12.2 vs. 12.5%; P < 0.01), milk urea nitrogen for all 14 weeks (8.42 vs. 8.82 mg/dL; P < 0.01), and tended to increase true protein yield (1.44 vs. 1.48 kg/d; P = 0.09). Feeding RPM tended to reduce milk lactose percentage (4.84 vs. 4.80%; P = 0.07). No effect was observed on MY (48.5 vs. 47.9 kg/d; P = 0.61), energy corrected milk (ECM, 49.9 vs. 50.5 kg/d; P = 0.31), milk fat yield (1.85 vs. 1.90 kg/d; P = 0.12), milk lactose yield (2.37 vs. 2.33 kg/d; P = 0.30), and total solids yield (6.14 vs. 6.17 kg/d; P = 0.61) for CON and RPM, respectively. Supplementing RPM pre- and postpartum improved lactation performance by increasing true protein percentage and yield, fat percentage, and total solids percentage, but did not affect total MY or ECM.

Key Words: methionine, milk production, protein

M292 Methane mitigation with corn oil and calcium sulfate, responses on whole animal energy and nitrogen balance in dairy cattle consuming reduced-fat distillers grains plus solubles. J. V. Judy*1, T. M. Brown-Brandl2, S. C. Fernando1, and P. J. Kononoff1, 1University of Nebraska-Lincoln, Lincoln, NE, 2USDA, ARS, US Meat Animal Research Center, Clay Center, NE.

Addition of fat and calcium sulfate to diets fed to ruminants has been shown to reduce methane production, but these factors have not shown effects on energy balance. A study using indirect calorimetry and 16 multiparous (8 Holstein and 8 Jersey) (78 ± 15 DIM; mean ± SD) lactating dairy cows was conducted to determine how mitigating methane by adding corn oil or calcium sulfate to diets containing reduced-fat distillers grains, affect energy and nitrogen balance in dairy cattle. A replicated 4 × 4 Latin square design with 35 d periods (28 d adaption and 7 d collections) was used to compare 4 different dietary treatments. Treatments were composed of a control (CON) diet which did not contain reduced-fat distillers grain plus solubles (RFDDGS), and treatment diets containing 20% (DM basis) RFDDGS (DG), 20% RFDDGS with 1.38% (DM basis) added corn oil (CO), and 20% RFDDGS with 0.93% (DM basis) added calcium sulfate (CaS). Addition of CaS reduced (P = 0.02) and addition of CO tended (P = 0.17) to reduce methane production compared with CON diet (421.6, 429.5, 394.7, and 381.4 ± 14.41
L/d for CON, DG, CO, and CaS respectively). Digestible energy was greater (P < 0.01) for DG and CO treatments compared with CON and CaS treatments (57.2, 61.5, 61.4, and 58.5 ± 1.13 for CON, DG, CO, and CaS, respectively). Metabolizable energy was greater (P < 0.01) in treatments containing RFDDGS compared with CON (50.5, 54.8, 55.0, and 52.3 ± 1.07 for CON, DG, CO, and CaS, respectively). Net energy of lactation per unit of DMI was greater in DG and CO than CON (1.44, 1.52, and 1.33 ± 0.04 Mcal/kg for DG, CO, and CON, respectively). Nitrogen balance was greater (P = 0.03) in DG than CO (91.1 vs 56.6 g/d for DG and CO, respectively). Added oil and calcium sulfate to diets containing RFDDGS may be a viable option to reduce methane emissions without affecting energy balance in lactating dairy cows.

**Key Words:** dried distillers grains and solubles, energy, methane

### M293

**Calves fed with oregano and green tea extracts alter slightly their blood redox status.** V. Fischer*1, M. de Paris1, S. C. B. Stivanin1, E. F. Vizzotto1, M. B. Zanela2, C. Klein1, V. Stone1, and C. J. Dairy Sci. Vol. 100, Suppl. 2

This work aimed to investigate the effect on antioxidant status in dairy calves fed milk with *Origanum vulgare* or *Camellia sinensis* extracts. Twenty-four calves were randomly assigned to one of the following treatments: control (CO, no plant extract), green tea extract (GTE), and oregano extract (OEG). Blood samples were collected by jugular vein puncture on d 1, 30, and 60 of life and further analyzed for redox status parameters. Data were submitted to variance analysis, using the Mixed procedure of SAS according to a completely randomized design, considering the effect of diet (n = 3), period (n = 3), diet by period interaction, and calves as random effect. Overall, calves of OEG group showed higher values for glutathione peroxidase (GPXs) activity than calves of GTE and OE groups. GPXs of the OEG group tended to show higher values for superoxide dismutase (SOD) activity than calves of GTE group. There were no differences for the remaining parameters evaluated between groups. On d 30 calves from groups GTE and OEG showed lower values for DCFP than calves of GTE and OE groups. (2111, 3242, 3468 nmol/mg, P < 0.05). Calves fed milk with green tea or oregano extracts show little changes in the blood redox status.

**Key Words:** plant extract, pre-weaning calf, redox

### M294

**Effects of a pulse dose of propionate on metabolic response in lactating dairy cows during the postpartum period.** K. M. Kennedy* and M. S. Allen, Michigan State University, East Lansing, MI.

Our long-term hypothesis is that hepatic oxidation of acetyl CoA is stimulated by anaplerosis of the tricarboxylic acid (TCA) cycle by propionate, causing an increase in hepatic energy charge. The objective of this study was to determine potential bottlenecks associated with propionate metabolism. Six cows (4 to 18 d postpartum) were used in a crossover design (3 d with 1 d rest). Cows were blocked from feed 1 h before treatment and received a pulse dose to the rumen of either 500 mL of water (control) or 2.0 moles of propionic acid (PA) in a 500 mL solution. Plasma and liver samples were collected immediately before dosing (T0) and at 10 (T10) and 20 (T20) min post-dosing. Plasma samples were analyzed for acetyl CoA (A-CoA), propionyl CoA (P-CoA), methylymalonyl CoA (M-CoA), succinyl CoA (S-CoA), succinate, fumarate, and malate and plasma was analyzed for propionate. Samples were standardized relative to T0 (T0 = 0). Data were analyzed with the Proc Mixed procedure in SAS (v.9.4). The PA treatment increased plasma propionate at T10 compared with control but propionate declined rapidly (interaction P < 0.04). PA tended to decrease A-CoA, did not affect P-CoA, tended to increase M-CoA and S-CoA, and increased succinate, fumarate and malate. Although interactions of treatment and time were not detected for liver metabolites, M-CoA and S-CoA were greater at T20 than T0 (P = 0.01 for both) and numerically greater than T10 indicating possible bottlenecks for metabolism of M-CoA and S-CoA.

**Table 1 (abstract M293).**

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Green Tea</th>
<th>Oregano</th>
</tr>
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<tbody>
<tr>
<td>SOD1</td>
<td>26.2 ± 1.63a</td>
<td>23.3 ± 1.68b</td>
<td>28.8 ± 1.86c</td>
</tr>
<tr>
<td>CAT1</td>
<td>3.45 ± 0.28a</td>
<td>3.35 ± 0.29a</td>
<td>3.31 ± 0.32a</td>
</tr>
<tr>
<td>GPX1</td>
<td>9.84 ± 0.7b</td>
<td>7.38 ± 0.7b</td>
<td>7.03 ± 0.8b</td>
</tr>
<tr>
<td>GSH2</td>
<td>0.07 ± 0.02a</td>
<td>0.095 ± 0.02a</td>
<td>0.05 ± 0.02a</td>
</tr>
<tr>
<td>Carbonyl2</td>
<td>1.93 ± 0.16a</td>
<td>1.74 ± 0.16a</td>
<td>2.02 ± 0.18a</td>
</tr>
<tr>
<td>Thiols2</td>
<td>0.63 ± 0.03a</td>
<td>0.66 ± 0.03a</td>
<td>0.70 ± 0.04a</td>
</tr>
</tbody>
</table>

*a,b Means in the same row followed by different letters differ (Lsmeans, P < 0.05) or tend to differ (P < 0.10).

1In U/mg.

2In nmol/mg.

**Table 1 (abstract M294).**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control</th>
<th>2 mol of PA</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T10</td>
<td>T20</td>
<td>T10</td>
<td>T20</td>
</tr>
<tr>
<td>Plasma propionate</td>
<td>−0.001</td>
<td>0.170</td>
<td>5.690</td>
<td>1.545</td>
</tr>
<tr>
<td>A-CoA</td>
<td>0.006</td>
<td>0.214</td>
<td>−0.475</td>
<td>0.063</td>
</tr>
<tr>
<td>P-CoA</td>
<td>1.232</td>
<td>0.206</td>
<td>1.228</td>
<td>0.271</td>
</tr>
<tr>
<td>M-CoA</td>
<td>−0.163</td>
<td>−0.191</td>
<td>0.265</td>
<td>1.655</td>
</tr>
<tr>
<td>S-CoA</td>
<td>−0.223</td>
<td>−0.319</td>
<td>0.132</td>
<td>1.406</td>
</tr>
<tr>
<td>Succinate</td>
<td>−0.048</td>
<td>0.004</td>
<td>0.247</td>
<td>0.488</td>
</tr>
<tr>
<td>Fumarate</td>
<td>−0.021</td>
<td>−0.162</td>
<td>0.419</td>
<td>0.349</td>
</tr>
<tr>
<td>Malate</td>
<td>0.013</td>
<td>−0.130</td>
<td>0.479</td>
<td>0.410</td>
</tr>
</tbody>
</table>

Means (SE)

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M-CoA requires vitamin B12 and its supplementation may improve efficiency of propionate metabolism by alleviating that bottleneck. Our research identified M-CoA and S-CoA as possible metabolic bottlenecks to examine in future work.

Key Words: propionate, metabolism, hepatic oxidation

M295  Milk and methane production in lactating dairy cattle consuming distillers dried grains and solubles or canola meal. M. A. Myers*,1, T. M. Brown-Brandl2, J. V. Judy3, K. J. Herrick3, and P. J. Kononoff3, 1Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE, 2USDA, ARS, US Meat Animal Research Center, Clay Center, NE, 3Poet Nutrition LLC, Sioux Falls, SD.

The use of co-products as an alternative feed source is a common practice when formulating dairy rations. A study using 12 multiparous (79 ± 16 DIM; mean ± SD) lactating Jersey cows, was conducted to evaluate the effects of dried distillers grains with solubles (DDGS) or canola meal (CM) on milk and methane production. A replicated 4 × 4 Latin square design was used to compare 4 different dietary treatments over 4 periods. Each of the 4 periods utilized 23 d for adaptation with 5 d of collection. Treatments were composed of a control (CON) containing corn and soybean meal and no co-products, a treatment diet containing 10% (DM basis) DDGS (DDGS),10% DDGS treatment with an alternative distillers grains source (ADDGS), and a 10% canola meal (CAN) treatment. Co-products were included in partial replacement for corn and soybean meal. Indirect calorimeters were used to sample methane. Dry matter intake and milk yield were similar (P > 0.55) between all treatments averaging 17.5 ± 0.78 kg/d and 24.1 ± 0.80 kg. Milk protein and fat percentage was similar across treatments (P ≥ 0.43) averaging 3.64 ± 0.04% and 6.18 ± 0.17%, respectively. Compared with CON, the addition of DDGS, CAN, or ADDGS did not affect total methane produced (P = 0.54) averaging 340.2 ± 19.59 L/d. When expressing methane per unit of DMI, all treatments were similar (P = 0.75) averaging 19.6 ± 1.26 L/kg/d. Heat production per day of metabolic body weight tended to be lowest in CON (P = 0.06) followed by DDGS, ADDGS and CAN (203.83, 210.73, 215.23, 227.70 ± 6.27 heat production/d/MBW, respectively). Milk urea nitrogen (MUN) was also affected by treatment (P < 0.01), CON and CAN were not different (20.7 and 19.9 ± 0.62 mg/dL, respectively) and DDGS and ADDGS were not different (18.1 and 18.1 ± 0.62 mg/dL, respectively). Results of this study indicate that milk production; milk components and methane production are not adversely affected when feeding the current inclusion rate of common co-products replacing both corn and soybean meal.

Key Words: dried distillers grains and solubles, methane, canola meal

M296  Increasing the diet concentrations of fat and hemicellulose on energy utilization and methane production in lactating Jersey cattle. O. R. Drehmel*1, T. M. Brown-Brandl2, J. V. Judy1, S. C. Fernando1, and P. J. Kononoff3, 1Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE, 2USDA, ARS, US Meat Animal Research Center, Clay Center, NE.

Feeding fat to lactating dairy cows may decrease methane production; however, these factors have not been studied simultaneously. Eight multiparous, lactating Jersey cows averaging 98 ± 30.8 DIM and BW of 439.3 ± 56.7 kg were used in a twice replicated 4 × 4 Latin square to determine the effects of fat and hemicellulose individually and together on energy utilization and methane production using a headbox type indirect calorimetry method. To manipulate the concentration of fat, porcine tallow was included at either 0 or 2% of the diet DM. The concentration of hemicellulose was manipulated by changing the inclusion rate of corn silage, alfalfa hay, and soybean hulls resulting in either 11.3% or 12.7% hemicellulose (DM basis). The factorial arrangement of treatments were low fat low hemicellulose (LFLH), low fat high hemicellulose (LFFH), high fat low hemicellulose (HFLH), and high fat high Hemicellulose (HFHH). Neither fat nor hemicellulose affected DMI (P ≥ 0.25) averaging 16.2 ± 1.18 kg/d across treatments. Likewise, treatments did not affect (P ≥ 0.51) milk production averaging 23.0 ± 1.72 kg/d. The inclusion of fat tended (P = 0.10) to decrease methane produced per kg of DMI from 24.8 to 22.7 ± 1.61 L/kg while hemicellulose had no effect (P = 0.37). Increasing hemicellulose increased (P = 0.01) NDF digestibility from 40.6 to 50.3 ± 2.91%. Methane per unit of digested NDF tended to decrease (P = 0.11) from 64.4 to 46.9 ± 0.70 L/kg with increasing hemicellulose while fat had no effect (P = 0.70). An interaction between hemicellulose and fat content on net energy intake was observed. Specifically, increasing hemicellulose in low fat diets tended (P = 0.08) to increase net energy intake but this was not observed in high fat diets. Results confirm methane production may be decreased with the inclusion of fat while energy intake of lactating dairy cows is improved by increasing hemicellulose in low fat diets.

Key Words: fat, hemicellulose, indirect calorimetry

M297  Increasing the concentration of linolenic acid in diets fed to Jersey cows in late lactation does not affect methane production. J. V. Judy4,1, T. M. Brown-Brandl2, S. C. Fernando1, and P. J. Kononoff3, 1University of Nebraska-Lincoln, Lincoln, NE, 2USDA, ARS, US Meat Animal Research Center, Clay Center, NE.

Although the inclusion of fat has shown to reduce methane production in ruminants, relatively little research has been conducted on comparing the source and profile of fatty acids on methane production in lactating dairy cows. A study using 8 multiparous (325 ± 17 DIM) (mean ± SD) lactating Jersey cows was conducted to determine effects of feeding canola/tallow vs. extruded byproduct containing flaxseed as a fat source on methane emissions and diet digestibility in late lactation dairy cows. A crossover design with 35 d periods (28 d adaption and 7 d collections) was used to compare 2 different fat sources. Diets contained approximately 50% forage mixture of corn silage, alfalfa hay, and brome hay with only the concentrate mixture changing between diets to include either 1) a conventional corn/soybean meal/canola meal with tallow, or 2) a conventional corn/soybean meal diet with an extruded byproduct containing flaxseed (EXF) as the fat source. Diets were balanced to decrease corn and canola meal and replace them with EXF to increase linolenic acid supply (31.2 vs 201.6 g/d) to the rumen. Methane production was measured using headbox-style indirect calorimeters. Milk production was similar (P = 0.38; 17.4 ± 1.04 kg/d) as well as DMI (P = 0.26; 15.4 ± 0.71 kg/d) across treatments. Milk fat was similar (P = 0.87; 5.88 ± 0.25%) and milk protein (P = 0.69; 4.08 ± 0.14%) across treatments. For methane, production was similar (P = 0.90) for total production (21.1 ± 0.7 L/kg). Methane production per unit of DMI was similar (P = 0.34) and averaged 10.5 ± 0.57 L/kg. Similarly, methane production per unit of energy corrected milk was similar (P = 0.30) for fat source and averaged 7.01 ± 0.68 L/kg. Heat production was similar (P = 0.98) averaging 21.1 ± 1.02 Mcal/d. Digestibility of NDF, CP, DM, OM, and starch were similar (P ≥ 0.22) by diet and averaged 53.6, 73.3, 67.5, 69.9 and 96.1 for NDF,
CP, DM, OM, and starch, respectively. Results indicate that increasing C18:3 may not affect methane emissions or digestibility of the diet.

**Key Words:** linolenic acid, methane, milk

### M298 The effects of feeding a high- or low-plane of milk pre-weaning on IGF-1 and IGFBP in dairy heifers.

J. Haisan1, M. Oba2, D. J. Ambrose2, and M. A. Steele1, 1University of Alberta, Edmonton, AB, Canada, 2Livestock Research Section, Agriculture and Forestry, Edmonton, AB, Canada.

The objective of this experiment was to determine the effects of a high-plane of milk before weaning on plasma insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding proteins (IGFBP) concentrations and their relationship in group-housed calves. Twenty-six female Holstein calves were fed 4 2-L feedings of colostrum for the first 2 d after birth and randomly assigned to either a HIGH (10 L/d; n = 13) or LOW (5 L/d; n = 13) plane of milk. All calves were allowed 2.5 L of pasteurized whole milk per meal until 7 weeks of life before a 10-d weaning transition began, where milk was reduced by 10% per day, resulting in all calves being weaned at 9 weeks. Calf starter and water were provided ad libitum throughout. Pre-weaning average daily gain was 0.90 kg/d for HIGH and 0.65 kg/d for LOW treatments, however, no difference was seen post-weaning. Blood samples were taken starting at 0830 h at 1, 3, 5, 7 and 9 weeks of life and analyzed for IGF-1 and IGFBP-2, -3, -4 and -5. Pre-weaning, IGF-1 concentrations were greater for HIGH than LOW (198.96 vs. 120.39 ng/mL, P < 0.05), with no difference post-weaning. There was no effect of treatment on concentrations of IGFBP-3, -4 or -5 post-weaning. However, a treatment by week interaction existed during the pre-weaning period for IGFBP-3. Overall, from wk 1 to 9, a treatment by week interaction existed for IGFBP-2 and the ratio of IGFBP-3 to IGFBP-2. These results suggest that a high plane of nutrition pre-weaning increased IGF-1 concentration before the weaning transition, and exerts effects on plasma concentrations of IGFBP.

**Key Words:** IGF, IGFBP, dairy calf

### M299 Interactions between levels of flaxseed oil and corn grain particle size on milk fatty acid profile in Jersey cows.

V. Brossillon1, A. F. Brito2, S. F. Reis2, D. C. Moura3, J. G. B. Galvão Jr.4, C. Côrtes1, and A. S. Oliveira5, 1InstitutoFederal de Educação do Rio Grande do Norte, 2Ecole Supérieure d’Agricultures, Angers, France, 3University of New Hampshire, Department of Biological Sciences, Durham, NH, 4Programa de Pós Graduação em Ciência Animal; Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil, 5Instituto Federal de Educação do Rio Grande do Norte, Ipanguaçu, RN, Brazil.

Flaxseed oil (FO) is the richest source of the essential n-3 fatty acid (FA) α-linolenic acid (ALA). We hypothesize that corn of 2 particle sizes [cracked corn (CC) vs. ground corn (GC)] and different degradation rates could interact with FO leading to shifts in ruminal biohydrogenation (BH) pathways that ultimately affect milk fatty acids (FA). Sixteen mid-lactation organically certified Jersey cows were randomly assigned to treatments in a replicated, factorial 4 × 4 Latin square design with 24-d periods. Treatments were fed as TMR (55:45 forage-to-concentrate ratio) consisting of (1) 0% FO + 28.3% CC, (2) 0% FO + 27.1% GC, (3) 3% FO + 28.3% CC, and (4) 3% FO + 27.1% GC. Diets averaged 19.5% starch, and 4.9 and 7.6% ether extract for 0 and 3% FO, respectively. Corn mean grain particle size averaged 2,047 µm (CC) and 580 µm (GC). Contrasts were used to compare: FO level, corn particle size, and oil × corn interaction. ALA was the only FA in milk that showed an oil × corn interaction (P = 0.02); cows fed 3% FO + GC showed the greatest proportion of ALA in milk (0.82 g/100 FA), and those fed 0% FO + CC the least (0.55 g/100 g FA). Except for c-9, c-12 18:2, all remaining milk FA increased with feeding 3% vs. 0% FO (Table 1). Increased milk 18-C FA with 3% FO may be explained by increased 18-C FA intake. Compared with cows fed CC, those fed GC had decreased 18:0 and c-9 18:1, and increased c-9, c-12 18:2, which may be linked to differences in FA profile and ruminal BH between corn sources. Overall, changes in milk FA profile appear to be largely influenced by level of FO supplementation or corn grain source, but not by the FO × oil interaction.

**Key Words:** corn grain, flax oil, milk fatty acid

### Table 1 (abstract M299). Milk FA profile

<table>
<thead>
<tr>
<th>FA, g/100 g</th>
<th>Flax oil</th>
<th>Corn grain</th>
<th>SEM</th>
<th>Contrasts (P &gt; F)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0%</td>
<td>3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>12.7</td>
<td>16.4</td>
<td>14.9</td>
<td>14.2</td>
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<tr>
<td>c-9 18:1</td>
<td>15.3</td>
<td>18.7</td>
<td>17.4</td>
<td>16.6</td>
</tr>
<tr>
<td>c-9, c-12 18:2</td>
<td>2.02</td>
<td>1.83</td>
<td>1.87</td>
<td>1.98</td>
</tr>
<tr>
<td>ALA</td>
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<tr>
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<td>0.36</td>
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</tbody>
</table>

### M300 Replacing conventional or brown midrib corn silage with brown midrib sudangrass silage in the diets of lactating dairy cows.

K. F. Kalscheur and G. E. Brink, U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.

Forages that use less water, but are high in digestibility, are sought as alternatives to traditional forages such as corn silage. Brown midrib (BMR) sudangrass is an example of alternative forage for corn silage. The objective of this study was to evaluate whether BMR sudangrass silage (SS) can replace 2 types of corn silage with differing fiber digestibilities [conventional (CONV) or BMR corn silage (CS)] in the diets of lactating dairy cows. Forty-eight Holstein cows in mid- to late-lactation were assigned to 1 of 4 treatments in a randomized complete block design. Cows were fed a common covariate diet for 2 weeks followed by 8 weeks of experimental diets. Diets were formulated to contain 40% CS, 20% alfalfa silage, and 40% concentrate on DM basis. Sudangrass silage was included in experimental diets at either 0 or 10% of the diet.
DM replacing either 10% CONV or BMR CS. All other ingredients (high moisture corn, canola meal, roasted soybeans, soyhulls, and minerals and vitamins) were included equally for all diets. Cow was the experimental unit. Dry matter intake (DMI) averaged 25.2 kg/d and was not affected by the type of CS used nor by the inclusion of SS in the diets (P > 0.05). Similarly, milk production (averaged 40.0 kg/d) and was not affected by type of CS nor SS inclusion. Milk fat percentage increased 0.15% for cows fed the addition of 10% SS compared with cows fed 0% SS. Milk protein, lactose, and total solids percentage was not affected by dietary treatments. Protein yield was greater (0.054 kg/d; P = 0.03) for cows fed 0% SS compared with cows fed 10% SS. Because the dietary CP% was slightly greater for diets containing 10% SS compared with 0% SS (17.2 vs 16.2%), MUN responded similarly (11.1 vs. 9.6 mg/dL; P = 0.001). Energy-corrected milk (ECM) and feed efficiency (defined as ECM/DMI) was not affected by changes in diet because of similar intake and performance. Overall, the inclusion of 10% SS as a replacement did not negatively affect lactation performance. BMR SS can be a successful replacement for CS where CS availability is limited.

Key Words: BMR sudangrass, corn silage, milk production

M301 The role of fat in distillers grains and solubles on the rumen bacterial community. E. Castillo-Lopez*,1, C. Jenkins2, N. Aluthge2, T. Wesley3, S. Fernando2, and P. Kononoff4, 1Universidad Nacional Autonoma de Mexico-FESC, Cuautitlan, Estado de Mexico, Mexico, 2University of Nebraska-Lincoln, Lincoln, NE.

This study evaluated the effects of feeding dried distillers grains with solubles (DDGS) or reduced-fat DDGS (RFDG) on the rumen bacterial community composition. Four ruminally cannulated Holstein dairy cows were used in a 4 × 4 Latin square over 28-d experimental periods. Treatments (DM basis) were (1) CONT, a diet with no distillers grains; (2) DG, inclusion of 20% DDGS; (3) rDDG, inclusion of 20% RFDG; and (4) MIX, inclusion of 10% DDGS and 10% RFDG. The DDGS and RFDG were included by replacing corn grain and soybean meal. At the end of each period, the rumen bacterial community was evaluated by sequencing the 16S rRNA gene. The phylum Bacteroidetes decreased and Firmicutes tended to increase when distillers grains and solubles were fed. No treatment effect was observed on the predominant bacterial families Prevotellaceae (P = 0.51), Lachnospiraceae (P = 0.67), Veillonellaceae (P = 0.69), Spirochaetaceae (P = 0.45) and Paraprevotellaceae (P = 0.87) with averages across treatments of 29.37 ± 1.430%, 7.23 ± 0.367%, 5.89 ± 0.963%, 3.04 ± 0.218%, and 2.21 ± 0.151%, respectively. However, the abundance of the genus Ruminococcus tended to increase (P = 0.06) with estimates of 1.72 and 2.22 ± 0.230% for CONT and treatments containing distillers grains and solubles, respectively. In addition, the abundance of the genus Butyribioaptera increased (P < 0.01) with estimates of 0.35 and 0.46 ± 0.027% for CONT and treatments containing distillers grains and solubles, respectively. The archaean genera Methanobrevibacter (P = 0.86) and Methanosphaera (P = 0.85) were not affected by treatment and averaged 1.64 ± 0.234% and 0.11 ± 0.023%, respectively, across treatments. Overall, this study demonstrates that DDGS or RFDG can be used to replace corn and soybean meal in dairy rations; however, in doing so, the abundance of certain bacterial taxa within the rumen are shifted.

Key Words: distillers grains, Illumina DNA sequencing, ruminal microbiome

M302 Effects of selected feed additives to improve growth and health of dairy calves. L. Salazar1, C. Cortinhas2, T. Acedo2, P. Rotta1, M. Fontes1, V. Morais1, A. Machado1, A. Sguizzato1, and M. Marcondes1, 1Federal University of Vicsosa, Vicsosa, MG, Brazil, 2DSM Produtos Nutricionais Brasil SA, Sao Paulo, SP, Brazil.

We aimed to evaluate the effect of supplementation of monensin (MON), probiotics or essential oils on performance and health of suckling dairy calves from 6 to 60 d of age, and its residual effect 15 d after weaning. Fifty Holstein calves were fed 5 L/d of raw milk and starter feed. The products were provided by DSM Brasil SA, and the treatments were control, MON (30 mg/kg of starter), probiotic E. faecium (PROB, 70 mg/kg of starter, cfu/kg 7.0E+09), essential oils (EO, 300 mg/kg of starter; composed by thymol, guaiacol, eugenol, vanillin, salicylaldehyde and limonene), and EOPROB (treatments PROB + EO). The DMI and fecal score (scored from 1 to 4) were daily measured, and animals were weighed every 15 d. Two 48 h digestibility trials were performed at d 20–28 (period 1) and 50–56 (period 2), with total feces collection. The experiment was designed in completely randomized blocks with 10 replications, and date of entrance was used as block. Treatment effects were tested using ANOVA and means were compared by Student’s t-test at P < 0.10. In digestibility trials, periods were included as repeated measures. The DMI before weaning was greater for EO (903.03 g/d) compared with MON (794.34 g/d) and EOPROB (783.12 g/d) (P < 0.05). The EO (P < 0.026) and MON (P < 0.010) improved health and decreased the incidence of diarrhea demonstrated by the lower fecal score. Average daily gain (ADG) did not differ among treatments (P > 0.10) before weaning. After weaning, EO had greater ADG (917.50 g/d) compared with control (615.80 g/d) and PROB (592.60 g/d). Feed efficiency of EO (0.72 g/g) improved over control (0.36 g/d), MON (0.49 g/d) and PROB (0.36 g/d). During the digestibility trials, greater intakes of DM (1097.80 g/d), CP (237.91 g/d), and NDF (268.59 g/d) were observed during period 2 compared with period 1 (P < 0.001). Animals fed PROB had greater intakes of DM (P < 0.022; 1184.56 g/d), CP (P < 0.022; 254.63 g/d) and NDF (P < 0.030; 320.36 g/d) than animals fed EOPROB. Digestibility of NDF was greater in animals fed MON (P < 0.012). In summary, EO can replace MON to improve the health of young dairy calves and can be used as an alternative to prevent diarrhea.

Key Words: essential oils, probiotic, dairy calf


The objective of this study was to determine the effect of varying undigested NDF at 240 h (uNDF240) and physically effective NDF (peNDF) content of fresh cow rations on metabolism. Multiparous Holstein cows (n = 56) were fed a common prepartum ration beginning 28 d before expected parturition and assigned randomly at calving to 1 of 2 postpartum diets differing in content of uNDF240 and peNDF. High fiber (HF; 35.3% NDF, 12.1% uNDF240, 25.0% peNDF; n = 27) and low fiber (LF; 32.8% NDF, 9.5% uNDF240, 21.4% peNDF; n = 29) treatment diets were formulated for equivalent metabolizable protein (110 g/kg DM) and starch (24.8% DM), with higher fiber levels achieved through the addition of straw. At 29 DIM, cows fed HF were switched to the LF diet and all cows were fed the LF diet through 42 DIM. Blood samples were collected 2×/wk prepartum, daily from d 0 through 7 DIM, 3×/wk through 21 DIM and 2×/wk to 42 DIM. Liver biopsies were obtained from a subset of 40 cows on d 7 ± 1.1 (mean ± SD) and 14 ± 1.0 postpartum and incubated in an in vitro system to determine liver capacity to convert [1-14C] propionate and [1-14C] palmitic acid to end products. Data were analyzed by repeated measures ANOVA with the
random effect of cow within treatment and fixed effects of treatment, time, and treatment × time. A treatment × time effect was observed for plasma NEFA and was higher for cows fed HF particularly from 21 to 31 DIM (P = 0.01), and plasma β-hydroxybutyrate was higher for cows fed HF from 12 to 31 DIM (P < 0.01), and plasma glucose was lower for cows fed HF from 9 to 27 DIM (P < 0.01) compared with cows fed LF. Cows fed LF tended to have greater liver oxidation of palmitate to CO2 (12.23 nmol/(g·h) vs. 10.94 nmol/(g·h); P = 0.15) and lower conversion to esterified products (226.9 nmol/(g·h) vs. 248.2 nmol/(g·h); P = 0.10) than cows fed HF. Conversion of palmitate to acid soluble products was not different between treatments and no effects on in vitro liver propionate metabolism were observed. Changes in plasma metabolites and liver fatty acid metabolism were consistent with lower dry matter intake of cows fed the HF diet.

Key Words: transition cow, fiber, metabolism

M304 Pre- and post-weaning performance and health of dairy calves fed complete pelleted calf starters formulated for three different starch levels. D. Ziegler*, H. Chester-Jones1, B. Ziegler2, and S. Schuling2, 1University of Minnesota, Waseca, MN, 2Hubbard Feeds Inc, Mankato, MN.

One-hundred twelve (2 to 5 d old) individually fed Holstein heifer calves (38.6 ± 0.56 kg) from 3 commercial dairy farms were randomly assigned to 1 of 4 calf starter (CS) treatments formulated for varying starch levels to evaluate pre- (d 1–42) and post weaning (d 43–56) calf performance and health. The study was conducted between August and October, 2016. All treatment diets included a non-medicated 20% CP:20% fat milk replacer (all milk protein) fed at 0.28 kg in 2 L of water 2× daily from d 1 to d 35 and 1x daily from d 36 to weaning at d 42; supplemented daily with neomycin sulfate and oxytetracycline at 22mg/kg BW for 14 d. Calf starter treatments were as follows 1) texturized starter (TS) formulated for 30% starch DM basis, (TS30); 2) complete pelleted starter (CPS) formulated for 18% starch DM basis, (CPS18); 3) CPS formulated for 24% starch DM basis, (CPS24); 4) CPS formulated for 30% starch DM basis, (CPS30); Water and CS were offered free choice from d 1 to 56. Pre-weaning (d 1–42), 0.55 vs. 0.48 kg/d and post weaning gains (d 43–56) 1.03 vs. 0.83 kg/d were greater for calves fed TS (P < 0.05) vs. CPS18, CPS24 and CPS30. Overall 56 d gain was greater for TS30 vs. CPS18, CPS24 and CPS30, 0.67 vs. 0.57 kg/d. Gain/feed (d 1–42) was greater for TS30 vs. CPS18, CPS24 and CPS30, 0.62 vs. 0.56 kg/d (P < 0.05). Overall gain/feed (d 1–56) was greater for TS30 vs. CPS18, CPS24 and CPS30, 0.57 vs. 0.52 kg/d. There were no differences in daily fecal scores or health costs. Under conditions of this study, calf performance was reduced with a CPS regardless of starch level compared with TS30. Cost savings with a CPS may still provide economical gains over TS30.

Key Words: calf performance, calf starter, starch.

Table 1 (abstract M305). Commercial farm forage and grain population descriptive statistics

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<tr>
<th>Feed</th>
<th>TFA mean (%DM)</th>
<th>SD</th>
<th>CV</th>
<th>RUFAL mean (%TFA)</th>
<th>SD</th>
<th>CV</th>
<th>RUFAL mean (%DM)</th>
<th>SD</th>
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</thead>
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<td>Legume/grass hay</td>
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<td>35.6</td>
<td>64.7</td>
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<td>14.2</td>
<td>0.74</td>
<td>0.32</td>
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</tbody>
</table>

M305 Total fatty acid and rumen unsaturated fatty acid load variation in commercial TMR, forages, and corn grain. J. P. Goeser*1,2, J. Karlen1, D. Meyer1, and A. L. Lock3, 1Rock River Laboratory Inc., Watertown, WI, 2University of Wisconsin-Madison, Madison, WI, 3Michigan State University, Lansing, MI.

Risk of dietary-induced milk fat depression and troubleshooting low milk fat tests may be partially alleviated when the fatty acid (FA) content and profiles for individual feeds are known. Our first objective was to expand on feed library FA data by describing population statistics for total FA content (TFA, % DM) and rumen unsaturated FA load (RUFAL) for commercial dairy farm feeds. Our second objective was to determine if knowing TFA and feed type was sufficient to predict RUFAL (% DM and % TFA). Commercial farm legume and/or grass hay (n = 69) and silage (n = 129), corn silage (n = 115), corn grain (n = 35), and small grain silages (n = 46) were selected for FA analyses from samples submitted from across the US for routine nutritional analysis based upon near-infrared spectral diversity. Total FA concentration and profile was determined by gas-liquid chromatography. TFA (% DM) was calculated by summing individual FA concentrations and RUFAL determined by summing unsaturated 18-carbon FA. RUFAL was expressed as both % DM and % TFA to explore the variation across feeds and within FA content. Data were analyzed by SAS JMPPro v11. RUFAL (% TFA and % DM) was related to TFA and feed type using backward elimination. Population statistics for TFA and RUFAL are presented in Table 1. RUFAL (% DM) was related to TFA (linear and quadratic effects), feed, a TFA interaction with feed (each with P < 0.001; model adj. R2 = 0.49), RUFAL (% TFA) was related to TFA (linear and quadratic effects) and feed (each with P < 0.001; model adj. R2 = 0.77). The coefficient of variation (percentage; Table 1) suggest that TFA varies greater than FA profile. Results suggest that feed TFA (% DM) can predict RUFAL (% DM), however relationships may not be linear and are feed dependent.

Key Words: milk fat, fatty acid, nutrition


Objectives were to evaluate the effects of feeding rumen-protected methionine (MET) from 23 d (±12) before calving until 98 DIM on lactation performance and DMI of dairy cows. Multiparous Holstein cows (n = 223) were enrolled before calving and housed in replicated close-up (n = 4; 10 cows each) and lactation pens (n = 6; 16 cows each) in a free-stall barn, milked 2× daily, pen-fed a basal diet formulated to contain 14.5% and 16.2% CP in close-up and lactation periods, respectively and...
M307 Effects of temporal supply of propionic acid on feeding behavior of cows in the postpartum period. G. Maldini*1, 2 and M. S. Allen1, 1 Michigan State University, East Lansing, MI, 2 CAPES, Brasilia, DF, Brazil.

Appetite suppression during the postpartum (PP) period is likely caused by a signal related to hepatic oxidation of fuels. Faster absorption and hepatic uptake of propionate is expected to accelerate hepatic oxidation, stimulating satiety sooner, reducing meal size. However, if excessive propionate absorption from the rumen results in less efficient hepatic extraction of propionate, effects on meal size might diminish and hepatic oxidation following meals might be extended decreasing meal frequency. The objective of this study was to determine the temporal effects of propionic acid (PA) infused intraruminally at initiation of meals on feeding behavior. Eight ruminally-cannulated, multiparous cows in the PP period were utilized in a 4x4 Latin square design experiment. Cows were blocked by parturition and randomly assigned to treatment sequence within square. Treatments were infusion of 1.25 (HIGH) or 0.5 mol (LOW) of PA at initiation of meals over 5 min (FAST) or 15 min (SLOW). Infusions were triggered at each meal for 12 h. A 24-h recovery period was allowed between infusion days to reduce carry-over effects of treatment. HIGH decreased DMI compared with LOW (7.2 vs 11.2 kg/12 h; P = 0.001) by decreasing meal frequency (5.8 vs 7.5 meals/12 h; P = 0.02). HIGH increased eating time (103 vs 127 min/12 h, P = 0.02) and yield (1.39 kg/d vs. 1.44 kg/d, P = 0.04), with no difference in ECM, 3.5% FCM, fat, lactose, somatic cells count and milk urea nitrogen. HIGH decreased DMI and feeding behavior parameters (P < 0.05) but tended to decrease meal size (109 vs 122 min/12 h; P = 0.06) and did not affect meal size (P = 0.68) or meal frequency (P = 0.16). No interactions of treatment were detected for DMI or feeding behavior parameters (P > 0.32). Treatment effects on milk yield were not detected. The lack of effect of infusion rate on meal size, along with the reduction in DMI by HIGH compared with LOW by decreasing meal frequency rather than meal size, suggests that propionate flux to the liver might have exceeded the liver’s capacity for first-pass extraction of propionate from the blood, likely extending hepatic oxidation longer after meals for the higher propionate dose.

Key Words: methionine, transition cow, milk protein

M308 Comparing choline bioavailability of two rumen-protected choline products using milk betaine as a biomarker in the lactating dairy cow. M. J. de Veth1, 1, M. Cooney2, and P. French3, 1 BioNarus LLC, Cary, NC, 2 phdR&D, Fort Atkinson, WI, 3 Feed Components LLC, East Troy, WI.

During the periparturient period, supplementation of rumen-protected choline (RPC) has reduced liver triacylglycerol accumulation and improved animal performance in the dairy cow. Recently milk betaine (Bet) was identified as biomarker for choline bioavailability, making it possible to compare the effectiveness of RPC products at delivering choline for absorption. Our objective was to compare the choline bioavailability of 2 commercial RPC products, ReaShure (RES; Balchem Corporation, New Hampton, NY) and Excential Rumenpass (ERP; Orffa, Werkendam, the Netherlands), using milk betaine as a biomarker for choline absorption. Nine lactating Holstein cows (173 ± 8 DIM) were used in a replicated 5 x 5 Latin square design (one square incomplete), with 7-d treatment periods and a 3-d interval between periods. Treatments were (1) control (0 g/d choline), (2) 12.5 g/d choline fed as RES, (3) 25 g/d choline fed as RES, (4) 12.5 g/d choline fed as ERP, (5) 25 g/d choline fed as ERP. Choline chloride was the choline form for both products; RES and ERP contained 22.2% and 19.8% choline, respectively. Cows were fed twice daily and RPC products mixed with 25% of TMR to ensure treatment consumption. Milk samples from d7 were analyzed for Bet using liquid chromatography-stable isotope dilution-multiple reaction monitoring mass spectrometry. No changes in DMI or milk yield were observed with choline treatment (P > 0.40). The concentration and yield of milk Bet increased with ERP relative to RES (P = 0.02). The concentration of milk Bet (control – 83.5 μM) increased linearly (P = 0.05) with ERP (84.8 and 99.6 μM for 12.5 and 25 g/d ERP, respectively). Similarly, the yield of milk Bet (control – 0.42 g/d) increased linearly (P = 0.02) with ERP (0.38 and 0.51 g/d for 12.5 and 25 g/d ERP, respectively). No relationship was found between RES dose and milk Bet concentration (P = 0.44; 78.1 and 74.8 μM for 12.5 and 25 g/d RES, respectively) or RES dose and milk Bet yield (P = 0.76; 0.38 and 0.38 g/d for 12.5 and 25 g/d RES, respectively). Overall, in this experiment the choline bioavailability of ERP was greater than RES when using milk betaine as a biomarker for choline absorption.

Key Words: choline, bioavailability, dairy cow

M309 Effects of supplementation with a combination of palmitic and stearic acids on dry matter intake, milk yield, and component production: a meta-analysis. M. D. Sellers*, T. L. Harris, and J. R. Lofton, Milk Specialties Global Animal Nutrition, Eden Prairie, MN.

To date, a cumulative meta-analysis of the effects of supplementation with a combination of prilled C16:0 and C18:0 fatty acids (PFA) that includes all study designs has not been completed. The objective of the current analysis was to examine dry matter intake (DMI), milk yield (MY), and milk component production responses when lactating cows were supplemented with PFA. Data were extracted from 25 peer-reviewed publications and the final data set included 73 treatments, with 39 treatments supplementing PFA and 34 non-supplemented control treatments (CON). Dietary nutrient concentrations (DM%; range) were 17.1% CP [12.0 – 20.1%], 26.1% starch [17.3 – 37.4%], and 33.7% NDF.
A random-effects model with the random effect of study was chosen to estimate the mean of the sampling distribution of possible effect sizes, and studies were weighted by the inverse of their variance. Weighted mean differences between PFA and CON treatments as well as standard errors of the differences between means are reported. The average amount of C16:0 and C18:0 supplementation across studies was $632 \pm 222.4$ g/d. DMI was not affected by PFA supplementation ($22.01 \pm 22.07 \pm 0.18$ kg/d; $P = 0.75$), while net energy intake increased with PFA supplementation ($37.59 \pm 35.46 \pm 0.62$ Mcal/d; $P < 0.01$). PFA increased MY ($33.78 \pm 32.55 \pm 0.26$ kg/d; $P < 0.01$) and milk fat percentage ($3.53 \pm 3.45 \pm 0.03$%; $P = 0.01$). There was no change in milk protein concentration ($3.12 \pm 3.14 \pm 0.02$%; $P = 0.34$) and a tendency for decreased milk lactose concentration ($4.85 \pm 4.89 \pm 0.02$%; $P = 0.06$) with PFA. Yields of milk fat and milk protein were increased ($1.17 \pm 1.11 \pm 0.01$ kg/d and $1.04 \pm 1.01 \pm 0.01$ kg/d, respectively; $P < 0.01$) with PFA supplementation, while milk lactose yield was unaffected ($1.83 \pm 1.78 \pm 0.03$ kg/d; $P = 0.16$). Supplementation with a combination of C16:0 and C18:0 fatty acids resulted in a significant increase in MY, milk fat and milk protein yield and net energy intake, while causing no appreciable decrease in DMI.

**Key Words:** palmitic acid, stearic acid, supplemental fat

**M310** Withdrown

**M311** Effects of supplementation with calcium salts of palm fatty acid distillate on dry matter intake, milk yield, and component production: A meta-analysis. T. L. Harris*, M. D. Sellers, and J. R. Loften, *Milk Specialties Global Animal Nutrition, Eden Prairie, MN.*

A cumulative meta-analysis examining the effects of all calcium salts of palm fatty acid distillate (CS) on production responses that includes all study designs is not available. The objective of the current analysis was to examine dry matter intake (DMI), milk yield (MY), and component production in lactating dairy cows supplemented with CS. Data included in the study were extracted from 31 peer-reviewed publications which included 74 treatment means that were supplemented with CS and 65 non-supplemented control (CON) diets. Dietary nutrient concentrations (DM%; range) were 17.4% CP [14 – 23.3%], 32.0% starch [23.4 – 42.2%], and 34.0% NDF [24.8 – 51.7%]. The average feeding amount of CS was 661.7 ± 275.5 g/d. A random-effects model with the random effect of study was chosen to estimate the mean of the sampling distribution of possible effect sizes, and studies were weighted by the inverse of their variance. Weighted mean differences between CS and CON treatments as well as standard errors of the differences between means are reported. Calcium salts decreased DMI (20.04 vs 20.97 ± 0.181 kg/d; $P < 0.01$), however, NE_{J}\text{ intake did not differ among treatments (36.85 vs 35.98 ± 0.550 Mcal/d; } P = 0.11). Milk yield increased (32.61 vs 31.82 ± 0.260 kg/d; $P < 0.01$) with CS supplementation. Milk fat percentage increased (3.48 vs 3.42 ± 0.023%; $P < 0.01$), milk protein percentage decreased (3.03 vs 3.12 ± 0.011%; $P < 0.01$), and milk lactose percentage did not differ (4.99 vs 4.97 ± 0.021%; $P = 0.40$) with CS supplementation. Yields of milk fat and lactose increased (1.13 vs 1.08 ± 0.012 kg/d; $P < 0.01$; 1.48 vs 1.38 ± 0.014 kg/d; $P < 0.01$; respectively), while protein yield was unchanged (1.00 vs 1.01 ± 0.011 kg/d; $P = 0.45$). Supplementation with CS in lactating cows has mixed effects on production, as evidenced by decreased DMI, milk protein percentage, and milk protein yield and increased MY, milk fat percentage and yield, and milk lactose yield.

**Key Words:** calcium salt, palm fatty acid distillate, supplemental fat

**M312** Replacing ground corn with liquid molasses decreases production performance in dairy cows offered low-starch diets. C. P. Ghedini1, A. F. Brito*,1 D. C. Moura2, A. S. Oliveira1, and R. A. V. Santana4, 1University of New Hampshire, Department of Biological Sciences, Durham, NH, 2Universidade Federal de Mato Grosso, Programa de Pós Graduação em Ciência Animal, Cuiabá, MT, Brazil, 3Universidade Federal de Mato Grosso–Campus Sinop, Instituto de Ciências Agrárias e Ambientais, Sinop, MT, Brazil, 4Instituto Federal de Educação, Ciência e Tecnologia do Norte de Minas Gerais–Campus Arinos, Arinos, MG, Brazil.

Production performance data regarding the use of liquid molasses (LM) or ground corn (GC) as the sole supplemental NSC source or in different combinations to dairy cows offered low-starch diets are lacking. The objective of this study was to evaluate the effects of feeding incremental amounts of LM on milk yield and composition, and apparent total-tract nutrient digestibility in Jersey cows. Sixteen multiparous Jersey cows (99 ± 41 DIM and 462 ± 38.2 kg of BW) were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 14 d for diet adaptation and 7 d for data and sample collection. Diets were fed as TMR and consisted of 52% grass-legume baleage, 43% (99 ± 41 DIM and 462 ± 38.2 kg of BW) were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 14 d for diet adaptation and 7 d for data and sample collection. Diets were fed as TMR and consisted of 52% grass-legume baleage, 43% grass hay, 8.5% soyhulls, 2.5% roasted soybean, and 15% flaxseed meal. Ground corn was replaced by incremental amounts of LM at 0, 4, 8, or 12% of diet DM. Diets averaged 9.7 and 0.0%, 7.0 and 1.7%, 4.3 and 3.3%, and 1.64 and 5.0% of starch and LM-added sugars, respectively; CP and NDF concentrations averaged 19.0 and 43.5% across diets. Results are presented in Table 1. Intake of DM, as well as MUN and yields of milk and milk fat and protein decreased linearly.

<table>
<thead>
<tr>
<th>Item</th>
<th>Molasses: Corn ratio (% diet DM)</th>
<th>SEM</th>
<th>L</th>
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<td></td>
<td>0:12</td>
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<tr>
<td>Milk protein, %</td>
<td>4.03</td>
<td>4.04</td>
<td>4.02</td>
<td>3.99</td>
</tr>
<tr>
<td>Milk protein, kg/d</td>
<td>0.76</td>
<td>0.70</td>
<td>0.72</td>
<td>0.63</td>
</tr>
<tr>
<td>MUN, mg/dL</td>
<td>16.8</td>
<td>16.4</td>
<td>15.8</td>
<td>16.0</td>
</tr>
<tr>
<td>OM digestibility, %</td>
<td>72.7</td>
<td>71.2</td>
<td>72.1</td>
<td>72.1</td>
</tr>
<tr>
<td>NDF digestibility, %</td>
<td>64.7</td>
<td>64.2</td>
<td>65.0</td>
<td>65.2</td>
</tr>
</tbody>
</table>

**Table 1 (abstract M312).** Production performance and nutrient digestibility.
with replacing GC with LM. Decreased milk yield is likely explained by the drop in DMI. Conversely, milk fat and protein concentrations, and OM and NDF total-tract digestibilities did not differ across treatments. Overall, it appears that the amount of LM-added sugars, particularly at the greatest level of supplementation depressed ruminal fermentation processes negatively affecting DMI and milk yield.

**Key Words:** dairy cow, ground corn, molasses

**M313** Effects of supplementation with palmitic acid-enriched fat products on dry matter intake, milk yield, and component production: A meta-analysis. M. D. Sellers, T. L. Harris, and J. R. Loften*, Milk Specialties Global Animal Nutrition, Eden Prairie, MN.

Recent research has demonstrated unique production responses to various supplemental fat products. Data on effects of supplementation with palmitic acid-enriched fat products (PA) on milk and component responses are limited. The objective of the current analysis was to examine dry matter intake (DMI), milk yield (MY), and milk component production responses in lactating dairy cows supplemented with PA. Data included in the study were extracted from 7 peer-reviewed publications, which included 12 treatment means that were supplemented with PA and 8 non-supplemented control (CON) diets. Dietary nutrient concentrations (DM%; range) were 16.3% CP [15 - 18.7%], 26.9% starch [16.1 - 29.3%], and 31.3% NDF [25.1 - 34.3%]. A random-effects model with the random effect of study was chosen to estimate the mean of the sampling distribution of possible effect sizes, and studies were weighted by the inverse of their variance. Weighted mean differences between PA and CON treatments as well as standard errors of the differences between means are reported. The average feeding amount of PA was 560 ± 92.5 g/d. Dry matter intake was decreased an average of 0.54 kg/d in cows supplemented with PA versus CON (26.07 vs 26.61 ± 0.169 kg/d; P < 0.01). Milk yield was not different between PA and CON (37.99 vs 37.76 ± 0.305 kg/d; P = 0.45). There were varying milk component responses to feeding PA. Milk fat percentage increased with PA supplementation (3.98 vs 3.74 ± 0.03%; P < 0.01), but milk protein percentage (3.15 vs 3.20 ± 0.015%; P < 0.01) and milk lactose percentage (4.76 vs 4.81 ± 0.0099%; P < 0.01) decreased with PA supplementation. Milk fat yield increased with PA supplementation (1.44 vs. 1.35 ± 0.01 kg/d; P < 0.01), while milk protein yield did not differ (1.20 vs 1.21 ± 0.013 kg/d; P = 0.28), and milk lactose yield tended to decrease (1.80 vs 1.82 ± 0.013 kg/d; P = 0.07) with PA supplementation. These results indicate that supplementation with highly enriched PA products decreases DMI and does not affect MY. Moreover, PA supplementation increases milk fat concentration and yield, but decreases concentrations of milk protein and lactose.

**Key Words:** meta-analysis, palmitic acid, supplemental fat


The objective of this study was to evaluate the effect of virginiamycin (VM) combined with crude glycerin (CG) on pH value, N-NH₃ (mg/100 mL) and VFA (mM). Rumen fistulated bulls (BW = 600 ± 34 kg) were used in a replicated 4 × 4 Latin square (21-d periods) with 2 × 2 factorial arrangement of treatments: diets without virginiamycin (VM-) or with 25 mg/kg of VM in DM (VM+) combined with diets without crude glycerin (CG-) or with 100 g/kg of CG (80% glycerol) in DM (CG+). The sugar cane bagasse was used as the exclusive roughage in the proportion of 20% in DM of diet and crude glycerin replace corn in the diet formulation. Diets were offered on an ad libitum basis at 0700 and 1600 h. Ruminal samples were taken immediately before feeding and at 3, 6, 12, 18 h post feeding on d20 and d21 of the sampling week. Data were analyzed in a replicated 4 × 4 Latin square with a 2 × 2 factorial using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Statistical model included the fixed effect of virginiamycin (1 degree of freedom, DF), crude glycerin (1DF) and all interactions. Random effects were period (3DF), bulls (3DF), and residual error. There were no CG × VM interactions for any variable measured (P > 0.05). Diets with CG or VM had similar values of pH (mean = 6.15; P > 0.05). CG+ had lower N-NH₃ concentration compared with CG- diets, independent of VM inclusion (24.26 vs. 28.69 mg/100mL; P > 0.05). Data showed that CG or VM did not affect the concentration of total VFA (116.92 mM; P > 0.05). The molar proportion of acetate was lower in CG+ compared with CG- diets (53.58 vs. 62.70% of total VFA; P < 0.01) and it was greater in VM- compared with VM+ diets (59.60 vs. 56.68%; P < 0.01). The molar proportion of propionate was greater in CG+ than CG- diets, independent of VM inclusion (24.47 vs. 18.54%; P = 0.0091). The molar proportion of isobutyrate and isovalerate were not affected by CG or VM (P > 0.05). Acetate:propionate ratio was lower in CG+ compared with CG- diets (3.57 vs. 2.36; P > 0.05). Valerate and butyrate proportion was greater in CG+ than CG- (P < 0.05). CG can replace corn at 100 g/kg of DM and VM in Nellore finishing diets without impairing fermentation.

**Key Words:** crude glycerin, finishing diet, virginiamycin

**M315** Comparative analysis of bacterial community composition from the different ruminal ecological niche of Alxa Bactrian camel. J. Zhao*1,2, Z. Yu2, and H. Wu1, 1Inner Mongolia University for Nationalities, Tongliao, Inner Mongolia, China, 2The Ohio State University, Columbus, OH.

Similar to ruminants, camels, as pseudoruminants, depend on the microbiota in their pseudorumen (a 3-chambered forestomach) to digest fibrous feed. Compared with the ruminal microbiome of cattle and sheep, the microbiome in the pseudorumen of camels is poorly understood. The objective of this research was to characterize the bacteria community compositions partitioned into different niches: liquid phase (LP), solid phase (SP), and epimural phase (EP), of the pseudorumen of Alxa Bactrian camel. Samples of the 3 phases were collected from 6 slaughtered Alxa Bactrian camels. Community composition of bacteria were determined through sequencing 16S rRNA gene amplicons of the V3-V5 hypervariable regions on the Illumina MiSeq platform. Weighted UniFrac analysis revealed that the bacterial community of LP was clearly different from that of SP and EP. From 619,517 quality-checked sequences, 774 operational taxonomic units (OTUs) were identified at a 97% sequence identity. As in the rumen, Bacteroidetes (46.6% of the total sequences) and Firmicutes (32.8%) were the 2 most predominant phyla, with other minor phyla also being represented: Verrucomicrobia (4.4%), Spirochaetes (3.3%), Proteobacteria (2.8%), Firmobacteres (2.6%), Tenericutes (2.4%), and Lentisphaerae (1.2%). Bacteroidetes was more predominant in LP than in SP and EP, while Firmobacteres was more predominant in SP than in LP. At genus level, a total of 117 taxa were observed across all the samples, but 48 taxa of them were belong to unknown genera. Only 0.1% of the total sequences were assigned to archaea. The results showed that the camel pseudoruminal microbiome was structurally similar but compositionally distinct from that of true...
rumen, reflecting the different dietary and genetic impact of camels on their pseudoruminal microbiome. These findings also demonstrated the compositional differences among LP, SP, and EP, indicating that those bacterial communities are specific and adapted to their niches.

**Key Words:** Alxa Bactrian camel, bacteria, microbiome

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**M316** Screening of chemically and physically treated corn stover and soybean meal pellet formulations for in situ digestibility in dairy cows. B. C. Dooley*1, C. S. Shouse1, M. A. Torres-Crespo1, R. Zeeck2, and H. A. Ramirez-Ramirez3, Iowa State University, Ames, IA, 2Pellet Technology USA, Greta, NE.

The objective of this screening study was to determine the in situ ruminal digestibility of dry matter (DMD), neutral detergent (NDFD), and acid detergent fiber (ADF) of 4 corn stover-based experimental formulations of blended material as potential feedstuffs for dairy cows. Each prototype formulation included approximately 42.5% ground corn stover (CS), 15% distillers solubles and varied proportions of soybean meal (SBM) ranging from 33 to 40% to allow inclusion of CaO as a chemical treatment or a custom mixture of fatty acids. Treatments were 1) untreated corn stover (U-CS; 75%NDF, 44% ADF); 2) untreated pelleted blend (U-BLN; 46% NDF, 25% ADF); 3) CaO-treated blend (T-BLN; 44% NDF, 25% ADF); 4) blend with no fatty acid supplementation (NFA; 33% NDF, 23% ADF); and 5) fatty acid-supplemented blend (FA; 29% NDF, 18% ADF). Ten 5-g samples of each prototype and untreated CS were evenly assigned and incubated in the rumen of 2 ruminally-cannulated lactating Holstein cows for 48 h. Data were analyzed using the GLIMMIX procedure of SAS with prototype formulation and cow as fixed effects. By design, U-CS resulted in the lowest (P < 0.01) DMD, 37.3 ± 1.64% followed by T-BLN, which was 65.5 ± 1.64%. Prototypes NFA and FA were similar (P = 0.27) and averaged 78.5 ± 1.64% and U-BLN had the greatest (P < 0.01) DMD at 82.0 ± 1.64%. Untreated CS had the least (P < 0.01) NDFD at 29.8 ± 2.28% followed by T-BLN at 44.4 ± 2.28%, NFA and FA were similar (P = 0.63) and averaged 50.4 ± 2.28% whereas NDFD of U-BLN was 67.2 ± 2.28%. Digestibility of ADF was different (P < 0.01) across treatments, 25.2 and 38.2 ± 2.47% for U-CS and T-BLN, respectively; NFA and FA were similar (P = 0.10) averaging 45.6 ± 2.47% and U-BLN had the greatest (P < 0.01) ADF at 59.6 ± 2.47%. Similarities between NFA and FA indicate that additional fatty acids did not alter digestibility. Although additional research is warranted to isolate the effects of chemical composition and mechanical processing, these results indicate that corn stover and SBM-based pellets are highly digestible and may be a suitable feedstuff for dairy cows.

**Key Words:** endoscopic biopsy, colon, rumen biopsy

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**M317** The development of methodology for ruminal and colon tissue biopsying of Holstein dairy bull calves during weaning. J. K. van Niekerk*, R. Zeeck2, and H. A. Ramirez-Ramirez3, 1Iowa State University, Ames, IA, 2Pellet Technology USA, Greta, NE.

The objective of this study was to develop methodology for biopsying the rumen and colon from dairy calves during weaning. Six Holstein dairy bull calves (45 ± 1.5 kg birth weight) were ruminally cannulated during the second week of life and were fully weaned on d 42. Colon and rumen tissue samples were collected on d 28, 35, 42, 49, 56 and 84. Calves were not sedated but restrained in a chute for sampling. The lubricated distal tip of the endoscope (100 cm length, 9.8 mm diameter) was gradually inserted into the calf’s anus. Six (±12.6 mg) colon tissue samples were collected (30–40 cm from the calf’s anus) every sampling with endoscopic biopsy forceps (single use capture hot biopsy forceps), which were inserted through the instrument channel. The instrument channel was washed with double distilled water and 70% ethanol between calves as well as outside of the endoscope. Furthermore, the endoscope was introduced through the ruminal cannula to harvest ruminal papillae. Endoscopic biopsy of the rumen with endoscopic biopsy forceps (capture hot biopsy forceps and alligator jaws with a needle biopsy forceps) was unsuccessful (85% of the time) because the endoscopic biopsy forceps were unable to excise rumen papillae due to connective tissue. Thereafter, an Allis clamp was used to retrieve the blind sac through the ruminal cannula to perform direct tissue biopsying. Surgical scissors were used to perform tissue biopsying after exteriorization of the blind sac through the ruminal cannula. Colon and rumen samples were washed in PBS and 2 samples submerged in formalin solution and stored at room temperature for light histology analysis and 4 samples were stored in RNA stabilization fluid for molecular biology analysis. The mean RNA integrity number for the rumen papillae and colon samples were 8.9 ± 0.13 and 8.7 ± 0.09, respectively. In conclusion, endoscopic biopsying can be used for tissue harvest in the colon in young calves. However, it was found that collecting rumen tissue through retracting the rumen and taking samples with surgical scissors was more successful than using an endoscopic biopsy.

**Key Words:** ionophore, milk fat
M319  Extracellular amino acids and lysine to methionine ratio affect cell signaling in mammary epithelial cells. P. S. Yoder*1,2, T. Ruiz-Cortes3, and M. D. Hanigan1, 1Virginia Tech, Blacksburg, V/A, 2Perdue AgriBusiness, Salisbury, MD, 3Universidad de Antioquia, Medellin, Colombia.

Extracellular amino acid (AA) profile may affect intracellular AA concentrations and profile as well as signaling proteins that regulate translation rate. The objective of this study was to assess the effects of various extracellular AA profiles and Lys to Met ratio to determine signaling protein sensitivity. Six AA profiles of DDEM, blood meal (BM), corn gluten meal (CM), casein (CS), blood plasma of cows milk (CW), and a negative control (NC) represented the profile a 3:1 ratio treatment versus other ML 3:1 ratio treatments despite similar extracellular concentrations indicating greater uptake. Within mTOR pathway, mTOR, ribosomal protein S6 kinase 1 (S6K1), and eukaryotic initiation factor 4 E binding protein 1 were induced by AAPROF (P<0.01) while only S6K1 was affected by ML (P=0.11). mTOR pathway proteins had greater phosphorylation for DMEM, BM, and CM tended to have higher phosphorylation than CW (P<0.01). Eukaryotic initiation factor 2 α subunit was unaffected by PROF and ML factors (P=0.23). Setting Lys/Met at a 3:1 ratio had a small positive effect on S6K1 regardless of AA profile. Changes in extracellular AA profiles largely translated to intracellular AA and these varying profiles in general stimulated mTOR pathway related proteins.

Key Words: amino acid, mTOR, translation regulation

M320  Milk enterolactone and fatty acid profile in dairy cows offered flaxseed meal and incremental amounts of liquid molasses. C. P. Ghedini1, A. F. Brito*1, D. C. Moura2, A. S. Oliveira3, and R. A. V. Santana4, 1Department of Biological Sciences, University of New Hampshire, Durham, NH, 2Programa de Pós Graduação em Ciência Animal, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil, 3Instituto de Ciências Agrárias e Ambientais, Universidade Federal de Mato Grosso–Campus Sinop, Sinop, MT, Brazil, 4Instituto Federal de Educação, Ciência e Tecnologia do Norte de Minas Gerais–Campus Arinos, Arinos, MG, Brazil.

Enterolactone (EL) is a mammalian lignan originated from the microbial fermentation of flaxseed in the rumen, and it has been linked to potential human health benefits. However, there is limited information about the impact of liquid molasses (LM) or ground corn (GC) on the microbial output of EL in the rumen and EL transfer to milk. There is also scarce information about the effect of LM or GC or LM:GC mixtures on milk fatty acid (FA). Our objective was to investigate the impact of replacing GC with incremental amounts of LM on milk concentrations of EL and FA. Sixteen multiparous Jersey cows were assigned to treatment in a

Table 1 (abstract M318).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>MON</td>
</tr>
<tr>
<td>Production, kg/d</td>
<td>29.2</td>
<td>29.5</td>
</tr>
<tr>
<td>3.5% FCM</td>
<td>28.7</td>
<td>28.3</td>
</tr>
<tr>
<td>Milk concentration, g/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>34.6b</td>
<td>32.5b</td>
</tr>
<tr>
<td>Protein</td>
<td>30.7</td>
<td>30.9</td>
</tr>
<tr>
<td>Lactose</td>
<td>45.9</td>
<td>46.2</td>
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<tr>
<td>DMI, kg/d</td>
<td>24.6a</td>
<td>23.0b</td>
</tr>
<tr>
<td>Milk yield/DMI</td>
<td>1.21</td>
<td>1.30</td>
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</table>

1Diet by time (wk) interaction effect >0.10 for all variables.

Table 1 (abstract M320). Milk enterolactone and FA (g/100 g)

<table>
<thead>
<tr>
<th>Item</th>
<th>Molasses:Corn ratio (% diet DM)</th>
<th>P &gt; F</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0:12</td>
<td>4:8</td>
</tr>
<tr>
<td>Enterolactone, nM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>246</td>
<td>324</td>
</tr>
<tr>
<td>c-9 18:1</td>
<td>11.0</td>
<td>10.7</td>
</tr>
<tr>
<td>c-9, c-12 18:2</td>
<td>13.6</td>
<td>13.1</td>
</tr>
<tr>
<td>c-9, t-11 18:2</td>
<td>1.35</td>
<td>1.36</td>
</tr>
<tr>
<td>c-9, c-12, c-15 18:3</td>
<td>0.54</td>
<td>0.55</td>
</tr>
<tr>
<td>Σ odd-chain</td>
<td>0.62</td>
<td>0.62</td>
</tr>
<tr>
<td>Σ branched-chain</td>
<td>2.04</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>1.93</td>
<td>1.94</td>
</tr>
</tbody>
</table>
replicated 4 × 4 Latin square design with 21-d periods. Diets were fed as TMR and consisted (DM basis) of 52% grass-legume baleage, 8% grass hay, 8.5% soyhulls, 2.5% roasted soybean, and 15% flaxseed meal. Ground corn was replaced by LM at 0, 4, 8, or 12% of diet DM. Diets averaged 9.7 and 0.0%, 7.0 and 1.7%, 4.3 and 3.3%, and 1.64 and 5.0% of starch and LM-added sugars, respectively; CP and NDF concentrations averaged 19.0 and 43.5% across diets. Results are presented in Table 1. Concentration of milk EL and Σ branched-chain FA was not affected by treatments. Milk 18:0, c-9 18:1, and c-9, c-12 18:2 decreased linearly, whereas c-9, t-11 18:2 and c-9, c-12, c-15 18:3 increased linearly with replacing GC with LM. These results may be associated with decreased intake of 18:2 or shifts in ruminal biohydrogenation. Σ odd-chain FA responded quadratically to LM supplementation, suggesting changes in ruminal microbiota. Overall, changing the dietary NSC profile in cows fed flaxseed meal did not alter milk EL, but affected milk FA.

Key Words: dairy cow, enterolactone, flaxseed meal

M321 Pre-ensiling addition of bacterial inoculant, amylase or both to rehydrated cracked corn. L. C. Solórzano*1, L. L. Solórzano2, and A. A. Rodríguez1, 1University of Puerto Rico, Mayagüez, PR, 2Independent Researcher, Fitchburg, WI.

Increasing starch digestibility benefits lactational and economic performance of dairy cows. Ensiling increases starch digestibility, but it is affected by the length of ensiling and intensity of fermentation. Increasing the intensity of fermentation by adding a homo-fermentative bacterial inoculant (HBI, 1 g/kg supplying > 9.1 × 10^10 cfu/g containing Lactobacillus plantarum, Enterococcus faecium, Lactococcus lactis, Pediococcus pentosaceus, P. acidilactici), a source of α-amylase (AMY, 1.1 g amylase/kg), both (HBI+AMY) or no additives (CTL) to rehydrated cracked corn (RCC; 66% DM) before ensiling was evaluated. Sixteen 1-L glass mini-silos (4/treatment) were vacuum-packed and stored for 90 d at 25°C. Nutritional and fermentation characteristics, in vitro starch digestibility (IVSD) and aerobic stability were determined. Data were analyzed as a completely randomized design. Means were separated using Tukey’s test. HBI tended to increase (P < 0.10) DM recovery (95.4%) compared with CT (95.0%) while the addition of AMY (94.2%) or HBI+AMY (95.5%) reduced it. HBI decreased (P < 0.05) pH and butyric acid (3.90, 0.01%) compared with CTL (4.03, 0.11%), AMY (3.95, 0.06%) or HBI+AMY (4.00, 0.04%). HBI lowered (P < 0.05) butyric acid compared with AMY or HBI+AMY. Ethanol was increased (P < 0.05) with AMY (0.38%) compared with CTL (0.30%). Adding HBI (0.23%) or HBI+AMY (0.29%) decreased (P < 0.05) ethanol compared with CTL (0.30%). HBI and HBI+AMY decreased NH₃-N (0.13 and 0.14% of CP) compared with CTL (0.22% of CP) or AMY (0.17% of CP). AMY and CTL differed between them in NH₃-N. HBI+AMY tended (P < 0.13) to increase IVSD (69.1% of starch) compared with CTL (67.5% of starch) while HBI or AMY (67.5 or 66.6% of starch) tended to decrease it. HBI+AMY decreased the stability (h) of RCC upon aerobic exposure vs. HBI (78 vs 138 h), AMY (>168 h) or CTL (>168 h). HBI or AMY positively influenced the nutritional and fermentation characteristics of RCC. Neither HBI nor AMY increased IVSD. The combination of HBI+AMY tended to increase IVSD but decreased aerobic stability of RCC.

Key Words: ensiling, inoculant, amylase

M322 Relationship between mineral composition of milk and lactation performance. A. R. Alfonso-Avila*1, E. Charbonneau1, P. Y. Chouinard1, G.F. Tremblay2, D. E. Rico1, and R. Gervais1, 1Université Laval, Quebec, QC, Canada, 2Agriculture and Agri-Food Canada, Quebec, QC, Canada.

Minerals could be implicated in cellular transport of milk constituents or their precursors in the mammary epithelial cell. Our objective was to examine associations between mineral composition of milk and lactation performance in dairy cows. A total of 120 observations from 2 separate randomized complete block design experiments comprising 60 early-lactation Holstein cows (38 ± 11 DIM; Mean ± SD) were used. In both experiments, treatment periods lasted 28 d and were preceded by a 1-wk pretreatment collection period. In both trials, diets had a forage-to-concentrate ratio of 40:60 (DM basis) and were supplemented, on a DM basis, with (1) 1.5% K₂CO₃, (2) 1.8% K₂CO₃, (3) 2.6% KHCO₃, (4) 2% KCl, (5) 1.4% Na₂CO₃, (6) 1.5% K₂CO₃ + 2% soybean oil, (7) 2% soybean oil, or (8) unsupplemented. Milk yield was recorded and samples collected on the last 3 or 5 d of each period, pooled by cow and period, and analyzed for fat, protein, lactose, and mineral concentrations (Na, K, Cl, S, Mg, P, Ca). Associations between minerals and milk yield and component concentrations were assessed using mixed model regressions, considering cow and experiment as random effects, and parity and individual mineral concentrations as fixed effects. Parity was removed from the model when not significant (P > 0.10). Milk concentrations of Ca, Mg, P, and S were positively associated with both fat (P < 0.01; R² > 0.28) and protein (P < 0.01; R² > 0.30) contents. Negative relationships were observed between Cl and fat (P < 0.02; R² = 0.48), protein (P < 0.01; R² = 0.40) as well as lactose (P < 0.01; R² = 0.71) contents, whereas K was negatively associated with lactose concentration (P < 0.01; R² = 0.62). Finally, Ca, Mg, and P were negatively related with milk yield (P < 0.01; R² > 0.86), whereas a positive association was observed with Cl (P < 0.01; R² = 0.79). The strong relationship of milk Cl concentration with milk yield suggests that this mineral is involved in milk synthesis. Mineral concentrations in milk are recognized to reflect their cellular levels, it is then possible to explore the biological role of minerals in the secretory mechanisms of milk constituents.

Key Words: chlorine, milk constituent, milk synthesis

M323 Effects of pre-ensiling additions of a bacterial inoculant with or without molasses on rehydrated cracked corn fermentation parameters. L. C. Solórzano*1, L. L. Solórzano2, and A. A. Rodríguez1, 1University of Puerto Rico, Mayagüez, PR, 2Independent Researcher, Fitchburg, WI.

Ensiling increases starch digestibility of corn, which is affected by the intensity of fermentation. Increasing the intensity of fermentation by adding a homo-fermentative bacterial inoculant (BI, 1 g/kg supplying > 9.1 × 10^10 cfu/g containing Lactobacillus plantarum, Enterococcus faecium, Lactococcus lactis, Pediococcus pentosaceus, P. acidilactici), or BI plus molasses at 1.1 g/kg (BI+MOL) or no additives (CTL) to rehydrated cracked corn (RCC; 66% DM) before ensiling was evaluated. Twelve 1-L glass mini-silos (4/treatment) were vacuum-packed and stored for 90 d at 25°C. Data were analyzed as a completely randomized design. Means were separated using Tukey’s test. DM recovery (95.4%) compared with CT (95.0%) while the addition of AMY (94.2%) or HBI+AMY (95.5%) reduced it. HBI decreased (P < 0.05) NH₃-N compared with CTL (0.13 vs. 0.11%). BI (0.23%) decreased (P < 0.05) butyric acid compared with CTL (0.01%). BI (3.9) decreased (P < 0.05) NH₃-N compared with CTL (0.13 vs. 0.14%). BI+MOL (0.38%) increased (P < 0.05) butyric acid compared with AMY or HBI+AMY. Ethanol was increased (P < 0.05) with AMY (0.38%) compared with CTL (0.30%). Adding HBI (0.23%) or HBI+AMY (0.29%) decreased (P < 0.05) ethanol compared with CTL (0.30%). HBI, AMY and HBI+AMY decreased NH₃-N (0.13 and 0.14% of CP) compared with CTL (0.22% of CP) or AMY (0.17% of CP). AMY and CTL differed between them in NH₃-N. HBI+AMY tended (P < 0.13) to increase IVSD (69.1% of starch) compared with CTL (67.5% of starch) while HBI or AMY (67.5 or 66.6% of starch) tended to decrease it. HBI+AMY decreased the stability (h) of RCC upon aerobic exposure vs. HBI (78 vs 138 h), AMY (>168 h) or CTL (>168 h). HBI or AMY positively influenced the nutritional and fermentation characteristics of RCC. Neither HBI nor AMY increased IVSD. The combination of HBI+AMY tended to increase IVSD but decreased aerobic stability of RCC.

Key Words: ensiling, inoculant, amylase
nor BI+MOL affected in vitro starch digestibility compared with CTL (66.2, 67.1, 67.5% of starch, respectively). BI+MOL tended (P < 0.10) to decrease DM recovery compared with CTL (83.8 vs. 89.9%) or BI (86.1%) after 168 h of aerobic exposure (AE). The average temperature during 168 h of AE was higher (P < 0.05) for BI+MOL (22.1°C) compared with CTL (19.7°C) or BI (21.3°C). BI+MOL decreased the aerobic stability (h) of RCC compared with BI (60 vs 138 h) or CTL (>168 h). Additives had some positive effects on fermentation characteristics, however, the use of a homo-fermentative BI, or BI+MOL is not recommended for ensiling of RCC due to their negative effects on DM recovery and aerobic stability upon opening of silos. Additives did not affect starch digestibility.

Key Words: inoculant, molasses, ensiling

M324 Supplementation of blackberry pomace during the transition phase may improve health and reproductive performance of dairy cows. K. Swanson*, S. Akers, K. Estenson, R. Wilson, M. Keller, and G. Bobe, Oregon State University, Corvallis, OR.

Dairy cows are during the transition period most susceptible to metabolic and infectious diseases, which adversely affect reproductive performance. Blackberry pomace, the waste product from blackberry processing, is rich in polyphenolic compounds that have anti-inflammatory and anti-oxidative properties. To evaluate the effect of supplementation of blackberry pomace during the transition period on health and reproductive performance of dairy cows, 24 multiparous dairy cows were fed either 0 (Control), 57, or 114 g/d of dried blackberry pomace as top dressing to the TMR from 28 d before to 28 d after calving. Blood samples were collected on approximately d 28, 21, 14, 7, 3, and 1 postpartum, while both blood and milk samples were collected on d 0, 1, 3, 7, 14, 21, and 28 postpartum. Upon completion of the study, serum samples were analyzed for BHB, glucose, FFA, BUN, calcium, and phosphorus concentrations. Reproductive data, including days open and number of times bred before confirmed pregnant, was collected. All data were analyzed using PROC MIXED and PROC GLIMMIX in SAS version 9.4. Fixed effects were blackberry pomace supplementation rate and breed and for repeated data within cows, time and the interaction of time with treatment. Compared with Control, supplementation with 114 g/d decreased the number of days until first heat (33 ± 5d vs. 48 ± 7d; P = 0.02), first breeding (58 ± 3d vs. 68 ± 8d; P = 0.04), and days open (73 ± 9d vs. 122 ± 22d; P = 0.002) and tended to improve pregnancy rate from first breeding (78 ± 17% vs. 16 ± 15%; P = 0.06). No significant group differences were observed for disease incidence (P = 0.34), feed intake (P = 0.58), milk yield (P = 0.36), or concentrations of serum for BHB (P = 0.31), glucose (P = 0.0525), FFA (P = 0.74), BUN (P = 0.65), calcium (P = 0.46), and phosphorus (P = 0.102). In conclusion, supplementation of blackberry pomace at 114 g/d during the transition phase may improve health and reproductive performance of dairy cows.

Key Words: blackberry pomace, dairy health, reproductive performance

M325 Evaluation of *Saccharomyces cerevisiae* fermentation products on production, metabolism, oxidative stress, and health of transition dairy cows. K. M. Glosson*, I. Yoon*, and J. K. Drackley1, 1University of Illinois, Department of Animal Science, Urbana, IL, 2Diamond V, Cedar Rapids, IA.

Yeast culture products have been used in the dairy industry to modify the rumen environment of lactating cows to improve production and reduce metabolic stresses in the transition period. The objective of this study was to determine the effects of feeding *Saccharomyces cerevisiae* fermentation products (Diamond V Original XPC and NutriTek) on the production and health of cows from −26 through 28 DIM. Multiparous Holstein cows (n = 100) were randomly assigned one of 4 top-dress treatments that were combinations of the products and ground corn to equal 50 g/d: 1) control, ground corn (CON); 2) 14 g XPC mix (XPC); 3) lower level, 19 g, NutriTek mix (NTL); or 4) higher level, 38 g, NutriTek mix (NTH). Cows were milked 3x/d and milk production and components were summarized by week. Blood samples were collected prepartum based on expected calving date at −26, −17, −14, −10, −7, −4 and −1 d, and postpartum at calving and 1, 3, 5, 7, 10, 14, 17, and 21 d for analysis of blood metabolites. Phagocytosis (PN/PM) and oxidative burst (OBN/OBM) activity of neutrophils (N) and monocytes (M) were used to evaluate immune status at −17, −7, 5, 14, and 28 d. Intake did not differ significantly among treatments through d 28, but cows supplemented with XPC or NTH tended to have greater ECM yield in this period (trt × time, P = 0.06) when compared with the other treatments (CON: 44.3 kg/d; XPC: 45.0 kg/d; NTL: 44.1 kg/d; NTH: 46.9 kg/d). Most blood metabolites and minerals were not different among treatments. Activities of PN (Trt, P < 0.01) and OBN (Trt, P < 0.01) were increased at d 5 for cows supplemented with NTH when compared with cows given CON or NTL (CON: 53%, 44%; XPC: 58%, 49%; NTL: 53%, 48%; NTH: 64%, 58%). While cows supplemented with NTL appeared to partition more energy toward other metabolic uses than milk production, the difference of innate immune system activity during the first week of lactation and the differences in ECM production shows a possible benefit of supplementing XPC or NTH to transition dairy cows.*

Key Words: *Saccharomyces cerevisiae* fermentation product, transition dairy cow

*Corrected abstract
**Table 1 (abstract M327).** Empirical equations commonly used to predict DMI for weaned Saanen goats

<table>
<thead>
<tr>
<th>Equation</th>
<th>Source</th>
<th>DMI (mean ± SD [CL90])</th>
<th>Overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: DMI (g/d) = 1748.7 – 495.7 × DE + 18.4 × BW + 3.0 × ADG</td>
<td>Lu and Potchoiba (1990)</td>
<td>0.802±0.219</td>
<td>77.8</td>
</tr>
<tr>
<td>2: DMI (g/d) = (76.7 × BW^{0.75}) × (–0.666 + 1.333 × ME – 0.266 × ME^{2})</td>
<td>AFRC (1998)</td>
<td>1.01±0.199</td>
<td>97.0</td>
</tr>
<tr>
<td>3: DMI (kg/d) = –0.0047 ± 0.03072 + (0.9637 ± 0.04928 × MEp/ME) – (70.27 ± 23.543 × ADG/BW) + (38.71 ± 12.224 × ADG/BW^{0.75}) – (243.4 ± 121.73 × (ADG/MEp/ME)^{2})</td>
<td>Luo et al. (2004)</td>
<td>0.738±0.187</td>
<td>67.4</td>
</tr>
<tr>
<td>4: DMI (g/d) = –0.308 ± 0.0435 × BW + 0.00370 × ADG</td>
<td>INRA (2007)</td>
<td>1.14±0.312</td>
<td>80.1</td>
</tr>
<tr>
<td>5: DMI (kg/d) = 0.04 × BW × (BW/MW) × [1.7 – (BW/MW)] × [1.0 – 1.7 × (0.8 – DMdmg)]</td>
<td>NRC (2007)</td>
<td>1.30±0.244</td>
<td>74.6</td>
</tr>
</tbody>
</table>

De = digestible energy, kcal/gDM; BW = body weight, kg; MW = mature BW (considered values reported by Almeida et al., 2016), kg; ME = metabolizable energy, Mcal/kgDM (eq.3) or MJ/kgDM (eq.4); qm = metabolizability; MEp = predicted ME requirements, MJ/d; ADG = average daily gain, g/d; DMdmg = DM digestibility, 0-1.

\*Predicted DMI, in kg/d or overlap % using the MCRA.
0.49). At approximately 1 mo before parturition, 10 crossbred dairy goats were divided into 2 groups (n = 5 each) including control (CNT, 31.4 ± 3 kg BW) and 2% coconut oil supplementation (CoO, 30.0 ± 2.0 kg BW). Both experimental formulations were isoenergetic and isonitrogenous (37.7% DM and 1.7 Mcal NEL/kg). During the late gestation, coconut oil supplementation did not affect daily dry matter intake (DMI) as compared with CNT (0.9 ± 0.1 and 0.8 ± 0.1 kg/d, P > 0.05). However, the light phase DMI (from 0700 to 1800) of CoO group was higher than from CNT group (0.8 ± 0.1 and 0.6 ± 0.1 kg/d, P < 0.05). During lactation period, there was again no effect of coconut oil on DMI. However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). During lactation period, there was again no effect of coconut oil on DMI. However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tended to be lower than from CNT group (CNT: 61.9 ± 2.0 kg; CoO: 60.7 ± 2.0 kg, P > 0.05). However, cumulative milk yield as 4%FCM (5 wk) from the CoO group tende...
were the F:C ratio resulting of adding concentrate (0, 0.5 or 1 kg/d, as fed) to a basal diet (1.8 Mcal NEL and 15.9% CP, DM basis): high- (HF, 70:30), medium- (MF, 55:45) and low-forage (LF, 40:60). Ewes were fed the HF diet during 4 wk and the experimental diets thereafter (wk 5 to 8). Milk recording was done at each milking and milk sampled on wk 3 and 7 for composition (NIR system; Foss, Nordersted, Denmark). No differences in total DM intake were detected by treatment (MN vs. LC, 2.57 ± 0.19 vs. 3.01 ± 0.19 kg DM/d, on average), but forage intake decreased 28 and 37% with the LF diet, in LC and MN ewes, respectively (P < 0.05). Reduction of forage intake was only detected between HF vs. MF diets in the MN ewes (−21%; P < 0.05). Milk yield varied by breed (MN vs. LC, 1.46 ± 0.09 vs. 2.12 ± 0.22 kg/d; P < 0.001), but no yield nor composition vary by treatment (P = 0.97 to 0.24). Nevertheless, milk fat content decreased numerically between HF and LF diets in both breeds (MN, −8%, P = 0.24; LC, −5%, P = 0.53). No effect was seen between HF and MF diets. Body weight and BCS markedly increased in the LC ewes (HF-MF vs. LF, 2.7 vs. 4.6 kg BW; P = 0.015), but not in the MN ewes. No effect of concentrate was detected on feces consistency, although fecal (P < 0.001) and urinary pH (MN, P = 0.24; LC, P = 0.10) decreased by adding concentrate. In conclusion, the use of high concentrate (>45%, DM basis) had not effect on milk yield and milk composition, producing remarkable BW gains during lactation. On the dairy ewes feeding practice, the final decision on the amount of concentrate to be used will be dependent on the feedstuffs prices and the body reserves of the ewes.

**Key Words:** dairy sheep, concentrate ratio, intake

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**M332 Improving goat sperm post-thaw quality using GameteGuard extender.** M. Shepherd*, C. Bennett, B. Baker, and L. Herickhoff, Membrane Protective Technologies Incorporated, Fort Collins, CO.

Post-thaw quality of goat sperm ranges from acceptable to poor using today’s current technology largely developed from bull industry standards. At the same time, the goat dairy and meat industries are expanding and, in turn, are requesting and requiring optimization in artificial insemination. Therefore, there is a need to develop a buck-specific methodology to enhance post-thaw sperm quality. In this study, GameteGuard was used as an alternative extender to improve post-thaw quality of goat sperm. GameteGuard, a unique blend of plant-derived antioxidants, has previously provided significant post-thaw membrane protection and acrosome integrity in the bovine model (Herickhoff et al., 2015). An experiment was conducted using split ejaculates from 9 bucks (4 La Mancha, 1 Boer, and 4 Nigerian Dwarf). Ejaculates were collected using commercial procedures, washed once and split into 1 of 3 extenders: control 22% Egg Yolk Citrate (EYC), 5% GameteGuard A in EYC (GGA), and 5% GameteGuard B in EYC (GGB). All extender contained 5% glycerol (i.e., ‘one-step’ extenders). After extension to 25 million/mL, sperm was allowed to equilibrate for 3 h at 4°C then loaded into 0.5 mL straws. Straws were equilibrated for an additional 2 h at 4°C, floated 4 cm over liquid N for 15 min then plunged into liquid N. Straws were thawed in a 37°C water bath for 2 min. Afterward, computer assisted sperm analysis (CASA) and flow cytometry data were collected for motility, progressive motility, V AP, membrane integrity (PI and SYBR-green fluorophores), and acrosome integrity (AlexaFluor 647-PNA fluorophore) immediately post-thaw (0 h) and after 3 h incubation at 37°C. Use of GameteGuard in the extender improved 0-h motility, progressive motility, and VAP by 19 ± 1, 36 ± 0, and 10 ± 0.4% respectively (P < 0.05) for both GGA and GGB. There was also an 11.3 ± 0.9% improvement in overall acrosome integrity (P < 0.05) in both GGA and GGB treatments compared with control. There was no effect of treatment × breed for motility or acrosome intactness. These results indicate that GameteGuard can be used as an alternative cryopreservation extender to improve post-thaw quality of goat sperm.

**Key Words:** goat, reproduction, sperm
Teaching/Undergraduate and Graduate Education

M333 Virginia Tech STEM Scholars program: Freshman academic performance influences subsequent academic success. R. R. Cockrum*, K. F. Knowlton, and M. D. Denbow, Virginia Polytechnic Institute and State University, Blacksburg, VA.

There is mounting pressure to develop a pipeline of talented animal biology graduates to address mounting societal and agricultural problems in food, energy and water. To better prepare students to enter into agricultural careers, researchers at Virginia Tech have developed the STEM Scholars program. The objectives of this study were to (1) determine effect of cohort on academic performance and (2) identify early academic parameters that influence overall academic success. Economically challenged but academically talented students (n = 21) were recruited their freshman year into the Virginia Tech STEM Scholars program from the dairy and animal sciences departments over 3 cohorts (C1, n = 7; C2, n = 8; C3, n = 6). Students remained in the program until graduation. The effects of cohort were tested using an ANOVA in SAS with the fixed effects of primary major and sex. A Tukey adjusted post-test analysis was used to adjust for multiple comparisons. Mean comparisons and SE are reported using least squares means. A Pearson correlation and multiple linear regression analyses in SAS were used to determine relationships among academic records across semesters. These included: primary major, gender, number of majors, number of minors, transfer credits, term GPA, academic class, and grades in STEM Scholar professional development courses. The professional development courses included “Journal Club in STEM,” “Career Success in STEM,” and “Effective Communication and Career Development.” Class scores were decreased for C2 (95.88 ± 0.86) compared with C3 (99.92 ± 0.94). There was a strong correlation (r ≥0.58) among semesters 1 through 3 with academic performance. Finally, academic performance in the second semester and performance in “Effective Communication and Career development” influenced 3rd semester academic performance. Students that were academically successful their first semester were more likely to be successful their sophomore year. Overall, we suggest that providing academic and career support within the first year of college will increase the likelihood that students will remain successful in their undergraduate program.

Key Words: academic, STEM, success
Fractionation of α-lactalbumin (a-la) and β-lactoglobulin (β-lg), the 2 major whey proteins (WP), is quite challenging to scale-up by using eco-efficient technologies due to their similar molecular weight (14.2 and 18.36 kDa, respectively). The proposed approach was to evaluate the difference of baro-sensitivity of a-la and β-lg under high hydrostatic pressure (HHP) treatment and their capacity to form specific aggregates when combined to casein (CN), used as a ligand in this study. The objectives were to (1) evaluate the impact of pressure and time intensity on WP, (2) compare the use of 2 different type of CN: micellar (MC) and isoelectric (IC) as ligand to generate specific interactions with WP, and (3) determine optimal parameters of pressure/time combination and type of CN to improve WP fractionation. Model protein solutions composed of a-la, β-lg and CN (IC or MC) (2.5 mg/mL for each protein) were pressurized (200, 400 and 600 MPa during 100, 200 and 300 s) using an hydrostatic pressurization unit (Hiperbaric 135, Burgos, Spain). Proteins were characterized by HPLC and PAGE. Protein aggregation was studied (size and composition) using a high-performance size exclusion chromatography (HPSEC). Response surface model demonstrated that an optimal pressure/time of 600 MPa – 300s allowed to generate the highest a-la enriched fraction (85.04% and 79.79% purity with a protein recovery of 77.39% and 82.76%, respectively for IC and MC). Analysis by HPSEC showed that 2 main categories of aggregates were generated: (1) αS1-CN, αS2-CN and a large portion of β-lg mainly linked by disulfide bonds, and (2) αS1-CN, αS2-CN and a low amount of β-lg supposedly linked by hydrophobic interactions. Our experiments performed on dairy model solutions demonstrated that using HHP, an eco-efficient process, was suitable to generate specific aggregation of β-lg with CN ligand due to the difference of baro-sensitivity. Optimal pressurization parameters allowed to generate an a-la enriched fraction with purity of 85.04%. The approach suggested that HHP could be used as pre-treatment for a new method of a-la fractionation from whey.

Key Words: high hydrostatic pressure, purification, whey protein

19 Measurement of casein as a percentage of true protein in milk by Kjeldahl and SDS-PAGE. L. Di Marzo* and D. M. Barbano, Department of Food Science, Northeast Dairy Food Research Center, Cornell University, Ithaca, NY.

Our objective was to compare the Kjeldahl (i.e., reference method) and the SDS PAGE methods for determination of milk casein as percentage of true protein (CN%TP). The CN%TP is used as an index of casein degradation (i.e., lower CN%TP and cheese yield capacity. Milks with lower CN%TP will have a higher frequency of off flavors. Our experiments were generated: (1) α-CN and a large portion of β-lg mainly linked by disulfide bonds, and (2) using the sum of CN and CNPP divided by the total of all protein bands. A t-test was performed to determine if there was difference between the mean CN%TP given by Kjeldahl versus the 2 estimates made using SDS-PAGE. The Kjeldahl CN%TP (mean = 81.05%, SD = 2.35) was higher (P < 0.05) than the SDS-PAGE CN%TP (mean = 75.80%, SD = 4.37). However, no difference (P > 0.05) was detected between Kjeldahl CN%TP (mean = 81.05%, SD = 2.35) and SDS-PAGE CN+CNPP%TP (mean = 80.42%, SD = 2.99). If the goal is to achieve agreement between results of Kjeldahl and SDS PAGE to estimate CN%TP for cheese yield, then the sum of the area of intact CN bands plus CNPP bands should be expressed as percentage of all protein bands. If the goal is to determine the extent of proteolytic damage that may relate to flavor defects and changes in functionality in dairy products, then the increase in SDS PAGE CNPP%TP is probably a more sensitive metric than Kjeldahl CN%TP.

Key Words: Kjeldahl, SDS-PAGE, casein

20 Identification of iron loci by scanning electron microscopy and iron recovery rate in iron fortified caprine milk Cheddar cheese. A. Siddique*, B. I. Davis, B. N. Vaidya, and Y. W. Park, Fort Valley State University, Fort Valley, GA.

Iron is deficient in the milk of most dairy species including cows and goats; therefore, iron fortification is desirable in milk and dairy products to increase dietary iron levels. Ferrous sulfate (FeSO4) is reportedly the most preferable form of iron salts for iron fortification as it provides high bioavailability. No report has been available for iron recovery and microstructural distribution of iron in FeSO4 fortified cheeses, especially in caprine cheeses. The purposes of this study were to determine the recovery of iron, and locate microstructural loci of iron in iron-fortified caprine milk cheeses. Three batches of 3 types of goat Cheddar cheeses were manufactured, and stored at 2 temperatures (4°C and −18°C) for 0, 2 and 4 mo. Three cheese types were control cheese (CC) without Fe addition, and 2 types of iron fortified cheeses with regular ferrous sulfate (RFS) and large microencapsulated ferrous sulfate (LMFS) by 8.23g and 9.03g per 9 kg cheese, respectively, considering 16% Fe in FeSO4 for both types of fortifications. All cheese samples were analyzed for microstructure and Fe loci of samples by scanning electron microscopy (SEM, S-3400N II, Hitachi, Japan). SEM samples were initially fixed with 2% glutaraldehyde and 1% of osmium tetraoxide (OsO4) in 0.05 M phosphate buffer for 10 min, followed by a series of acetone dehydrations with increased concentrations and times, placed in liquid CO2 for critical point drying, and then were gold coated by sputter coating. Results showed that iron contents of CC, RFS and LMFS cheeses were 0.0162, 0.822, 0.932 mg Fe/g cheese, respectively, indicating that Fe levels substantially increased iron in both fortified cheeses. Respective Fe recovery rates for LMFS and RFS cheeses were 73.5 and 71.9%. Cheese microstructures revealed that LMFS contained smaller, elongated and sharp-edged Fe particles, while RFS showed bigger, wider and rectangular Fe particles. The aggregated iron particles became clearly visible from proteolyzed casein networks as storage time advanced. It was concluded that SEM analysis was able to identify iron loci and its concentration in the Fe fortified caprine cheeses.

Key Words: goat cheese, iron fortification, scanning electron microscopy
21 Effect of sugars and protein sources on expression of genes involved in exopolysaccharide production by *Streptococcus thermophilus* ASCC1275. A. Padmanabhan*, Q. Wu, and N. P. Shah, The University of Hong Kong, Hong Kong, China.

*Streptococcus thermophilus* ASCC1275 (ST1275), a dairy starter bacterium, is known to produce high amount of exopolysaccharide (EPS). The genomic study of ST1275 revealed the presence of a novel EPS gene cluster containing 2 pair of chain length determining genes epsC – epsD, along with an efficient proteolytic and stress response system. Due to the complex and unique nature of the EPS gene cluster in this bacterium, it will be interesting to unravel the EPS biosynthetic pathway under different conditions. In this study, we investigated the influence of various sugars (glucose, sucrose and lactose) and protein sources (lactalbumin hydrolysate, casein hydrolysate, whey protein isolate) on the growth, EPS production and EPS gene expression of ST1275 in M17 media and in reconstituted skim milk (RSM), respectively. The EPS production by ST1275 was studied at different time intervals from 0 to 48 h. When grown in M17 supplemented with different sugars, ST1275 produced significantly high amount of EPS (630 mg/L) in sucrose (1%) supplemented M17 in 12 h at 37°C when compared with glucose and lactose supplemented M17. Interestingly, the pH was found to remain stable at 5.5 in lactose supplemented media from 12 h, when the pH dropped to 4.5 in the presence of other sugars. The lactic acid production was further validated using HPLC. In case of protein sources, EPS production was significantly increased when RSM was fortified with 0.5% whey protein isolate (826 mg/L) and casein hydrolysate (740 mg/L) in 12 h at 37°C in pH uncontrolled fermentation. The gene expression studies were also performed using q-PCR to investigate the regulatory genes involved in EPS production. It was observed that the expression of genes that resulted in amino sugar synthesis like galactokinase (GK), glutamine-fructose-6-phosphate transaminase, and UDP-glucose pyrophosphorylase have significantly increased in the presence of sucrose and WI. The upregulation of galactokinase, a key enzyme involved in Leloir pathway, highlights the involvement of this pathway for EPS production in ST1275.

**Key Words:** exopolysaccharide, gene expression, q-PCR

22 In vivo digestion of a model infant formula in piglets: Protein digestion pattern and physiological responses. N. R. Jaeggi2, M. E. Johnson2, and J. A. Lucey1,2, 1University of Wisconsin-Madison, Madison, WI, 2Wisconsin Center for Dairy Research, Madison, WI.

High pressure processing (HPP) and low temperature storage (super-chilling at 0°C) were investigated as an alternative to freezing to extend the performance shelf life of low moisture part-skim (LMPS) mozzarella. Batches (n = 5) of reduced Na LMPS mozzarella (mean composition 46.4 ± 0.4% moisture, 23.5 ± 0.3% fat, 25.5 ± 0.2% protein, 1.0 ± 0.1% salt) were manufactured using camel chymosin, stored for 2 weeks at 4°C, divided into 2 groups: control (non-HPP) and HPP (600 MPa, 3 min), then each group subjected to 3 storage temperature treatments (+4°C, 0°C, and −20°C) for a year. Analyses were performed at 3, 5, 7, 9, and 12 mo of storage. Performance properties were observed using texture profile analysis (TPA) and dynamic low-amplitude oscillatory rheology. Levels of pH 4.6 soluble N were used to track primary proteolysis. Changes in flavor, texture, shred properties, and pizza performance were evaluated using quantitative descriptive analysis (QDA) with 12 trained panelists using a 15-point scale. HPP initially decreases TPA hardness (P < 0.05) but no further decrease is observed time, while non-HPP samples lose hardness over 12 mo to lower values when stored at 4°C, similar values to HPP samples when stored at 0°C, no change when stored at −20°C. Primary proteolysis over 12 mo was slightly reduced (P < 0.05) through the combination of both, and stopped when stored at −20°C, compared with only storage at 4°C. HPP and 0°C storage alone, moderately reduced (P < 0.05) through the combination of both, and stopped when stored at −20°C, compared with only storage at 4°C. HPP and 0°C storage reduced blister quantity development and strand thickness loss observed in the refrigerated control, HPP or 0°C storage alone show no significant difference, and both attributes remained unchanged in all samples stored at −20°C. No impacts of flavor due to HPP treatment were observed until after 9 mo of storage. Although freezing LMPS mozzarella to −20°C gives the best performance results over the 12 mo of study, the combination of HPP and superchilling to 0°C achieved an acceptable performance shelf life up to 9 mo; a significant increase compared with refrigeration at 4°C alone.

**Key Words:** performance shelf life, high pressure processing, superchilling

The warm climate in the southeastern United States allows for extended grazing seasons; however, there is limited information on the nutritional quality of forage mixtures to sustain pasture-based organic dairy production. A study was conducted to evaluate the nutritional quality of 9 forage mixtures designed for this region. Mixtures of cool-season perennial and annual legumes (red clover, alfalfa, crimson clover), cool-season perennial and annual grasses (tall fescue, orchard-grass, annual rye-grass), warm-season annual grasses (sorghum-X sudan-grass hybrids, crabgrass), and warm-season annual legumes (annual lespedeza, cowpea) were established in a randomized complete block design at the East Tennessee Research and Education Center-Organic Crops Unit. Plots (2 × 10 m, 4 replicates per plot) were harvested twice at the appropriate growth stages. Grab samples were analyzed using near-infrared spectroscopy (Foss-DS2500, Foss America, Eden Prairie, MN) to determine crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, and in vitro dry matter digestibility for 48 h (IVTDM48h). Data were analyzed in SAS 9.4 using the GLIMMIX procedure and the model included the fixed effects of forage mixture, replica, time, and their interactions. Data from the first set of harvests indicate that average CP ranged from 8.2 to 15.0%, with greatest concentrations in mixtures that contained legume species (P < 0.01). Concentrations of ADF ranged from 40.2 to 45.2%, with the lowest concentration in monoculture orchard-grass stands. Concentrations of NDF ranged from 54.1 to 67.6%, with the lowest concentrations in mixtures containing red clover (P < 0.01). Lignin content ranged from 4.2 to 8.5%, with the lowest content in red clover stands (P < 0.01). Prediction means of IVTDM48h ranged from 63.5 to 68.8%, with the greatest digestibility in orchard-grass-red clover mixtures (P < 0.01). Results indicate that mixed grass-legume stands should provide nutrients to enhance organic dairy production in the Southeast. Additional information is needed over multiple seasons in combination with dairy cow responses to mixed stands.

Key Words: organic, forage, dairy cow

25 Productivity of lactating dairy cows fed diets with teff hay as the sole forage. B. Saylor*, D. Min, and B. Bradford, Kansas State University, Manhattan, KS.

Groundwater depletion is one of the most pressing issues facing the dairy industry today. One strategy to improve the industry’s drought resilience involves feeding drought tolerant forage crops in place of traditional forage crops like alfalfa and corn silage. The objective of this study was to assess the productivity of lactating dairy cows fed diets with teff hay (Eragrostis tef) as the sole forage. Teff is a warm-season annual grass native to Ethiopia that is well adapted to drought conditions. Nine multiparous Holstein cows (185 ± 31 d in milk; mean ± SD) were randomly assigned to 1 of 3 diets in a 3 × 3 Latin square design with 18-d periods (14 d acclimation and 4 d sampling). Diets were either control (CON), where dietary forage consisted of a combination of corn silage, alfalfa hay, and prairie hay, or 1 of 2 teff diets (TEFF-A and TEFF-B), where teff hay (13.97 ± 0.32% CP, DM basis) was the sole forage. All 3 diets were formulated for similar dry matter (DM), crude protein (CP) and starch concentrations. CON and TEFF-A were matched for concentrations of neutral detergent fiber (NDF) from forage (18.23 ± 0.15% of DM), and TEFF-B included slightly less, providing 16.63% NDF from forage. Dry matter intake (DMI), milk and component production, body weight (BW), body condition score (BCS), as well as DM and NDF digestibility (DMD and NDFD) were monitored and assessed using mixed model analysis. Treatment had no effect (P = 0.76) on DMI (28.14 ± 0.75 kg/d). Similarly, treatment had no effect (P = 0.65) on milk production (40.68 ± 1.79 kg/d). Concentrations of milk fat (3.90 ± 0.16%) and lactose (4.68 ± 0.07%) were also unaffected by treatment (P > 0.10). TEFF-A and TEFF-B increased milk protein concentration (P < 0.001) compared with CON (3.07 vs. 3.16 ± 0.09%). Treatment had no effect on energy-corrected milk (ECM) yield (43.37 ± 1.26 kg/d), BW, or BCS change (all P > 0.10). Additionally, treatment had no effect (P = 0.47) on total-tract DM or NDF (P = 0.58) digestibility. Results from this study indicate that teff hay has potential to replace alfalfa and corn silage in the diets of lactating dairy cattle without loss of productivity.

Key Words: drought, teff hay, dairy cattle

26 Transient effects of supplemental potassium and magnesium in lactating dairy cattle. A. W. Tebbe* and W. P. Weiss, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH.

Magnesium is absorbed in the rumen but is antagonized by high dietary K. Because the kidney regulates Mg, urinary Mg can be used as an index of Mg absorption. The rumen environment also affects Mg absorption and milk fatty acid (MFA) profile. Therefore, the objective of this experiment was to investigate the interaction of dietary K and Mg on transient Mg metabolism and milk fatty acid profile in dairy cows. Thirty-two multiparous cows (215 ± 32 DIM) were placed into 8 blocks based on milk yield (33.1 ± 8.8 kg/d). Cows were fed a common diet (0.24% Mg and 1.74% K on a DM basis) for 7 d and then abruptly switched to 1 of 4 treatments for 1 wk: basal Mg, basal K (Basal; 0.21% Mg and 1.73% K); basal Mg, High K (HiK; 2.71% K), High Mg, basal K (HiMg; 0.43% Mg) and High Mg, High K (HiK+Mg; 0.44% Mg and 2.81% K). Supplemented diets contained 2.0% K2CO3 and/or 0.36% and MgO. Spot urine samples were taken on d0, 2, 4 and 7 to measure Mg and creatinine and predict excretion of urinary Mg. Milk was collected on d7 for MFA profile. Data were analyzed with a model containing the fixed effects of K, Mg, K x Mg, and the random effect of block. For urine measures, the fixed effect of day (repeated) was added with d0 as a covariate. In milk fat, K decreased the proportion of C4 and C6 (P < 0.01) while Mg increased iso 16:0 (P < 0.05) and tended to increase trans-10,cis-12 18:2 and total iso-FA (P < 0.10). Anteiso 15:0 increased in HiK (5.61 mg/kg) and HiMg (5.58 mg/kg) but was not different between HiK+Mg (5.14 mg/kg) and basal (5.28 mg/kg). A K × Mg × day interaction on urinary Mg excretion (P < 0.05) was found. Excretion of Mg did not change over time for basal diet (8.7 g/d), but supplementing K without Mg decreased excretion on d 2 and 4 (5.2 g/d) and then increased on d 7 (6.6 g/d). Feeding supplemental Mg linearly increased Mg excretion over time. When both K and Mg were supplemented, Mg excretion increased on d 2 (11.3 g/d) and then remained unchanged on d 4 and 7. Therefore, Mg excretion was only transiently affected by an abrupt increase in dietary K whereas the effect of increased dietary Mg is more persistent. Treatment induced changes in MFA suggest both dietary Mg and K affected rumen ecology.

Key Words: magnesium, potassium, milk fatty acid profile
The objectives of this experiment were to determine the effects of feeding supplemental 25-hydroxyvitamin D$_3$ (25D) on concentrations of 25D and minerals in serum, lactation performance, and mastitis resistance in dairy cows. Sixty Holstein cows (multiparous, pregnant, lactating, SCC <165,000 cells/mL) were blocked by milk yield and, within each block, randomly assigned to receive a daily dietary supplement containing 1 mg vitamin D (1mgD), 1 mg 25D (1mg25D), 3 mg vitamin D (3mgD), or 3 mg 25D (3mg25D) for 28 d (n = 15/group). Blood and milk were sampled at 0, 7, 14, and 21 d for measurement of vitamin D metabolites, minerals, and energy metabolites in serum. At 21 d, cows fed 1mgD and 3mg25D received an intramammary Streptococcus uberis challenge. Data were analyzed by ANOVA with mixed models using the MIXED procedure of SAS. Significance was declared at $P < 0.05$. The 1mg25D and 3mg25D cows had greater serum 25D concentrations at 7, 14 and 21 d, greater serum 24,25-hydroxyvitamin D (24,25D) at 21 d, and lower vitamin D at 21 d compared with cows fed 1mgD and 3mgD (25D = 62 ± 7, 66 ± 8 ng/mL, 135 ± 15, and 232 ± 26 ng/mL; 24,25D = 4.9, 4.0, 11.8, and 30.6 ± 2.8 ng/mL; vitamin D = 7.6, 15.7, 1.9, and 3.1 ± 1.6 ng/mL for 1mgD, 3mgD, 1mg25D and 3mg25D, respectively, at 21d). The 3mg25D cows had greater concentrations of Ca and P at 21 d compared with other treatments, which did not differ (Ca = 2.38, 2.4, 2.37, 2.48 ± 0.02 mEq/L; P = 1.69, 1.87, 1.88 and 2.10 ± 0.08 mEq/L for 1mgD, 3mgD, 1mg25D and 3mg25D, respectively). Milk yield and components, DMI, BW, NEFA, BHBA, glucose, 1,25-dihydroxyvitamin D and Mg did not differ between treatments. The 3mg25D cows had less severe mastitis at 60 and 72 h after challenge with S. uberis compared with 1mgD cows. The 3mg25D cows also had slightly lower (P = 0.06) rectal temperature compared with 1mgD cows during the challenge period (38.9 vs. 39.1°C). Feeding 25D increases serum 25D more effectively than supplemental vitamin D, resulting in increased serum mineral concentrations and less severe mastitis in lactating dairy cows.

Key Words: vitamin D, dairy cows, mastitis

29 The development of a decision support tool to determine optimal economic treatment decisions by causative mastitis pathogen. D. T. Nolan* and J. M. Bewley, University of Kentucky, Lexington, KY.

Mastitis is a complex disease, caused by a variety of pathogens that affect the cow in different ways. The complexity of mastitis makes treatment decisions and cost estimates difficult to make. A decision support tool was developed allowing dairy producers to calculate the cost of a case of mastitis when making optimum treatment decisions based on infecting pathogen using herd data. Mastitis costs were modeled using a stochastic decision tree (Palisade Corporation, Ithaca, NY) that represents decisions and probabilities that producers face with a mastitis case. A stochastic decision tree allowed researchers to model the cost of mastitis treatments while changing market, production, and pathogen characteristic values by running multiple model iterations. A dashboard (SAP SE, Weinheim, Germany) was developed, allowing for producer interaction with the model. The dashboard presents treatment options for a case of mastitis including a 2-, 5-, and 8-d intramammary treatment, no treatment, or culling and allows dairy producers to choose a treatment option and infecting pathogen. To demonstrate how the model works, baseline values were set using averages obtained from an Dairy Records Management Systems (Dairy Records Management Systems, Raleigh, NC) data. Dairy producers can change inputs to make production values herd specific. The following is an example dashboard output using the base model for a second lactation cow with a Staph aureus infection. If the producer selected a 2-d treatment, the mastitis case cost was $388.18. The 2-d intramammary treatment would be economically optimal in 100% of iterations compared with both a 5- and 8-d treatment regimen. However, the 2-d treatment was economically optimal for 13% and 0% of iterations with culling or no treatment as the treatment option, respectively. Making the optimum treatment decision when faced with a mastitis case is complicated. The University of Kentucky Southeast Quality Milk Initiative Mastitis Treatment Dashboard (https://afs.ca.uky.edu/dairy/decisiontools/mastitistreatment) aids producers in comparing treatment options using real farm data.

Key Words: mastitis, economics, decision support

30 Nutrient restriction increases circulating and hepatic ceramide in dairy cows displaying impaired insulin tolerance. A. N. Davis*, J. L. Clegg, D. K. Henry, C. A. Perry, and J. W. McFadden, West Virginia University, Morgantown, WV.

The progression of insulin resistance in dairy cows represents a maternal adaptation to support milk production during heightened energy demand; however, excessive adipose tissue lipolysis can develop. In diabetic monogastrics, the mechanisms that mediate insulin resistance involve the sphingolipid ceramide (cer). We tested the hypothesis that ceramide accumulates in dairy cows experiencing lipolysis and insulin resistance. Nine non-pregnant, nonlactating dairy cows were utilized in a replicated 3 × 3 Latin square design. Cows were ad libitum fed, nutrient-restricted (NR), or NR with nicotinic acid (NA; 5 mg NA/h per kg BW i.v.) for 34 h. Ad libitum fed cows were provided a mixed ration to meet or exceed requirements. Intake for NR or NR + NA cows was limited to vitamins and minerals. At the end of treatment, a liver biopsy and insulin challenge were performed. Before and after treatment, all cows were provided ad libitum access to feed. Serum was analyzed using colorimetry and mass spectrometry. Data were analyzed using a mixed model with repeated measures. As previously established, NR progressively increased serum fatty acid levels (69 vs. 725 µmol/L at h 32; P < 0.01) and decreased glucose clearance rate (CR$_{40\min}$; 1.76 vs. 1.36%/min; P < 0.01). Feed removal increased liver lipid content by 35% (P < 0.01). Similar responses were observed for 16:0-, 18:0-, 20:0-cer, or fatty acids. Notably, NR increased serum 16:0- and 24:0-cer 75 and 78% by h 32 (P < 0.01). Similar responses were observed for 16:0-, 20:0-, and 22:0-cer (P < 0.05). NA was unable to prevent the rise in ceramide. NR with or without NA increased hepatic ceramide (56 to 116%; P < 0.01) relative to feeding. Re-feeding lowered ceramide levels (P < 0.05). We detected an inverse relationship between very long chain ceramide levels and insulin sensitivity (e.g., serum and hepatic 24:0-cer vs CR$_{40\min}$; r = −0.51, P < 0.01), a response not observed for 16:0-, 18:0-, 20:0-cer, or fatty acids. Our data support a role for very long chain ceramides as antagonists of insulin sensitivity in dairy cows experiencing energy deficit.

Key Words: ceramide, insulin resistance, nutrient restriction
This study was conducted to evaluate the interaction between the amount of concentrate provided in an AMS and the energy density in a PMR on DMI, milk and milk component yield, and ruminal fermentation. Eight ruminally cannulated Holstein cows (replicated 4×4 Latin square), housed in a guided-traffic-flow barn, were fed a PMR containing either low (54:46; L-FOR) or a high (63:37; H-FOR) forage to concentrate ratio. Within each PMR, cows were provided to achieve either a low (2 kg/d; L-AMS) or a high (6 kg/d; H-AMS) concentrate in the AMS. Data were analyzed using the mixed model in SAS (version 9.4) with significance declared when \( P < 0.05 \). Variation in AMS concentrate intake among days was greater for H-AMS than L-AMS (0.83 vs 0.22 kg/d; \( P < 0.01 \)), while there were no changes in PMR intake. Total DMI (PMR+AMS) was not affected by treatments, averaging 27 kg/d. Cows fed the H-AMS consumed 3.5 kg less PMR than cows fed L-AMS (\( P < 0.01 \)), regardless of the PMR energy content. A greater number of voluntary visits were observed in cows fed H-AMS than L-AMS (3.25 vs 3.20%; \( P = 0.04 \)) compared with H-FOR. Milk protein content was greater for cows fed the H-AMS than the L-AMS (3.25 vs 3.20%; \( P = 0.04 \)); while, milk fat content tended to be reduced (\( P = 0.09 \)) with H-AMS compared with L-AMS (3.51 vs 3.64%). Ruminal pH variables were not affected by treatments. However, short-chain fatty acid concentration was greater for cows fed L-FOR PMR and H-AMS than cows fed H-FOR PMR and L-AMS (112.95 vs 108.43 mM; \( P = 0.03 \)). These data suggest that feeding a greater quantity of concentrate in the AMS increases variability in concentrate consumption but may also improve voluntary visits and milk yield. When considering iso-caloric diets, these results suggest no benefit of providing more concentrate in the AMS.

**Key Words:** partial mixed ration, automated milking system, milk yield.

### 32 Using in-line milk progesterone data to characterize luteal activity parameters associated with reduced fertility in dairy herds. T. C. Bruinje1, M. G. Colazo2, M. Gobikrashanth1, and D. J. Ambrose1,2

Using milk progesterone (mP4) data, this observational retrospective cohort study investigated characteristics of (1) commencement of luteal activity (CLA), (2) luteal phase (LP) length, (3) mP4 concentration pre-AI, and (4) interval from cessation of LP to AI, influencing AI outcomes. Records of mP4 (n = 153,156) obtained by an in-line milk analysis system (Herd Navigator, DeLaval) from 1,240 Holstein cows (2,354 lactations) in 4 Alberta farms were evaluated. Sampling started at 21.5 ± 4.0 DIM (Mean ± SD) and repeated every 2.3 ± 2.0 d. Luteal activity (LA) was determined by mP4 level ≥5 ng/mL and LP as period of LA until an mP4 decline (<5 ng/mL). Outcomes of AI were presumed based on interval from AI to mP4 decline of ensuing LP as not pregnant (≤30 d), pregnant (≥50d) or pregnancy loss (PLOSS; 31 to 50 d). Days to CLA and first LP length were evaluated for 1,439 first AI. Length of pre-AI LP, mP4 concentration pre-AI (at mP4 decline), and interval from mP4 decline to AI were evaluated in 4,526 AI. Data were analyzed using PROC GLIMMIX (SAS) and models included parity, milk yield and DIM as fixed and farm as random effects, with cow as repeated measure. Only significant (\( P \leq 0.05 \)) results are presented. Overall proportions of pregnancy per AI (P/AI) and PLOSS were 24.8% (17–48% among farms) and 29.8% (17–32% among farms). Odds of P/AI was lower in multiparous than in primiparous cows (odds ratio [OR] = 0.34). Having CLA ≥56 DIM decreased P/AI (OR = 0.15) and increased PLOSS (OR = 6.67) compared with earlier CLA. Having first LP of >17 d decreased P/AI (OR = 0.18) and increased PLOSS (OR = 9.09) compared with shorter LP. Pre-AI LP of >16 d reduced P/AI (OR = 0.43) and increased PLOSS (OR = 6.67) compared with pre-AI LP ≤16 d. Pre-AI mP4 > 0.5 ng/mL reduced P/AI (OR = 0.68) and increased PLOSS (OR = 1.64) compared with lower mP4. Interval from mP4 decline to AI >1.5 d reduced P/AI (OR = 0.61) compared with shorter interval. Based on mP4 profiles, we identified characteristics of LA affecting fertility as: (1) delayed CLA, (2) prolonged first or pre-AI LP, (3) high mP4 pre-AI, and (4) delayed AI after mP4 decline.

**Key Words:** estrous cycle, progesterone profile, reproductive efficiency.

### 33 Genome-wide association analysis and genomic heritability for anti-Müllerian hormone in Holstein dairy heifers. M. Y. Nawaz1, F. Jimenez-Kassel1, J. P. Steibel1, Y. Lu1, A. Bakula1, N. Vukasinovic1, S. K. DeNise1, L. Neuder2, J. L. H. Ireland1, J. J. Ireland1, and R. J. Templeman1

Anti-Müllerian hormone (AMH) is a growth factor produced by granulosa cells that has an important role in regulation of ovarian follicle growth. Recent studies have shown a positive association between circulating AMH concentrations with number of follicles and oocytes in ovaries (ovarian reserve), response to superovulation and embryo production, fertility and herd longevity in dairy and beef cattle. The objectives of this study were to estimate the genomic heritability of AMH and identify candidate genes associated with AMH production. Serum concentrations of AMH were determined in 3092 dairy Holstein heifers (12–14 mo of age) from Green Meadows dairy farm in Elsie, Michigan. A total of 2939 Holstein heifers were genotyped for single nucleotide polymorphisms (SNP) using the Zoetis 70K SNP Panel. The genotypes were imputed to standard USDA 60,671 bovine SNP set; 54,519 SNP markers remained after standard editing procedures. A total of 2914 cows with both genotypes and phenotypes (AMH) were used for the analysis. A linear mixed model was used to model the random effects of sampling day and genomics on the logarithm of AMH. Results showed that the genomic heritability (±SEM) of AMH was 0.36 ± 0.03. We identified 12 significant SNP markers on chromosomes 11 and 20 based on a 5% false discovery rate which were mapped to 6 candidate genes using the ENSEMBL database implying these genes may have a role in AMH regulation. These candidate genes are linked to polycystic ovarian disease (DENND1A), steriodogenesis (NR5A1), embry development (NR6A1), cell cycle regulation (PPP6C, RABGAP1), T cell development (LRRC8A). The high heritability and positive association of AMH with well-established reproductive traits implies that AMH can be used as a biomarker to improve reproductive potential in dairy cattle.

**Key Words:** anti-Müllerian, reproductive potential, genome wide association.

### 34 Growth and metabolic pathways are impacted by milk replacer dietary energy in pre-weaned Holstein heifers. C. E.
Protein and fat content in milk replacer influences tissue growth and metabolism; however, the intrinsic influence on performance has yet to be fully evaluated. We hypothesized that feeding milk replacer varying in dietary energy content would elicit differential expression of genes within pathways associated with growth and metabolism. The objectives of this study were to (1) identify transcripts differentially expressed in tissues related to growth and metabolism in pre-weaned dairy heifers, and (2) determine the growth and metabolic pathways influenced by these transcripts. Pre-weaned Holstein heifers (n = 12; age 6 d ± 0.02) were randomly assigned to 1 of 2 milk replacer diets: Enhanced (E; 28% Fat, 25% CP; n = 6), or Restricted (R; 20% Fat, 20% CP; n = 6). After 8 wks, samples from longissimus dorsi (LD), adipose (A), and liver (L) tissues were collected, snap frozen and stored at −80°C. Libraries were constructed from extracted RNA for RNA-Seq analyses. Average daily gain (ADG) and gain-to-feed ratio (G:F) were calculated for each calf. Analysis of ADG and G:F was performed using a PROC GLM in SAS with diet as the main effect; E calves had increased ADG and G:F. RNA-Seq analysis was performed using CLC Genomics Workbench and the Robinson and Smith Exact Test was used to identify differentially expressed genes between diets. There were 238 differentially expressed genes in A, 227 in LD, and 40 in L. Of the differentially expressed genes, 10 appeared in at least 2 tissues. PANTHER was used to identify functional categories of differentially expressed genes. The majority of genes were associated with metabolic processes (A = 112, 26.7%; L = 16, 32.0%; LD = 81, 34.0%) or cellular processes (A = 93, 22.1%; L = 13, 26.0%; LD = 73, 30.7%). In E calves, upregulated genes included those regulating NADH dehydrogenation (LD = 17, A = 5; i.e., ND1, ND4), gluconeogenesis (LD = 2, A = 6; i.e., ALDOB, PCK2), and cell proliferation (LD = 2, A = 3; i.e., GADD45A, CDKN1A). This change in regulation of cell cycle and ATP synthesis in response to dietary energy could explain the change in ADG between diets.

Key Words: calf management, RNA sequencing, growth and metabolism pathways

The effect of limit-feeding hay on rumen development in pre-weaned Jersey calves. D. E. McCurdy* and A. H. Laarman, University of Idaho, Moscow, ID.

The objective of this study was to determine the effect of limiting hay on the development of the rumen in pre-weaned Jersey calves. Jersey bull calves (n = 13) at 4 weeks of age were individually housed and randomly assigned to one of 2 treatments in a randomized complete block design. One group was limit-fed (LF) alfalfa hay at 90g/d while the second group was fed alfalfa hay ad libitum (AL). All calves were fed 1300 g of milk replacer (26% CP/18% Fat) and were fed a texturized calf-starter ad libitum. Weekly, calves were weighed and a blood plasma sample was taken for metabolite analysis. After a calf consumed 680g/d of starter for 3 consecutive days, calves were ruminally dosed with a rumen pH logger and ruminal pH was recorded every 2 min for 7 d. Data were analyzed using the Mixed procedure of SAS 9.4. Average daily gain was not significantly different between AL and LF (0.87 ± 0.03 vs. 0.85 ± 0.04 kg/d, respectively; P = 0.55). Mean rumen pH did not differ between AL and LF treatments (6.23 ± 0.17 vs. 6.13 ± 0.22, respectively; P = 0.68) and there was no difference between duration of time rumen pH <5.8 between AL and LF (259 ± 178 vs. 318 ± 228 min/d, respectively; P = 0.83). Standard deviation of rumen pH was greater in LF than AL (0.33 ± 0.02 vs. 0.25 ± 0.02, respectively; P = 0.05). Additionally, hay intake was negatively correlated to standard deviation of rumen pH (r = −0.93; P = 0.02). This study demonstrates that limit-feeding hay can help maintain mean rumen pH in pre-weaned dairy calves. Although mean rumen pH is similar between AL and LF treatments, the higher standard deviation indicates rumen pH is more variable when hay is limit-fed.

Key Words: rumen pH, weaning, hay
36  Significance of cow cooling practices and bulk tank milk quality parameters in southeastern United States dairy farms. Z. Mason*, D. T. Nolan2, P. D. Krawczel1, G. M. Pighetti3, C. S. Petersson-Wolfe1, A. E. Stone1,2, J. M. Bewley2, and S. H. Ward2, 1Mississippi State University, Starkville, MS, 2University of Kentucky, Lexington, KY, 3University of Tennessee, Knoxville, TN, 4Virginia Polytechnic Institute and State University, Blacksburg, VA, 5North Carolina State University, Raleigh, NC.

The objective of this investigation was to identify heat abatement management practices (HAMP) that significantly influence mean annual bulk tank somatic cell count (SCC) and score (SCS) and standard plate count (SPC) in dairy farms participating in the Southeast Quality Milk Initiative project (USDA-NIFA-AFRI grant no. 2013–68004–20424). Producers from Mississippi, Kentucky, Tennessee and Virginia (n = 7, 96, 84, and 96; respectively) completed On-farm surveys from July 8, 2014 to August 3, 2015. Mean annual bulk tank SCC and SPC were calculated from 2014 Federal Order data. A total of 283 surveys were procured for analysis. The GLM procedure of SAS (Version 9.4 SAS Institute, Inc., Cary, NC) was used to perform one-way ANOVA to determine significance of HAMP. Use of fans and evaporative cooling (EC) significantly influenced SCC (P = 0.001 and P = 0.015), SCS (P = 0.008 and P = 0.046), and SPC (P = 0.041 and P < 0.0001, respectively). Method used to decide when to activate HAMP did not influence parameters. When ambient temperature was used as the decision tool, threshold temperature when fans were activated significantly influenced SCC (P = 0.044), but not SCS (P = 0.053) nor SPC (P = 0.241). Threshold temperature when EC was activated significantly influenced SCC (P = 0.008) and SCS (P = 0.046), but not SPC (P = 0.732). Coefficients of correlation ranged from 0.007- 0.109, 0.009- 0.089 and 0.002- 0.136 for SCC, SCS and SPC respectively. This suggests HAMP may not be able to recover milk quality parameters as well as might be hoped. Identifying management practices correlated to improved milk quality helps producers and consultants in meeting regional, national and international demand for high quality dairy products.

Key Words: bulk tank somatic cell count, somatic cell score, standard plate count

37  Effects of feeding hull-less barley on production performance, milk fatty acid composition, and nutrient digestibility of lactating dairy cows. Y. Yang*, G. Ferreira1, C. L. Teets1, B. A. Corl1, W. E. Thomason2, and C. A. Griffey3, 1Department of Dairy Science, Virginia Tech, Blacksburg, VA, 2Department of Crop and Soil Environmental Science, Virginia Tech, Blacksburg, VA.

The objectives of this study were to evaluate production performance, milk fatty acid composition, and nutrient digestibility in high-producing dairy cows consuming diets containing corn or hull-less barley. Eight primiparous (580 ± 41 kg of BW and 49 ± 25 DIM) and 16 multiparous (650 ± 60 kg of BW and 59 ± 25 DIM) Holstein cows were assigned to 1 of 4 diets in a replicated 4x4 Latin square design with 21-d periods. Cows were fed once daily (1200 h) by means of a Calan gate system. Treatments consisted of diets containing 100% corn (CON), 67% corn and 33% hull-less barley (33B), 33% corn and 67% hull-less barley (67B), and 100% hull-less barley (100B) as the grain source. Total-tract nutrient digestibility was estimated using lanthanum chloride (LaCl3) as an external marker. The statistical model included the effects of square (fixed; df = 5), treatment (fixed; df = 3), square by treatment interaction (fixed; df = 15), period (random; df = 3), and cow within square (random; df = 18) and the random residual error. Dry matter intake differed quadratically among treatments (P < 0.01), being lowest for 67B (24.3 kg/d) and highest for 0B (26.9 kg/d). Feeding hull-less barley did not affect milk yield (41.4 kg/d, P > 0.98). Milk fat concentration differed cubically among treatments (P < 0.03), being lowest for 0B (3.43%) and highest for 67B (3.91%). Neither the concentrations in milk of protein (3.02%, P < 0.33) and lactose (4.82%, P < 0.58) nor the yields of protein (1.24 kg/d, P < 0.23) and lactose (1.98 kg/d, P < 0.30) differed among treatments. The proportion of de novo synthesized fatty acids in milk did not differ among treatments (P < 0.43). The apparent total-tract digestibility of dry matter (61.5%, P < 0.82), crude protein (61.1%, P < 0.28), and neutral detergent fiber (37.3%, P < 0.38) did not differ among treatments. The apparent total-tract digestibility of starch increased quadratically (P < 0.02), being lowest (97.3%) for 0B and highest (97.9%) for 67B, although these differences are biologically minor. In conclusion, hull-less barley grain is as good as corn grain as an energy source when formulating diets for high-producing dairy cows.

Key Words: hull-less (hulless) barley, digestibility, milk fatty acid

38  Effects of milk replacer feeding levels on performance and metabolism of pre-weaned dairy calves during summer. R. M. Orellana*, G. H. Komori, V. V. Beihling, T. N. Marins, J. K. Bernard, and S. Tao, University of Georgia, Tifton, GA.

Holstein calves (n = 39; initial body weight [BW] = 37.6 ± 5.2 kg) were used to evaluate the effects of 3 milk replacers (MR) feeding programs on performance and metabolism during summer. Calves were randomly assigned to 3 treatments including: control (CON, 0.57 kg/d of a 20% CP and 20% fat MR), intermediate (IL, 0.68 kg/d of a 26% CP and 17% fat MR [26:17]), or high (HL, 0.79 kg/d of 26:17), at 3 d of age (DOA). Calves were managed similarly and housed in polyethylene hutches on sand. Milk replacer (12% solids) was offered twice daily until 42 DOA when MR was fed once a day to reduce its intake by 50%. Calves were weaned at 49 DOA and remained in hutches until 56 DOA. Calf starter and water were offered ad libitum. Ambient temperature and relative humidity in and outside hutches were assessed hourly to calculate temperature-humidity index. Starter and MR intakes were recorded twice daily. Respiration rate and rectal temperature were determined thrice weekly. Body weight was measured at 3 DOA and at 2, 4, 6, 7, and 8 wk of age. Plasma was collected at 2, 5, 10, 14, 28, 42, 43, 45, 47, 49, 51, and 56 DOA to analyze glucose, β-hydroxybutyrate, triglycerides, and nonesterified fatty acids. Data were analyzed using the mixed procedure of SAS. During the trial, temperature-humidity index averaged 75 in and outside the hutches. There was no treatment effect on starter intake, rectal temperature or respiration rate (P > 0.57). By 7 DOA, calves fed IL and HL diets consumed the same amount of MR but higher than CON, but on wk 2 calves from all treatments had similar MR consumption before returning to the projected intake by design (treatment × time, P < 0.01). The IL and HL calves had similar BW but were heavier than CON on wk 6, 7, and 8 (treatment × time, P < 0.01). Calves fed IL and HL diets had similar average daily gain which was higher than CON (P < 0.05). There was no difference among treatments in plasma metabolites (P > 0.38). In summary, feeding an intermediate level of MR during summer improved calf growth than control diet, but a higher MR allowance did not support further improvements in calf performance.

Key Words: milk replacer, calf, heat stress
39 Effects of varying prepartum DCAD and calcium concentrations on pre- and postpartum mineral and metabolite concentrations. A. L. Diehl*, J. K. Bernard1, S. Tao1, T. N. Smith1, D. J. Kirk2, D. J. McClean2, and J. D. Chapman2. 1University of Georgia, Tifton, GA, 2Phibro Animal Health, Corp., Teaneck, NJ.

The objective of this trial was to determine the effects of feeding prepartum diets varying in DCAD and dietary Ca concentrations on blood metabolites and Ca status postpartum. Multiparous Holstein cows (82) were enrolled 28 d before calving and remained on trial through 63 DIM (91 d total). Cows were individually fed 1 of 4 prepartum dietary treatments in a randomized block design experiment with a 2 × 2 factorial arrangement. Treatments provided 2 DCAD concentrations: <22 (NEG) or ≥3 mEq/100 g DM (NEU); and 2 dietary Ca concentrations: 1.0 or 1.5% of DM. After calving cows were fed the same lactation ration. Urine and blood samples were collected once weekly before calving. Additional blood samples were collected 2 d before expected calving date, immediately after calving, and on 1, 2, and 3 DIM. Data were analyzed using PROC MIXED that included block, DCAD, Ca, interaction of DCAD and Ca, week, and interactions of week and treatments. Significance was declared at P < 0.05 and trends when P > 0.05 and <0.10. Urine pH was lower (P < 0.0001) for NEG compared with NEU and tended to be lower (P = 0.06) for 1.5% compared with 1% Ca. Prepartum concentrations of urine Ca were higher (P = 0.02) whereas urine creatinine and urine K were lower (P < 0.01) for NEG compared with NEU. Fractional excretion of Ca (5.91 and 2.86%) and Mg (21.36 and 14.96%) was greater (P < 0.0001) for NEG than NEU, respectively. Plasma total P was higher (P = 0.01) for 1% Ca compared with 1.5% Ca from 0 to 3 DIM, plasma total protein, albumin, total Mg, and ionized Mg concentrations were lower (P < 0.05) for NEG compared with NEU. Interactions of DCAD and DIM (P < 0.05) were observed for plasma total Mg and ionized Mg, as concentrations were similar on 0 and 3 DIM but were higher for NEU on d 1 and 2. An interaction of Ca and DIM was observed for plasma total Ca due to higher concentrations (P < 0.05) at 1 DIM for 1.5%, whereas concentrations were not different at 2 or 3 DIM. These results suggest that feeding <22 mEq/100 g DM prepartum alters plasma and urine mineral concentrations to improve calcium status by 3 DIM compared with feeding ≥3 mEq/100 g DM. Feeding 1.5% Ca prepartum improved plasma Ca at 1 DIM only.

Key Words: DCAD, calcium, plasma minerals

41 Evaluation of low concentrations of rumen degradable protein in the diet of lactating dairy cows: A meta-analysis. J. D. Kaufman* and A. G. Rius, The University of Tennessee, Knoxville, TN.

The objective of the present study was to conduct a meta-analysis that summarizes the effect of reducing dietary rumen degradable protein (RDP) proportions on dry matter intake (DMI) and milk production in dairy cows. The data set was identified using 2 search engines comprising of 41 studies with 109 observations. Means were weighted by the inverse of their variance. Treatments of RDP (% of DM) were organized as very low (<8% RDP), low (8% ≤ RDP <9%), medium (9% ≤ RDP <10%), and high (≥11% RDP) and compared with a control (10% ≤ RDP ≤11%). Comprehensive Meta-Analysis v3 (Biostat, Englewood, NJ) was used to evaluate the raw mean difference (RDP treatment – RDP control) in a random-effects model. Variables evaluated were DMI and yields of milk and milk protein. Moderators included were experimental design of the studies (change-over or continuous), concentration of dietary energy [medium (<1.6 Mcal of net energy of lactation per kg of DM) or high (≥1.6 Mcal of net energy of lactation per kg of DM)], and concentration of dietary rumen undegradable protein (RUP; < 6 or ≥6% of DM). Statistical significance was identified at P ≤ 0.05. Heterogeneity was present, and publication bias was absent. High RDP did not affect DMI and production (n = 47). Compared with control RDP, low RDP sustained DMI and milk production (n = 12), and medium RDP sustained yields of milk and milk protein (n = 40). However, medium RDP reduced DMI in diets with medium and high energy content (0.45 kg/d; P < 0.02). Low and high RDP reduced DMI and milk production in continuous design trials (n = 56; P < 0.05); whereas, RDP did not affect the evaluated variables in change-over trials. Trials with RUP <6% (n = 26), medium RDP decreased DMI (0.50 kg/d; P < 0.03), but at RUP ≥6%, lowering RDP did not affect production. In summary, lowering RDP proportions may not always reduce DMI and milk production in dairy cows. However, experimental design of the study, dietary energy, and dietary RUP may influence production responses to lowering RDP.

Key Words: meta-analysis, milk production, rumen degradable protein
42 Comparing summer to winter ratios of milk production and SCS among states in the southeast United States. J. Guinn*1, D. Nolan1, P. Krawczel2, C. Petersson-Wolfe3, G. Pighetti2, A. Stone1,4, S. Ward4,5, and J. Bewley1. 1University of Kentucky, Lexington, KY, 2University of Tennessee, Knoxville, TN, 3Virginia Polytechnic Institute and State University, Blacksburg, VA, 4Mississippi State University, Starkville, MS, 5North Carolina State University, Raleigh, NC.

The southeastern United States experiences hot, humid summers causing heat stress, resulting in reduced milk production and milk quality. The objective was to compare summer:winter ratios for milk production and somatic cell score (SCS) among states in the Southeast United States. Data from DHIA were collected from 2007 to 2016 for herds in Florida (n = 91), Georgia (n = 205), Kentucky (n = 413), Mississippi (n = 47), Tennessee (n = 183), and Virginia (n = 479). The summer:winter ratio was developed as a metric to estimate alleviation of heat stress by cow cooling strategies. This study used the ratios to compare milk production or SCS in summer (June 21-September 21; numerator) vs. winter (December 21-March 19; denominator) using test day averages for herds within state. The underlying assumption is that the closer the ratio is to one, the less heat stress is experienced by the cows within the herd. The GLM procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC) was used to analyze the lsmeans of milk production and SCS summer:winter averages and ratios for herds within state. Results are shown in Table 1. Results show that the milk production summer:winter ratio is highest for Virginia, and lowest for Mississippi, indicating that cows in Virginia herds experience less heat stress. Ratios for SCS indicate that cows in Florida herds experience the most heat stress in the summer. The summer:winter ratios could be widely available for producers and consultants as a tool to assess on-farm heat abatement and encourage improvement in their cow cooling strategies. Further analyses will investigate effects of cow cooling strategies specific to herds in this region in relation to THI, on summer:winter ratios.

Table 1 (abstract 42).

<table>
<thead>
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<th>Winter (W)</th>
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<tr>
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<td>0.93 ± 0.009b</td>
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Key Words: summer:winter ratio, heat stress, milk production
Animal Behavior and Well-Being I

43 Which data recorded by automated calf feeders can help to detect sick calves? C. Medrano-Galarza1,2, L. J. LeBlanc1,3, T. J. De Vries4, J. Rushen1, A. M. de Passillé1, A. Jones-Bitton1, M. I. Endres4, and D. B. Haley1,3, 1Department of Population Medicine, University of Guelph, Guelph, ON, Canada, 2Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, 3Faculty of Land and Food, University of British Columbia, Vancouver, BC, Canada, 4Department of Animal Science, University of Minnesota, St. Paul, MN, 5Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, ON, Canada.

Automated calf feeders continuously record individual calf feeding behavior data that could help detect illness before clinical signs and monitor recovery. The aim of this study was to evaluate the association between calf health status and milk feeding behavior. Thirty-seven dairy calves with diarrhea or respiratory disease from 2 farms in Ontario, using high milk allowance (9 to 10 L/d), were pair-matched to healthy calves by days on the feeder. Data collected by the feeder for each calf were daily milk intake, drinking speed and duration of each visit, and frequency of visits. The day producers treated a sick calf corresponded to d0. Data from 7 d before and after d0 were analyzed. Differences in daily feeding behavior between sick and healthy calves were evaluated using a repeated measures general linear mixed model. Day, health status, farm, and the interaction between day and health status were included as fixed effects. Matched-pair identification was included as a random effect. Compared with healthy calves, sick calves drank less milk in the 5 d before treatment (d−5: −0.9L, P < 0.03; d−4: −1.1L, P < 0.01; d−3: −1.6L, P < 0.001; d−2: −1.7L, P < 0.001; d−1: −2.4L, P < 0.001), d0 (d0: −2.1L, P < 0.001), and the 2 d following treatment (d+1: −1.1L, P < 0.01; d+2: −1.0L, P = 0.01). Among healthy calves, there were no differences in milk intake between days, whereas for sick calves, there was a drop (P < 0.001) in milk intake from d−5 (6.8L/d) to d0 (5.2 L/d), and an increase (P < 0.001) after treatment day until d+5 (7.4L/d). Sick calves drank more slowly than healthy calves (mean speed: 0.4 vs. 0.6 L/min, P < 0.001). Among sick calves, drinking speed dropped between d−1 and d0 (0.06 L/min; P = 0.01), and increased after treatment by 0.07 L/min on d+1 (P = 0.01), and by 0.1 L/min on d+5 (P < 0.001). There was no association of day and no health status × day interaction for the frequency of visits to the feeder or visit duration. In summary, sick calves started to decrease milk intake 5 d before treatment, and drank more slowly only on the day before treatment. Therefore, milk intake could be a useful indicator to help detect sick calves and monitor recovery.

Key Words: calf, health, feeding behavior

44 Daily milk consumption, number of visits, drinking speed and weight gain of preweaned calves in Midwest US farms with automated feeders. M. Peiter*, M. Jorgensen, and M. I. Endres, University of Minnesota, St. Paul, MN.

It is becoming more common in the United States to house preweaned dairy calves in groups and feed them using computerized automated calf feeders. However, limited research has been conducted in the USA to describe behaviors of calves when using these feeders. The objective of this observational study was to characterize daily milk consumption, calf drinking speed, number of calf visits to the feeder (rewarded and unrewarded), and calf daily weight gain in 25 farms in the Upper Midwest using automated feeders to feed their preweaned calves; data were collected for a period of approximately 18 mo. We used PROC MEANS in SAS to calculate means and SD for each variable across all farms. Experimental unit for the analysis was calf/day recorded by the feeder software. We found that drinking speed (mL/min) was 793.6 ± 324.0 (n = 54,747) with a mean/farm ranging from 441.5 to 1,112.5 mL/min. The average daily milk allowance (L/calf) was 8.72 ± 2.29; calves consumed 87.0 ± 20.6% (n = 62,548) of their milk allowance resulting in an estimated daily milk intake of 7.59 L/calf. Mean estimated daily milk intake/farm ranged from 5.5 to 11.6 L/calf. The number of daily rewarded visits (visits when calf is entitled to receive milk) was 4.77 ± 3.40 (n = 53,798); mean/farm ranged from 2.45 to 6.86 visits; however, most farms averaged between 4 and 6 visits. The number of unrewarded visits (visits without milk) was 6.52 ± 7.73 (n = 53,798); mean/farm ranged from 0.96 to 9.94 visits. Daily weight gain (g/d) was 803.5 ± 262.9 (n = 60,205); mean/farm ranged from 568.7 to 1,130.6 g/d. The farm with the greatest milk allowance had the greatest daily weight gain per calf. These behavior and weight gain measurements are most likely influenced by differences in housing and management practices across farms. In addition, some of the feeding behavior measurements recorded by the autofeeder software have been shown to be associated with health outcomes in previous university farm studies. Can we detect these associations across farms in this observational study? Further analysis will explore these relationships.

Key Words: calf feeder, feeding behavior, drinking speed

45 Assessment of the effects of oral administration of acetylsalicylic acid on biomarkers of inflammation and stress in dairy cows after parturition. A. A. Barragan*,1 L. M. Bauman1, J. Lakritz2, J. F. Coetzee2, J. Velez3, J. D. Rozo Gonzalez3, G. M. Schuenemann1, and S. Bas1, 1Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 2Department of Animal Sciences, The Ohio State University, Columbus, OH, 3Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, 4Department of Anatomy and Physiology, Kansas State University, Manhattan, KS, 5Aurora Organic Farms, Boulder, CO.

Dystocia (DYS), defined as a difficult birth resulting from prolonged calving or severe assisted extraction, not only affects the calf, but also negatively impacts the health, survival and productivity of the dam. Furthermore, DYS has been subjectively described by bovine veterinarians as one of the most painful and stressful events that dairy cattle can experience, and is characterized by a severe inflammatory process. The objective of this study was to assess the effect of an oral treatment with acetylsalicylic acid after calving on biomarkers of inflammation and stress in lactating dairy cows. Animals from 3 organic dairy herds were included in the present study. Cows were blocked by parity and calving ease (eutocia [EUT]; DYS) at parturition, and were randomly assigned to 2 groups: (1) ASP (n = 76): at ~12 h after parturition cows received 4 treatments with acetylsalicylic acid (100 mg/kg; 2 boluses) at approximately 12 h intervals; or (2) PLC (n = 76): at ~12 h after parturition cows received 4 consecutive treatments with gelatin capsules (2 capsules) containing water every 12 h. Blood samples were collected immediately before treatment, at 12 h, 24 h, 36 h and 48 h, and at 7 ± 3 DIM for assessment of circulating concentration of biomarkers of inflammation (haptoglobin; HP) and stress (cortisol). The data were analyzed using ANOVA by the MIXED procedure of SAS as a randomized block design with repeated measures. Cows in the ASP group had
lower ($P < 0.05$) concentration of HP at 24 h after parturition ($ASP = 65 \pm 10.11 \mu g/mL$; $PLC = 110 \pm 9.86 \mu g/mL$) and tended ($P = 0.09$) to have lower concentration of HP at 36 h after parturition. Furthermore, cows with DYS had higher ($P < 0.05$) concentration of HP at 24 h, 36 h, 48h and 7 d after parturition compared with EUT cows. No difference in circulating concentration of cortisol was observed between treatment groups. The results from this study suggest that a short duration anti-inflammatory therapy after calving may decrease inflammatory response in dairy cows early in the postpartum, when the discomfort from parturition may be the highest.

Key Words: parturition, acetylsalicylic acid, haptoglobin

46 Holstein calf behavioral responses to acidified milk. A. Adams Progar*, A. Deml, R. Pernu, H. A. Young, and J. Callanan, Washington State University, Pullman, WA.

Feeding acidified milk to preweaned dairy calves is a popular method to reduce bacterial contamination in milk and promote ad libitum feeding, especially in group housing systems. The objective of this study was to evaluate calf drinking behavior when offered acidified milk versus non-acidified milk. Ten one-day-old Holstein heifer calves were offered either acidified or non-acidified milk on alternating days, with milk on day 1 randomly assigned. The study concluded upon weaning (6 wk of age). Calves were offered 4 L of milk (per feeding) daily at 5:30 and 14:00. The amount of milk refusals were recorded at every feeding for each calf. Calf behavior was recorded during each feeding using video cameras and body weights were recorded every 7 d until weaning. Video footage was analyzed to document the amount of time required to finish drinking and the number of breakaways (calf stopped drinking, remained in the feeding area, and then proceeded to drink) per feeding for each calf. Data were analyzed as a randomized complete block design with repeated measures using SAS. The type of milk offered during Day 1 did not affect overall ADG ($P = 0.77$). When offered acidified milk, calves refused $2.50 \pm 0.12$ L more milk ($P < 0.0001$) and demonstrated $0.45 \pm 0.11$ more breakaways ($P < 0.0001$) during feedings than when offered non-acidified milk. As calves aged, they refused less milk ($P < 0.0001$) and demonstrated fewer breakaways during feedings ($P < 0.0001$). Holstein calves in this study exhibited more aversive behaviors toward acidified milk than non-acidified, unpasteurized milk; however, the frequency of these behaviors decreased as calves aged. When feeding ad libitum acidified milk to group-housed calves, it is important to monitor individual calf behavior to ensure all calves are drinking sufficient amounts to promote good health and growth.

Key Words: acidified milk, calf behavior, milk refusals

47 Clinical trial of local anesthetic protocols for acute pain associated with caustic paste disbudding in dairy calves. C. Winder*, L. S. LeBlanc, D. Haley, K. Lissimore, M. Godkin, and T. Duffield, 1Dept. of Population Medicine, University of Guelph, Guelph, ON, Canada, 2Ontario Ministry of Agriculture, Food, and Rural Affairs, Elora, ON, Canada.

Caustic paste disbudding is becoming more commonplace in North America, but few studies have evaluated pain control for this procedure. The objective of this clinical trial was to evaluate the effects of either a lidocaine cornual nerve block or a topical anesthetic incorporated into caustic paste on the acute pain of caustic paste disbudding. Seventy-two Holstein-Friesian calves housed in groups with an automated milk feeder were enrolled into 18 replicates balanced on age and assigned to one of 4 treatments: sham (S), a placebo paste and a saline cornual block; topical (T), a novel caustic paste containing lidocaine and prilocaine, and a saline cornual block; cornual block (B), commercial caustic paste and a lidocaine cornual nerve block; and positive (P), commercial caustic paste and a saline cornual block. All calves received 0.5 mg/ kg meloxicam SC at the time of the block. Researchers were blinded to treatment group. Primary outcomes were validated pain behavior responses, and pain sensitivity measured by algometry. Secondary outcomes consisted of respiratory and heart rate, latency to approach the evaluator, play behavior, feeding behavior, and standing and lying bout characteristics. Data were analyzed using linear, Poisson, and negative binomial regression models. Cornual-blocked calves had less pain sensitivity to 180 min after disbudding than all other groups ($P < 0.001$); T and P calves had more pain sensitivity than S calves for the same period ($P < 0.001$). Compared with T and P calves, B and S calves had fewer pain behaviors until 120 min post-disbudding ($P < 0.05$) and decreased respiratory and heart rates ($P < 0.01$). S calves exhibited more play behavior than other groups ($P < 0.001$) and tended to have a shorter latency to feed than T or P calves ($P < 0.10$). Caustic paste appears to be acutely painful for at least 180 min, and this is reduced by a cornual nerve block but not by our novel paste. We recommend that calves disbudded with caustic paste receive local anesthetic by a cornual nerve block to mitigate acute pain.

Key Words: cornual nerve block, pain control, behavior

48 Effects of acute lying and sleep deprivation on behavior and productivity of Holstein dairy cows. J. A. Kull*, G. M. Pighetti, K. L. Produnfoof, J. M. Bewley, B. F. O’Hara, K. D. Donohue, and P. D. Krawczel, 1Department of Animal Science, The University of Tennessee, Knoxville, TN, 2Department of Preventive Veterinary Medicine, The Ohio State University, Columbus, OH, 3Department of Animal and Food Sciences, University of Kentucky, Lexington, KY, 4Department of Biology, University of Kentucky, Lexington, KY, 5Department of Electrical and Computer Engineering, University of Kentucky, Lexington, KY.

The objective of the study was to determine the effects of sleep or lying deprivation on the behavior and productivity of dairy cows. Data were collected from 8 multi- and 4 primiparous cows (DIM = 199 ± 44 (mean ± SD); days pregnant = 77 ± 30). Each cow experienced: 1) 24 h sleep deprivation from noise or physical contact and 2) 24 h lying deprivation imposed by a wooden grid placed on pen floor that prevented a recumbent position. An 11-d collection period (from 2 d before the first treatment (trt) to 8 d after trt) was followed by 12-d washout periods. Study days were organized from 2100 to 2059. During habituation (d −2 and −1 before trt), baseline (d 0), and trt (d 1), housing was individual stalls (mattress with no bedding). After trt, cows returned to sand-bedded freestalls for a 7-d recovery period (d 2 to 8). Lying behaviors were recorded by accelerometers attached to the hind leg. Milk yield was recorded 2 × daily. NEFA and glucose concentrations were evaluated from serum sampled at 0300, 0900, 1500, and 2100 on d 1 and 2. Data were analyzed using a mixed model in SAS including fixed effects of trt, day, and their interaction with significant main effects separated using a PDIF test ($P \leq 0.05$). Interactions between trt and day were evident for lying time and bouts ($P < 0.001$). Lying time decreased during trt and increased on the first day of recovery for lying deprivation compared with sleep (d 1: 1.9 vs. 8.4 ± 0.7 h/d (mean ± pooled SE); $P < 0.001$; d 2: 16.8 vs. 13.6 ± 0.7 h/d; $P = 0.002$). Lying bouts were greater during sleep deprivation (d 1: 7.6 vs. 4.1 ± 0.8 n/d; $P = 0.01$). Milk yield decreased during lying deprivation compared with sleep on d 2 (31.8 ± vs. 35.3 ± 2.4 kg/d; $P = 0.002$). NEFA and glucose were not affected by trt ($P \geq 0.70$), but did vary by time ($P \leq 0.03$).
The combination of sustained energy balance, behavioral changes, and decreased productivity suggest cows shifted resources toward increased maintenance during periods of deprivation. Collectively, this suggests lack of access to resting resources rather than the relative comfort of that resource once accessed may have greater long-term effects on the welfare of dairy cows.

**Key Words:** behavior, dairy cow, deprivation

Dairy cow preference for outdoor access in summer and winter. A. M. C. Smid*, E. E. A. Burgers¹,², D. M. Weary¹, E. A. M. Bokkers², and M. A. G. Von Keyserlingk¹, ¹University of British Columbia, Vancouver, BC, Canada, ²Wageningen University, Wageningen, Gelderland, the Netherlands.

Freestall-housed dairy cows, when given the option during the summer, have a strong preference to access pasture during the night but remain indoors for the majority of the day. Given that pasture access is not feasible for all farms or in all seasons, there is merit to investigate alternative forms of outdoor access. This study tested the preference of freestall-housed dairy cows to access an outdoor deep-bedded bark mulch pack (versus remaining inside the barn) in summer and winter. For both seasons, 72 lactating pregnant cows were assigned to 6 groups of 12 animals. Groups were formed and allowed to stabilize for 5 d, followed by 2 d of baseline recording to assess location and feeding behavior. Habituation to the outdoor pack took place for the next 2 d during which the cows were moved outside at 5 set times each day (if cows were not already outdoors). Cows were then provided free choice access to the outdoor pack continuously for 5 d (Choice phase). During the Choice phase, the location of each cow (i.e., in the free-stall pen or the outdoor pack) was noted using 5-min scan sampling. Data were analyzed using a mixed model with group treated as the experimental unit. Cows spent a mean (±SE; min - max) of 21.7% (±4.6; 9.0 – 37.0) of the time outside in summer and 11.1% (±6.1; 0.1 – 33.4) in winter. Time spent in the outdoor pack was higher during the night (i.e., between 20:00 and 07:00 h) than during the day (between 07:00 h and 20:00 h), both in the summer (day: 3.3% ± 1.5; 0.8 – 9.2 vs. night: 43.6% ± 9.1; 19.0 – 72.1) and the winter (day: 6.1% ± 3.6; 0.3 – 23.2 vs. night: 17.0% ± 9.6; 0 – 49.0). Total daily time spent feeding indoors did not differ between the Baseline and Choice phases for both the summer and winter. In summary, cows displayed a partial preference to be outside on a bark mulch pack when given the opportunity, especially at night in both summer and winter.

**Key Words:** animal welfare, outdoor area, free range
The main objectives of the current study were to estimate the bulk tank milk prevalence of 4 main mastitis pathogens (Staphylococcus aureus, Proteotheca, Streptococcus agalactiae and Mycoplasma bovis) and determine the characteristics of herds that test positive. As part of the national study 374 farms were randomly selected from 10 provinces and visited between May and August in 2015 where an assessment of milking hygiene and other farm characteristics was undertaken. A sample of bulk tank milk was collected by the respective milk carriers between December 2015 and January 2016 and submitted for testing at CanWest DHI milk laboratory (Guelph, ON, Canada). All samples were tested using PathProof Mastitis Major 4 PCR Assay (Thermo Fisher Scientific Inc., Waltham, MA) according to manufacturer’s instructions. Production data for all Canadian farms were obtained from the provincial marketing boards. Based on the results of 372 milk samples, the apparent prevalence for each pathogen at the national level was as follows: 172 farms tested positive for S. aureus (46.2%; 95% CI: 41.4–51.6), 24 farms were positive for Proteotheca (6.4%; 95% CI: 3.9–8.9), 2 farms were positive for M. bovis (0.5%; 95% CI: −0.2–1.3), and one farm was positive for S. agalactiae (0.3%; 95% CI: −0.3–0.8). Risk factor analysis for farms testing positive for S. aureus, based on multivariate logistical regression, determined that statistically significant characteristics were: pipeline milking systems (odds ratio 2.6; P < 0.0001), not tagging chronically infected animals (odds ratio 2.10; P = 0.010), and not fore-stripping (odds ratio 1.81; P = 0.039) while using dry cow therapy on every quarter of every cow was protective (odds ratio 0.45; P = 0.035). This is the first time Canada has been able to determine national prevalence data on these 4 common mastitis pathogens. The apparent prevalences are lower than those reported previously by individual regions or using non-random sampling.

Key Words: bulk tank milk, Canadian National Dairy Study, mastitis


It is generally known that the milk electrical conductivity measurement could be used for mastitis detection. REM test (Rapid electronic mastitis test) which was developed in our institute is a handheld apparatus which can measure EC of milk from each quarter of the udder. The accuracy of measurement of REM test was tested in laboratory and subsequently in practical conditions. The results have shown the accuracy was excellent (1% from measurement range). The statistical value of electrical conductivity of healthy cows was 4.8 mS/cm (sd 0.3 mS/cm) and cows with mastitis was 6.5 mS/cm (sd 0.8 mS/cm). The difference between them was significant (P < 0.01). In practical conditions absolute EC values of healthy cow changed up to 10 mS/cm between milking but the differences of EC between quarters was less than 0.6 mS/cm. Absolute value of milk EC of single quarter has substantially lesser sensitivity for mastitis detection then IQD (inter quarter difference). The differences of EC between quarters in the healthy udder were under 0.6 mS/cm. If EC of some quarter was higher than 0.6 mS/cm (opposite of the quarter with the lowest value) then the quarter was suspicious of mastitis. When EC values of all quarters in the udder were higher than 6.0 mS/cm and differences between them were less than 0.6 mS/cm, than the cow was suspicious of another illness than mastitis. The correlation coefficient between absolute value of EC and SCC of single quarters was r = 0.63. The sensitivity of the mastitis detection by means of milk EC measurement was calculated as 87% and the reliability 92%. It was evaluated for IQD with threshold difference 0.6 mS/cm and for cows with mastitis which have SCC > 500 000. Development of REM test was possible through projects APVV of the Slovak Research and Development Agency Bratislava (0632–10 and 15–0060), and the project CEGEZ 26220120073 supported by the Operational Programme Research and Development funded from the European Regional Development Fund.

Key Words: mastitis, milk components, activity

Laboratory evaluation of on-farm culture-based mastitis tests and the potential effect on treatment decisions. K. Griffioen*1, L. A. LagerwerF2, R. P. Achterberg3, J. B. W. J. Cornelissen4, D. J. Mevius1, F. J. van der WalF2, R. Dijkman2, A. E. Heuvelink2, M.
Using on-farm tests could improve mastitis treatment decisions. Therefore this study aimed to evaluate on-farm mastitis tests using milk samples submitted for bacteriological culture (BC) with BC as the reference method. Further, the consequences of using on-farm tests to make selective treatment decisions in case of clinical mastitis (CM) were determined. For that 842 milk samples submitted to GD Animal Health laboratory in April–July 2016 were used, each cultured for BC and on the 4 evaluated on-farm tests. Of each test the sensitivity (Se), specificity (Sp), positive predictive value, negative predictive value and accompanying 95%CI’s were determined based on the (dis)agreement with BC for the result categories gram-positive, gram-negative, no growth (NG) and mixed. The Se of the on-farm tests for the result categories gram-positive and gram-negative were high (range 83–92% and 80–89% resp.), as well as the Sp for gram-negative, NG and mixed (range 89–90%, 88–96% and 92–98% resp.). The Sp for gram-positive results was lower (range 33–46%) just as the Se for NG and mixed (range 24–46% and 7–18% resp.). Assuming that gram-positive results would be treated with first-choice antibiotics, gram-negative and mixed results with second-choice antibiotics and NG would not be treated with antibiotics, 68–79% of all CM cases would be treated with first-choice antibiotics, versus 47% if the BC result would be used for treatment decisions. The use of second-choice antibiotics would decrease (>6%), but overall more cases would be treated with antibiotics if the decision would be based on the on-farm test result compared with if the BC result would be used. The low Se of the on-farm tests for NG would result in on average 8% of the CM cases left untreated while they had to be treated with an antibiotic. In conclusion, the Se and Sp of the on-farm mastitis tests for gram-negative results were high. The low Sp and Se of gram-positive results and NG resp., could result in an increased number of antibiotic treatments compared with if BC would be used. The low Se of the on-farm tests for NG would result down to 2 × 104 cfu/mL. Control milk did not show a signal in any of the assays tested. Acu-PCR assays were able to detect the presence of Staph. aureus down to 1 × 104 cfu/mL and the STREP assay detected dilutions of Strep. uberis down to 2 × 104 cfu/mL. Control milk did not show a signal in any of the assays tested. Acu-PCR assays were able to detect the presence of Staph. spp., Staph. aureus and other Staph. spp. (STAPH, all Acumen Detection LLC). The STAPH assay detected dilutions of Staph. aureus down to 1 × 104 cfu/mL and the STREP assay detected dilutions of Strep. uberis down to 2 × 104 cfu/mL. Control milk did not show a signal in any of the assays tested. Acu-PCR assays were able to detect the presence of Staph. spp., Staph. aureus, Strep. dysgalactiae and Strep. uberis in clinical samples confirmed by MALDI TOF. Ongoing testing is assessing specificity and sensitivity for each assay. The results show that the Acu-PCR Milk Prep Kit and the tested Acu-PCR assays are capable of detecting their respective targets in clinical milk samples.

**Key Words:** mastitis, diagnostic, quantitative real-time PCR

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**55 In silico identification of natural product inhibitors of Staphylococcus aureus threonyl-tRNA synthetase.** M. Li1,2, N. Zheng1,2, S. L. Li1,3, S. G. Zhao1,4, F. Wen1,3, Y. D. Zhang1,4, and J. Q. Wang*1,2, 1Ministry of Agriculture-Key Laboratory of Quality & Safety Control for Milk and Dairy Products, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2Ministry of Agriculture-Laboratory of Quality and Safety Risk Assessment for Dairy Products, Beijing, China, 3Ministry of Agriculture-Milk and Dairy Product Inspection Center, Beijing, China, 4State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

Gram-positive bacterium *Staphylococcus aureus* (*S. aureus*) is one of the most common pathogens causing bovine mastitis, which is a worldwide problem in dairy herds. The emergence and spread of methicillin-resistant and other antibiotic-resistant strains warrants the need for developing inhibitors based on novel targets. Previous research has shown that bacterial threonyl-tRNA synthetase is a promising target for inhibitors development. In this study, a 3-dimensional structure of *S. aureus* threonyl-tRNA synthetase (*SaThrRS*) was obtained from RCSB Protein Data Bank (PDB ID: 1NYR). The ZINC natural product database including 11247 compounds was used for virtual screening against the ATP binding site of the target using AutoDock Vina program. The lead compounds were selected based on their affinities to the target. Since the binding free energy of selective inhibitor (borrelidin) was −9.3 kcal/mol, 173 compounds whose binding free energy was not greater than −10 kcal/mol were selected as potential inhibitors using excel in microsoft office program. The top 5 hits, with the binding free energy lower than −11 kcal/mol, are ZINC14778984 (−11.3 kcal/mol), ZINC67911489 (−11.1 kcal/mol), ZINC77269158 (−11.1 kcal/mol), ZINC33861464 (−11.0 kcal/mol), and ZINC77269178 (−11.0 kcal/mol). Among them, ZINC14778984 (−11.3 kcal/mol) was best lead because of its highest inhibitory activity. The binding site of ZINC14778984 on SaThrRS was analyzed using Discovery studio 2.5 program. ZINC14778984 was located in a pocket consisted of 28 residues, which were MET334, ASN335, CY5336, MET363, ARG365, GLU375, GLN376, ARG377, VAL378, MET381, LEU383, ASP385, SER386, HIS387, LYS471, ASP473, THR485, LEU486, THR488, GLN490, HIS517, ARG518, GLYS519, SER522, THR523, GLUS525, and ARG526. This study demonstrates the success of the screening method resulting in 5 novel SaThrRS inhibitors with potential for treating bovine mastitis.

**Key Words:** *Staphylococcus aureus*, threonyl-tRNA synthetase, molecular docking
Clinical mastitis (CM) is the most prevalent and costly disease in dairy cows. Our objective was to develop for biomarker and pathway discovery a robust analytical workflow that can identify global serum metabolomic changes in healthy, close-up dairy cows that subsequently did (MastitisPost; n = 8) or did not (Control; n = 9) develop CM. Using a nested case-control design, we measured weekly serum signatures during the prepartum transition period and directly after calving. For analysis, we combined ethanol extraction, ultra-performance liquid chromatography coupled with quadrupole ion mobility time-of-flight mass spectrometry for data acquisition, and self-organizing maps for visualization of high-dimensional data. A repeated measures-in-time analysis of natural log-transformed data was conducted in PROC MIXED of SAS version 9.4. Fixed effects were collection time, group, and their interaction. Repeated measures within cows were modeled using a first-order heterogeneous variance-covariance matrix. Metabolomic analysis revealed global measures within cows were modeled using a first-order heterogeneous variance-covariance matrix. Metabolomic analysis revealed global changes in amino acids and their metabolites, phospholipid precursors, acylcarnitines, and conjugated bile acids; their signal intensities were higher 21 d before calving and decreased stronger until calving in MastitisPost vs. Control cows. Complete separation of groups were observed at −21, −14, −7 d before and directly after calving between MastitisPost vs. Control cows for 18, 7, 0, and 1 of a total of 81 annotated metabolites, respectively. Free carnitine, trimethyllysine, proline, tyrosine, choline, phosphocholine and methylethanolamine phosphate had complete separation at the first 2 time points. The most consistent single biomarker was 3′ sialyl-lactose (signal intensities were 14.4-, 4.1-, 2.6-, and 2.3-fold higher at 21, 14, 7 d before and directly after calving, respectively, in MastitisPost vs. Control cows). We conclude that LC-MS metabolomic serum signatures indicate that global challenges in protein and lipid metabolism precede CM in transition dairy cows.

**Key Words:** clinical mastitis, early disease indicator, metabolomics

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58 **Effects of the environmental origin of milk microbiota on milk biodiversity.** K. B. Fehr*, H. Derakhshani, S. Sepehri, J. C. Plaizier, and E. Khafipour, University of Manitoba, Winnipeg, MB, Canada.

Bovine mammary gland inflammation, known as mastitis, is one of the most costly infectious diseases to the dairy industry. The goal of this research is to identify whether the environmental origin of milk microbiota influences milk biodiversity and mammary inflammation under different farm systems. To achieve this goal a cross-sectional survey was carried out on 3 dairy farms each using a different bedding type [straw (ST), SSand, (SA) and recycled bedding material (RBM)]. To identify the diversity of microbiota in milk and in environmental sources, milk, teat-end swab and fecal samples were collected from each selected cow (n = 137), along with used bedding samples (n = 50) representative of each farm. Mammary gland inflammation was also estimated for each cow using milk somatic cell count (SCC). Using a Bayesian approach (SourceTracker), it was estimated that the overall environmental origin of the milk microbial communities (including teat-end, bedding and fecal sources) for farm RBM and farm SA was 47% and 43.5% respectively, while for farm ST was only 25%. Additional evidence of a difference in environmental contribution to milk between farms is that the bacterial diversity of milk was significantly different (and lower) than that of the teat-end for only farm ST (Shannon index, P < 0.05). In addition, farm ST had a lower bacterial diversity compared with farm RBM and SA (Shannon index, P < 0.05). Interestingly, both the proportion of milk microbiota originating from feces and the teat-end were positively correlated with milk bacterial diversity (Shannon index) (P < 0.01, r > 0.39 for all farms). Meanwhile, the proportion of microbiota in milk originating from bedding only influenced milk bacterial diversity (Shannon index) (r = 0.31, P < 0.05). While the proportion of microbiota from these sources was not correlated with milk SCC at the time of sampling (P > 0.05), milk bacterial diversity may be important to long-term stability of mammary inflammatory status. These results indicate that differences in milk biodiversity between farms is related to the level of microbiota originating from environmental sources such as bedding material.

**Key Words:** mastitis, microbiota, Escherichia coli

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Mammary inflammation, known as mastitis, is a major constraint to milk production and quality. Previous research suggests that changes in the bacterial composition of bovine milk are associated with changes in
mammary inflammation, and are influenced by factors such as a cow’s parity and farm management, which includes bedding material used. A goal of this research was to find evidence in support of this notion. To achieve this, a cross-sectional study was carried out to compare bacterial diversity of aseptically sampled milk from cows (n = 137) in different parities (multiparous vs. primiparous) housed on 3 farms each using a different bedding material [sand (SA), straw (ST), and recovered bedding material (RBM)]. Composite milk somatic cell count (SCC) was measured and used as an indicator of mammary inflammation. A $\beta$-diversity analysis using UniFrac distances revealed that milk microbial communities were distinct between farms ($P (PERMANOVA) < 0.0001$) and parities ($P (PERMANOVA) = 0.045$). In addition, fewer bacterial species were identified in milk of primiparous compared with multiparous cows ($P < 0.05$); also, fewer bacterial species were identified in milk of primiparous compared with multiparous cows ($P < 0.05$). The predominant phyla identified, *Proteobacteria*, was proportionally more abundant ($q_{FDR} < 0.0001$) in farm ST (72 ± 19.0%) compared with farm SA (50 ± 20.5%) and farm RBM (51 ± 21.7%). Furthermore, within farms ST and SA, milk SCC was positively correlated with the proportion of *Staphylococcus*. On farm ST only, milk SCC was also negatively correlated with the proportion of some genera (*Methylobacterium, Raistonia*, unclassified Beta proteobacteria, unclassified *Comamonadaceae* and unclassified *Rhodospirillaceae*) indicating potential for these microbiota to help limit mammary inflammation. While cows sampled from farm RBM had a higher SCC than the other 2 farms ($P < 0.05$), no genera could be correlated with milk SCC for this farm. Our results are in support of the idea that there are between-farm differences in milk bacterial diversity and in how milk microbiota are associated with mammary inflammatory status.

**Key Words:** mammary gland, microbiota, mastitis

60 Casein hydrolysate for involution of a single mastitic quarter in dairy cows. D. J. Wilson*, J. E. Britten, and K. A. Rood, Utah State University, Logan, UT.

Chronic mastitis in a single quarter can cause high SCC, clinical signs, or death in dairy cows. Casein hydrolysate (CH) is an intramammary infusion treatment reported to induce mammary involution. Objectives were to study cessation of milk production after CH infusion of one mastitic quarter for remaining lactation, subsequent 3-quartered cow milk production and SCC. Following the next calving, cow and quarter milk production, SCC and bacterial cure were evaluated. Criteria for study cows were: target quarter SCC >10^6/mL, total milk SCC <500,000/mL, target quarter producing >20% (front) or >25% (rear) of cow milk, cow producing >22.7 kg of daily milk, 75–190 d until next due date, and mycoplasma-negative. Cows were blocked based on parity and farm management, which includes bedding material used. Casein hydrolysate (CH) is an intramammary infusion treatment reported to induce mammary involution. Objectives were to study cessation of milk production after CH infusion of one mastitic quarter for remaining lactation, subsequent 3-quartered cow milk production and SCC. Following the next calving, cow and quarter milk production, SCC and bacterial cure were evaluated. Criteria for study cows were: target quarter SCC >10^6/mL, total milk SCC <500,000/mL, target quarter producing >20% (front) or >25% (rear) of cow milk, cow producing >22.7 kg of daily milk, 75–190 d until next due date, and mycoplasma-negative. Cows were blocked based on parity and farm management, which includes bedding material used.

**Key Words:** casein hydrolysate, mammary involution, dairy cattle


This study was conducted to assess the farm prevalence of extended-spectrum $\beta$-lactamase (ESBL)-/AmpC-producing *E. coli* on dairy farms 2 years following a first study conducted in 2011 and to assess the animal prevalence for calves, dairy cows, and young stock. Fecal samples from 196 Dutch dairy herds were screened for *E. coli* with non-wild type susceptibility for cefotaxime and isolates were confirmed as ESBL/AmpC-producing by the combination disc diffusion test using cefotaxime and ceftazidime with and without clavulanic acid and cefoxitin. Identification of ESBL/AmpC genes was performed by microarray, PCR and sequence analysis. All data were combined and analyzed using Stata 14 at 5% level of significance. Fecal samples collected from 58% of the dairy farms contained ESBL/AmpC-producing *E. coli*. The between-farm prevalence based on samples collected from the slatted floors (18%) was statistically significantly lower than in 2011 (33%). The animal prevalence among calves was 33%, and only 1 and 2% among dairy cows and young stock, respectively. Plasmid-mediated resistance was predominantly caused by AmpC, encoded by the ACT gene. The predominant ESBL gene type was CTX-M-1. The farm prevalence of ESBL/AmpC-producing *E. coli* in dairy farms, based on pooled fecal samples of the slatted floor, was significantly reduced in 2013 compared with 2011. However, the farm prevalence based on the fecal samples from cows, young stock and calves is relatively high. Furthermore, the prevalence of ESBL/AmpC-producing *E. coli* for young calves is high, whereas the prevalence for cows and young stock is low. Moreover, most fecal samples contained a low number of ESBL/AmpC-producing *E. coli* per gram feces.

**Key Words:** extended-spectrum $\beta$-lactamase (ESBL), dairy farm, prevalence
Breeding and Genetics Symposium: Inbreeding in the Genomics Era

62 Computational aspects of characterizing genomic inbreeding in livestock. J. T. Howard*, F. Tiezzi, and C. Maltecca, North Carolina State University, Raleigh, NC.

The widespread incorporation of genomic information in dairy genetic evaluations allows for the opportunity to develop and implement methods to manage populations at the genomic level. While for the most part genome-wide metrics are currently employed in managing livestock inbreeding, genomic information offers the ability to identity region-specific homozygosity causing inbreeding depression or with reduced levels of diversity. Here we emphasize the importance of dedicated simulation tools to better understand the impact of alternative management schemes on genetic diversity, inbreeding depression and the fitness of a population. Also, methods to understand inbreeding depression have so far primarily focused on the effect of a region being in a run of homozygosity (ROH), which doesn’t account for the region potentially containing multiple unique ROH genotypes with variable effect. We discuss alternative heuristic methods to identify haplotypes contained within a ROH that give rise to reduced performance. This class of algorithms comprises multiple steps that scan the genome for unfavorable haplotypes, which are then contrasted using a linear mixed model. The identified haplotypes are then used to generate a functional inbreeding load metric across individuals (ILL). We present results from simulated scenarios combining different levels of linkage disequilibrium (LD) and number of loci impacting a quantitative trait and show that with increased LD in the population, these algorithms identify a greater proportion of the true unfavorable ROH effects (mean for high LD scenario: 41% of highly unfavorable haplotypes). In real data the accuracy of predicting phenotypes based on ILL across milk yield traits in Jersey cattle is different from 0 (mean ± SD: 0.20 ± 0.02), suggesting the method is capable of capturing functional inbreeding. Results from previous analysis are then discussed in the context of characterizing the expected progeny genome based on the observed parental genotypes. The results illustrate how genomic information can be utilized to manage the genetic diversity and degree of inbreeding depression that exists within a population.

Key Words: inbreeding, genetic diversity, genomics

64 Inbreeding depression. I. Curik*, M. Ferencakovic¹, and J. Sohlkner², ¹Department of Animal Science, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia, ²Department of Sustainable Agricultural Systems, Division of Livestock Sciences, University of Natural Resources and Life Sciences Vienna, Vienna, Vienna, Austria.

Negative consequences of inbreeding have been subject to intensive research for over a century. Yet, major improvements of knowledge on identifying detrimental mutations and estimating inbreeding depression have been achieved only recently as a result of high-throughput DNA sequencing developments. The main objective of this review was to explain methodological changes following as a consequence of new molecular developments. Regression of phenotypes on pedigree inbreeding coefficients ($F_{ped}$) has been the standard procedure applied in quantifying inbreeding depression in animals and humans. Being a genome wide expectation, $F_{ped}$ neglects stochastic variations and the impact of selection on the regional autozygosity. This further implies that regression analysis based on $F_{ped}$ suits well for traits that can be approximated by the infinitesimal model with dominance. In contrast, runs of homozygosity (ROH) inbreeding coefficient ($F_{ROH}$) is able to quantify autozygosity of chromosomal regions, or even a single SNP. For genetic analysis this is quite a large conceptual difference where $F_{ROH}$ opens new standpoints in identifying or/and estimating the effects of chromosomal regions, genes, or even a single SNP mutations that sizably contribute to the inbreeding depression. Here, we systemized the methods used in the identification or/and estimation of negative consequences of inbreeding with respect to the genetic architecture of the analyzed trait. Emphasis was given to (1) ROH-based mapping of loci contributing to inbreeding depression as a concept that has already been applied in livestock and human research, and to (2) SNP-wise dissection of inbreeding depression as a new theoretical concept. While the first concept is targeted toward the identification and estimation of detrimental mutations segregating in a population, the second concept is derived to improve the understanding of the molecular basis of inbreeding depression (functions of genes, dominance versus overdominance, distribution of gene effects). At the end, we discuss the potential use of predicting detrimental load from the whole-genome sequences, as well as of applying the gene editing in reducing the inbreeding load.

Key Words: autozygosity, inbreeding depression, runs of homozygosity

144 J. Dairy Sci. Vol. 100, Suppl. 2
What is the optimal measure of genomic inbreeding? A. C. Sørensen*, Center for Quantitative Genetics and Genomics, Department of Molecular Biology and Genetics, Aarhus University, Tjele, Denmark.

Genetic gain and rate of inbreeding are the 2 most important parameters to assess in livestock breeding. Genetic gain is defined as the increase in population-mean true breeding value over time. Rate of inbreeding has traditionally been estimated from pedigree information. The underlying assumption of this measure is that pedigree rate of inbreeding is a good proxy for inbreeding in the genome. While pedigree rate of inbreeding is the expected proportional loss of genetic variance at a neutral locus, it is not always a good proxy of loss of genetic variation at genomic regions harboring genes under selection. Therefore alternative measures of loss of genetic variation, or equivalently rate of inbreeding, have been proposed. The measures are developed for different information sources: SNP sets from chips or sequence data; and they use this information in different ways: assuming independence of SNP or specifically modeling the dependence between loci. The best of these depends on the purpose: is it for monitoring or is it for assisting decision making. The G-matrix was suggested by VanRaden in 2008 and has since been used with minor modifications in prediction of breeding values. While the different versions of the G-matrix appear to result in equally good predictions, the interpretation of its elements is not straightforward. Moreover, the use of a G-matrix instead of a relationship matrix based on pedigree information (A) in an optimum-contribution selection framework can result in less genetic gain at similar rates of inbreeding. The major short-coming of this method is that it does not use the information that the SNP included are mutually dependent due to the inheritance of segments of chromosomes carrying multiple loci. Therefore, theoretically, methods that use information on the linkage disequilibrium between SNP should give better results. One major issue that needs to be solved is what the gold standard is. For simulation purposes, we should be able to agree upon a best criterion, parallel to true breeding value in terms of evaluating genetic gain. Having agreed upon such a best criterion, the question remains, which estimator obtainable from real data is the best proxy for this best criterion.

Key Words: inbreeding, relationship

Inbreeding in the genomics era the flip side: Crossbreeding. E. Amuzu-Aweh1,2, P. Bijma3, H. Bovenhuis3, and D. de Koning1,2. 1Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden, 2Department of Animal Breeding and Genetics, Wageningen University, Wageningen, the Netherlands.

Reliable methods for predicting heterosis will improve the efficiency of crossbreeding schemes by reducing their dependency on expensive field-tests of multiple pure-line combinations. When heterosis is due to dominance, heterosis is proportional to the squared difference in allele frequency (SDAF) between parental pure-lines (not necessarily homozygous). Hence, a linear model including regression on SDAF partitions crossbred phenotypes into pure-line values and heterosis, even without pure-line phenotypes. We regressed the phenotypes of crossbreds on the SDAF between parental lines. We used 53,421 SNP genotypes of 3,427 White Leghorn sires, allele frequencies of 6 White Leghorn dam-lines and cage-based records on egg number and egg weight of ~210,000 crossbred hens. SDAF predicted heterosis for egg number and weight with an accuracy of 0.5. Heterosis predictions allowed preselection of pure lines before field-testing, saving 50% of field-testing cost with only 4% loss in heterosis. The concept of using SDAF to predict heterosis can be extended to predict heterosis for individual sires. This will quantify genetic variation between sires from the same pure-line, and can increase heterosis in crossbred populations. We derived the theoretical expectation for heterosis due to dominance in crossbred offspring of individual sires: “heterozygosity excess.” Next, we predicted heterosis by regressing offspring performance on the heterozygosity excess. Between-line differences accounted for 99.0% of the total variance in predicted heterosis, while within-line differences among sires accounted for 0.7%. Despite the limited effect on these traits, the individual sire differences for expected heterosis could provide an additional tool for optimizing breeding strategies in crossbreeding programs. Finally, we ran single-SNP GWAS for crossbred hens in 3 ways: (1) across-line GWAS with phenotypic data on all crosses that were produced by the 4 sire-lines; (2) line-level GWAS for the crosses produced by each of the 4 sire-lines; and (3) within-cross GWAS for the sire-lines that showed significant SNPs. Heterosis in crossbred populations can be predicted from genotype data on the parental lines. Selecting SNPs that have dominance effects may improve this prediction.

Key Words: crossbreeding, heterosis, genomics

Genomic inbreeding from an industry perspective. S. A. E. Engle*, M. F. Costello, B. M. Haines, and D. G. Wilson, CRV USA, Madison, WI.

While the genomic era revolutionized the dairy cattle breeding industry with its promise of rapid genetic gain, it also brought along the fear of an equally rapid decrease of population diversity. With genomic selection integrated, statistics on inbreeding rate gain in US AI studs are reflecting what was feared. Moreover, the gain in genetic merit appears to be the driving force behind the current unprecedented increase in inbreeding, with selection in the US primarily being focused toward a few total merit indexes. The industry experiences an inner conflict between potentially putting the US Holstein population in harm’s way, and continuing to capitalize on delivering a desired, yet inbred, product. From an outsider’s perspective, there seems to be enough information on the risks and management of inbreeding. Numerous scientific studies present detrimental effects and describe algorithms to manage (genomic) inbreeding rate. Multiple parameters such as Expected and Genomic Future Inbreeding are calculated for industry and producers to use to their benefit. So why don’t we see more commercial producers actively managing the relatedness of their herd? The daunting issue of inbreeding in the genomic era reflects a gap in dairy cattle breeding between the scientific community, the industry and the commercial producer. Concern among US dairy producers may be growing but the ‘pain’ that is the result of this increased inbreeding is not made tangible. The sacrifice in giving up genetic growth to reduce unknown costs is thereby unattractive. A paucity in education and data on how inbreeding truly affects the profitability of a herd is apparent, and until such research gives the industry value in selling out-crossed bulls, the situation is unlikely to change. Genomic relationship coefficients have the ability to open up the pool of potential matings and make use of the additional discovered diversity. Local inbreeding would prep the industry to rather talk about increasing homozygosity in desired regions. There is the potential to manage genomic inbreeding in a way that benefits both industry and producer. But for these prospects to become common practice, research needs to be translated into values and tools that (1) industry can sell; and (2) producers can understand.

Key Words: inbreeding, dairy cattle, genomics
The aim of this study was to determine the effects of wilting and additives on fermentation quality and nutritional value of round baled rice straw silages. A 2 × 4 factorial experimental design was used. Four treatments were allocated both on fresh and 24-h wilted rice straw harvested on the end of October. The dry matter content were 36.5% and 42.3% for fresh and wilted straw, respectively. The 4 treatments were control, 2.5% molasses (FM basis), silage inoculant, and both of 2.5% molasses and silage inoculant. The inoculant was applied at twice the recommended rate of 10 mg/kg fresh forage to supply 2 × 10⁶ cfu/g of a mixture of homofermentative Lactobacillus plantarum and Pediococcus acidilactici. Each treatment was applied in triplicate. After rice straw was round baled and ensiled for 50 d on open cement floor at 18°C to 19°C, each silage round bale was open for sensory quality evaluating and sampling for fermentation parameters and nutrient composition analysis. Results showed that regardless of condition of rice straw it was hard to achieve good silage, one silage bale in 3 with visible white mold and light mushy odor. Molasses or inoculant alone could not improve fermentation quality and nutritional value of rice straw silages, except that inoculant decreased NH₃-N/TN and molasses decreased structural carbohydrate content. Combining additives increased acetic acid, propionic acid, and CP content, but structural carbohydrate content decreased. The same treatment increased butyric acid concentration in the wilted rice straw. Wilting decreased dry matter recovery of rice straw silage, with increases in CP content and ammonia-N concentration. In all, additives such as molasses or inoculant alone could contribute rice straw ensiling success. It needs further study to evaluate and determine whether rice straw should wilt or add both molasses and inoculant before ensiling with low moisture content.

Key Words: rice straw silage, wilting, molasses or inoculants
Grazing Damascus goats consume tannin-rich Mediterranean browse. *Pistacia lentiscus* is one of the major dietary components of grazing Damascus goats in Israel. The aim of the present study was to evaluate the pasture effect on productivity and milk quality in Damascus goats. Further, we isolated the *Pistacia lentiscus* effect on lactation performances by replacing the hay in goats ration in confinement with *Pistacia lentiscus*. The study consisted of 2 experiments: the first was designed to compare the effect of grazing vs confinement dietary regimen on milk quality, and the second was designed to elucidate the effect of the major dietary constituent of grazing Damascus goats on productivity and quality of milk. The first experiment compared milk produced by Damascus goats grazed on Mediterranean brushland (Pasture –P) with Damascus goats fed clover (*Trifolium alexandrium*) hay indoors (Hay –H, n = 6). Milk composition and fatty acid profile were measured, and individual DM intake and nutritional composition was estimated by fecal NIRS using a published equation calibrated for dietary percentage of concentrate, and knowing the concentrate allowance. Treatments did not affect milk yield but the P group yielded milk richer in protein (3.55 vs 3.28%, \( P < 0.01 \)) and fat (6.02 vs 4.07%, \( P < 0.001 \)) and lower in urea (0.048 vs 0.029 mg/dL, \( P < 0.001 \)). The concentration of omega 3 fatty acids was 25 percentage points higher in milk from animals grazing compared with those fed indoor. Moreover, the results suggest that *Pistacia lentiscus* can serve as proxy for Mediterranean browse in the investigation of pasture effects on milk composition.

**Key Words:** Damascus goats, milk quality, pasture

### Table 1 (abstract 70).

<table>
<thead>
<tr>
<th>Treatment Period</th>
<th>TMR+0h</th>
<th>TMR+4+4h</th>
<th>TMR+8h</th>
<th>SEM</th>
<th>Treatment Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI (kg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lucerne</td>
<td>0(^a)</td>
<td>8.18(^a)</td>
<td>6.99(^b)</td>
<td>0.266</td>
<td>0.0001</td>
</tr>
<tr>
<td>TMR</td>
<td>23.26(^a)</td>
<td>13.51(^b)</td>
<td>12.43(^b)</td>
<td>0.801</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total</td>
<td>23.26(^a)</td>
<td>21.7(^ab)</td>
<td>19.41(^b)</td>
<td>0.839</td>
<td>0.0259</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>24.15</td>
<td>26.22</td>
<td>24.81</td>
<td>2.010</td>
<td>0.5835</td>
</tr>
<tr>
<td>3.5% FCM, kg/d</td>
<td>27.42</td>
<td>28.69</td>
<td>27.59</td>
<td>2.153</td>
<td>0.7040</td>
</tr>
<tr>
<td>Fat, kg/d</td>
<td>1.05</td>
<td>1.07</td>
<td>1.04</td>
<td>0.083</td>
<td>0.8508</td>
</tr>
<tr>
<td>Protein, %</td>
<td>0.89</td>
<td>0.91</td>
<td>0.88</td>
<td>0.065</td>
<td>0.8402</td>
</tr>
<tr>
<td>Protein, kg/d</td>
<td>3.77</td>
<td>3.54</td>
<td>3.57</td>
<td>0.105</td>
<td>0.2410</td>
</tr>
<tr>
<td>Lactose, kg/d</td>
<td>1.16</td>
<td>1.26</td>
<td>1.20</td>
<td>0.100</td>
<td>0.6198</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.77</td>
<td>4.78</td>
<td>4.81</td>
<td>0.037</td>
<td>0.6678</td>
</tr>
<tr>
<td>Feed efficiency(^1)</td>
<td>1.24</td>
<td>1.35</td>
<td>1.42</td>
<td>0.121</td>
<td>0.3335</td>
</tr>
</tbody>
</table>

\(^{a,b,c}\)Within a row, means with different superscripts are different (\( P < 0.05 \)).

\(^{1}\)3.5% FCM yield (kg/d)/DMI (kg/d).

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**71 Damascus goats grazing on Mediterranean bushland or fed *Pistacia lentiscus* have improved milk quality.** N. Argov-Argaman\(^{1}\), O. Hadaya\(^{1}\), T. Glasser\(^{3}\), H. Muklada\(^{3}\), L. Dvash\(^{2}\), and S. Y. Landau\(^{2}\), \(^{1}\)Hebrew University, Rehovot, Israel, \(^{2}\)Volcani Center, Rishon LeZion, Israel, \(^{3}\)Ramat Hanadiv Nature Park, Zikhron Yaakov, Israel.

Increased global demand for soybean and rapeseed meal, and associated fluctuations in their availability and price has resulted in greater interest in home grown, protein-rich forage sources for ruminants. Alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*) are of interest due to their high protein content, but often contain insufficient digestible undegradable protein to meet cow requirements. Tannins can protect protein in forages from microbial degradation by forming a complex in the rumen that dissociates in the abomasum. The effect of the addition of hydrolysable tannins to alfalfa and red clover silage at ensiling on the intake, performance and whole tract digestibility were therefore studied. Twelve multiparous dairy cows received one of 4 dietary treatments in each of 4 periods of 28 d duration, in a Latin square design with measurements taken in the final 7 d of each period. The proportion of alfalfa (A) or red clover (RC) to corn silage was 40:60 (DM basis), and all dietary treatments contained 55.45 forage to concentrates (DM basis). There were 4 dietary treatments; A or RC silage ensiled without or with hydrolysable tannins. Hydrolysable chestnut tannin was added at ensiling at 25 g/kg DM. Cows were fed once daily at 0800h and milked twice daily. Acid insoluble ash was used as an indirect marker for whole tract digestibility. Data were analyzed as a Latin square design using Genstat (v. 17). DMI was highest (\( P < 0.01 \)) in cows when fed A (mean values of 22.1 and 19.8 kg DM/d for A and RC respectively). There was no effect (\( P > 0.05 \)) of treatment on milk yield, fat or protein content with mean values of 38.1 kg/d, 41.5 and 33.7 g/kg respectively. Dietary treatment had no effect (\( P > 0.05 \)) on OM or nitrogen digestibility, with mean values of 0.687 and 0.629 kg/kg respectively. In conclusion, cows offered red clover had a similar level of performance as those fed alfalfa.

**Key Words:** Damascus goats, milk quality, pasture

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silage but ate 2.3 kg DM/d less, and the inclusion of hydrolysable tannins in alfalfa and red clover silage had no benefit on performance or whole tract digestibility.

Key Words: tannin, forage, digestibility

73 Relations between silage composition, its metabolome, and preference shown by goats. R. Scherer*, K. Gerlach, and K.-H. Südekum, Institute of Animal Science, University of Bonn, Bonn, Germany.

The interrelationships between silage preference of ruminants and compounds that may affect dry matter (DM) intake (DMI) are not yet fully enlightened. Metabolic studies in addition to conventional chemical analysis and preference trials provided new insights. Six silage treatments each of alfalfa (ALF) and red clover (RC) with different DM (222–391 g/kg), silage additives and intended addition of soil were produced to obtain a range of qualities. After 120 d of ensiling, silages were sampled for chemical analysis, vacuum-packed and refrigerated for subsequent preference trials with goats. Within 21 d, each possible combination of 2 silages (n = 21) was presented for 3 h for ad libitum intake to goats (Saanen-type wethers, n = 8, body weight 105 ± 2.7 kg). The mean value comparisons (3 h DMI) were made using a variance analysis including terms for treatment and animal and the Waller-Duncan k-ratio t-test to separate means (SAS 9.3). Although treatments led only to few differences in fermentation acids and crude protein composition, preference behavior was strongly divergent. The most preferred and avoided treatments of ALF and RC silage amounted to 863, 858, 226, and 282 g DMI/3 h, respectively. Fermentation acid analysis showed the most obvious differences in acetic acid. To further explore relations between preference and silage composition a metabolome analysis was implemented. Metabolites (all low molecular weight molecules) were analyzed by a non-targeted metabolite profiling in the range of 50–1,700 Da. In 2 treatments of each plant species (those which were most different in preference) more than 6,400 compounds were detected and 2,010 were identified. For an initial overview of all analyzed samples a principal component (PC) analysis (PCA) was conducted; PC 1 clearly separated the different plant species, explaining 40.7% of all variances in the data set; PC2 separated the silage treatments explaining 22.9% of all variances. Between preferred and avoided treatments 934 compounds differed in RC and 1,860 in ALF. 475 of those were altered in both plant species (251 were reduced and 186 were increased; only 38 behaved contrarily). The database provides a useful foundation for the approach of explaining silage intake behavior by ruminants.

Key Words: silage, preference, metabolome

74 Modification of ruminal fermentation and methane production by adding legumes containing condensed tannins to an orchardgrass diet in continuous culture. A. I. Roca-Fernandez*,1,2, S. L. Dillard1, M. D. Rubano1, M. Baldin3, C. J. Dell1, J. MacAdam4, and K. J. Soder1, 1USDA-Agricultural Research Service, University Park, PA, 2Universidad de Santiago de Compostela, Lugo, Galicia, Spain, 3Penn State University, University Park, PA, 4Utah State University, Logan, UT.

Different legume sources containing varying levels of condensed tannins (CT) may alter ruminal fermentation and enteric CH4 production. Research is needed on how increased CT levels from different legume sources may affect nutrient digestibility, volatile fatty acid (VFA) production, bacterial protein synthesis, fatty acid (FA) flow, protozoa and CH4 production. A 4-unit continuous culture fermentor system was used to assess the effects of legume source containing varying levels of CT with orchardgrass on ruminal fermentation. Legumes were: alfalfa (ALF; control); birdsfoot trefoil (BFT; low CT); crown vetch (CV; intermediate CT); and sericea lespedeza (SL; high CT). Treatments consisted of orchardgrass/legume (50:50). The experimental design was a 4 × 4 Latin square. Forage, effluent and microbial samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) to determine nutrient digestibilities. Effluent samples were taken for VFA concentrations, bacterial protein synthesis, FA flow and protozoa count. Gas samples for CH4 analysis were recorded by a photoacoustic gas analyzer. Data were analyzed by PROC-GLIMMIX of SAS. Apparent and true DM and OM digestibilities were lower (P < 0.01) in SL than ALF and BFT, with CV in an intermediate level. Apparent NDF and ADF digestibilities were lowest (P < 0.01) in SL. Total VFA concentrations were least (P < 0.001) in SL and greatest in ALF and BFT. Greater dietary N (P < 0.05) and lower NH3-N (P < 0.001) were found in SL and BFT than in ALF and CV. Bacterial N efficiencies per kg of truly digested DM and OM were least (P < 0.05) in SL and greatest in BFT. Changes in FA biohydrogenation process were found with SL due to decreases in conjugated linoleic acid flow and in the ratio between stearic and vaccenic acids. The SL had the greatest numbers of protozoa (P < 0.05), the least total CH4 production (P < 0.001) and the least CH4 production per unit of OM truly digested (P < 0.001) which offset increased protozoal number. In summary, introducing SL into an orchardgrass diet reduced CH4 production but exerted adverse effects on ruminal feed digestion and fermentation.

Key Words: tannin, fermentor, methane

75 Effect of rehydrating and ensiling dry ground corn with varied concentrations of wet brewers grain on fermentation profile and ruminal in vitro starch digestibility. W. I. Silva Filho, H. Sultana, and L. F. Ferraretto*, University of Florida, Gainesville, FL.

The objective of this study was to evaluate the effect of rehydrating and ensiling dry ground corn (DGC) with varied concentrations of wet brewers grain (WBG) on fermentation profile and ruminal in vitro starch digestibility (ivSD; 7-h incubations on dried and 4-mm ground samples; Tilley and Terry, 1963). Samples of DGC and WBG were weighed separately and mixed following these treatments: 100% WBG (WBG); mix of DGC and WBG targeting for 60% (RC60), 65% (RC65) or 70% (RC70) of DM; and DGC rehydrated with distilled water targeting for 70% of DM (REH). Samples were ensiled in vacuum-sealed bags and allowed to ferment for 0, 1, 3, 7, 14 and 28 d. The experiment consisted of 30 treatments (5 mix of DGC and WGB × 6 ensiling times) and 120 mini-silos (4 silos per treatment). All samples were analyzed for fermentation profile and WSC. Except for WBG, samples from d 0 and 28 were analyzed for ivSD. Data were analyzed using Proc Glimmix of SAS with the Fixed effects of treatment, ensiling time and their interaction. Content of DM was greater (P < 0.05) for REH (70.0%), followed by RC70 (69.2%), RC65 (63.9%), RC60 (58.4%) and WBG (17.5%) on d 0, with a decrease (1–2 percentage units) observed for all treatments until d 28. Measurements of pH were (P < 0.05) greatest for REH (6.19) and lowest for WBG (4.68) on d 0, but all other treatments were lower than WBG on d 14 and 28 (3.83 vs. 4.14, on average). Except for WBG, all treatments had (P < 0.05) a gradual increase in lactate concentration from d 0 to 28. In contrast, butyrate gradually increased (P < 0.05) from d 0 (0.25%) to 28 (2.16% of DM) in WBG but not the other treatments. Fermentation patterns are related to WSC concentration which were greater (P < 0.05) for all treatments than WBG from d 0 (1.41% on average vs. 0.38% of DM, respectively) to d 28 (0.37%
on average vs. 0.19% of DM, respectively). Greater ivSD was observed ($P < 0.001$) for all treatments on d 28 than on d 0 (48.0% vs. 38.1% of starch, respectively) but magnitude of the difference was greater for REH and RC70 (14.5%-units on average). Rehydration and ensiling of DGC with WBG resulted in adequate fermentation and enhanced starch digestibility.

**Key Words:** rehydrated corn, wet brewers grain, starch digestibility

### 76 Development of a berry processing score for sorghum silage

J. R. Johnson*, J. P. Goeser2, and M. J. Brouk1, 1Kansas State University, Manhattan, KS, 2Rock River Laboratories, Watertown, WI.

This study was done in an effort to develop a berry processing score (BPS) for sorghum silage, similar to the kernel processing score (KPS) currently used for corn silage. Sorghum silage samples were collected from 3 commercial dairies and processed through a roller mill using 1 of 3 different roll gap settings to give 4 differently processed samples: unprocessed, 1.5, 1.0, or 0.5 mm. After drying, samples were placed into a Ro-Tap particle separation machine fitted with sieves containing square apertures of 9.50, 6.70, 4.75, 4.00, 3.35, 2.80, 2.36, 1.70, 1.18, and 0.6 mm (and a pan). Samples were placed into the Ro-Tap machine for 10 min until the whole sample was separated, allowing for the determination of mean particle size (MPS) and percent material retained on each sieve by weight. Whole samples, as well as separated fiber and whole berry portions were sent to Rock River Laboratories (Watertown, WI) and analyzed for percent starch retained on each sieve. As the roll gap setting was reduced, MPS tended to be reduced (2.16, 2.15, 2.07, and 2.00 ± 0.05 mm for unprocessed, 1.5 mm, 1.0 mm, and 0.5 mm, respectively; $P = 0.09$). Whole berries per g of sample weight were reduced ($P < 0.01$) from 10.0 to 0.3 ± 1.2 whole berries per g as the roll gap spacing was reduced, indicating successful processing of the samples. Percent starch passing through the 1.7 mm sieve was greater ($P < 0.01$) at the 0.5 mm roll gap for the whole sample (18.90, 22.31, 29.45, and 36.92 ± 2.93 for unprocessed, 1.5 mm, 1.0 mm, and 0.5 mm, respectively). The percent starch of the whole berry sample retained on the 2.8 mm sieve was 49.31 and 5.26 ± 6.07% while percent starch retained on the 1.7 mm sieve was 4.2 and 48.04 ± 6.07% for unprocessed and 0.5 mm, respectively ($P < 0.01$). Thus, we have determined that the appropriate sieve to use in determining a BPS for sorghum silage is the 1.7 mm sieve. A BPS for any sorghum silage sample can be calculated by analyzing the whole sample for starch content and percent starch retained on each sieve.

**Key Words:** sorghum silage, processing, starch digestion

### 77 Dual-flow continuous culture fermentation of organic BMR sorghum-sudangrass and teff grass to determine digestibility of forages in an organic dairy grazing system

K. J. Ruh*, B. J. Hein, M. D. Stern, and R. Gardner, University of Minnesota, St. Paul, MN.

The objective of this study was to compare 2 warm-season annual grasses to cool season perennial pasture (CSP) and alfalfa for ruminal nutrient digestibility and nitrogen synthesis in a dual-flow continuous culture rumen fermentation system. Eight dual-flow continuous culture fermentors were used during 2 consecutive 10-d periods. Dietary treatments were (1) alfalfa, (2) CSP, (3) brown midrib sorghum-sudangrass (BMRSS), and (4) teff grass from the organic dairy production system at the University of Minnesota organic dairy in Morris, MN. The CSP comprised orchardgrass, meadow fescue, bromegrass, red clover, and white clover. Treatments were randomly assigned to fermentors with 7 d for diet adaption and 3 d for data and sample collection. Fermentor samples for pH, NH$_3$-N, and VFAs were collected on d 8, 9, and 10. Apparent dry matter, organic matter, neutral detergent fiber, and acid detergent fiber digestibility were lower ($P < 0.05$) for BMRSS, teff, and CSP. (49.8%, 33.3%, 58.4%, and 60.8%, respectively) compared with alfalfa (69.4%, 54.1%, 75.5%, and 75.5%, respectively). The BMRSS, teff grass, and CSP were not different from each other. True dry matter and organic matter digestibility were lower ($P < 0.05$) for BMRSS, teff, and CSP (65.4% and 47.2%, respectively) compared with alfalfa (85.8% and 69.2%, respectively). Fermentor pH and total volatile fatty acids were not affected by forage. Ammonia N concentrations (mg/dl) were greater ($P < 0.05$) for alfalfa (22.5) compared with CSP (7.5), BMRSS (7.4), and teff (8.9), respectively. Crude protein degradation was not affected by forage treatment. The flow of NH$_3$-N was greatest ($P < 0.05$) for alfalfa (26.5%) compared with the other forages (11.6%), reflecting the greatest NH$_3$-N concentration. The flow of total N was greatest ($P < 0.05$) for alfalfa (1.99 g/d), lowest for CSP (1.50 g/d) and BMRSS (1.51 g/d), and intermediate for teff grass (1.70 g/d). Overall, the fermentation of the warm season grasses was similar to the CSP, indicating the potential for successful use of warm season grasses for grazing systems.

**Key Words:** organic, fermentation, pasture

### 78 Effects of foliar fungicide on whole-plant BMR and floury corn varieties at vegetative tassel and reproductive stages of growth

M. E. Weatherly*,1 R. T. Pate1, L. Hedges2, S. Mideros3, G. M. Fellows3, M. Akins4, M. R. Murphy1, and F. C. Cardoso1, 1Department of Animal Sciences, University of Illinois, Urbana, IL, 2Department of Crop Sciences, University of Illinois, Urbana, IL, 3B.A.S.F. Corporation, Research Triangle Park, NC, 4University of Wisconsin-Madison, Marshfield, WI.

Fungal disease can have a negative impact on yield, health, and performance of corn plants used for corn silage. The objective of this study was to determine the effect of foliar fungicide (FUNG) application on 2 whole plant corn varieties. Brown midrib (BMR) and floury (FLY) varieties were planted in 6.76 ha (3.38 ha per variety). Treatments were assigned to 16 plots in a completely randomized block split-plot design with 4 replicates per treatment and 0.42 ha per plot. Treatments were: BMR without FUNG (BMR/CON), FLY without FUNG (FLY/CON), BMR with FUNG (BMR/FLY/FUNG), and FLY with FUNG (FLY/FLY/FUNG). Fungicide (Headline AMP; BASF Corp.) was applied on both varieties at vegetative tassel (VT) stage. Samples of whole corn plants were collected from each treatment at VT and reproductive stage (R5). At each collection, corn was separated into leaves, stalks, flag leaf, and cobs for chemical analysis. Statistical analysis was conducted using the MIXED procedure in SAS (v9.4). No main effects or interactions of treatment were observed for any parameter. Leaves from BMR tended to be lower in CP than FLY (18.3 vs. 19.0 ± 0.23% DM; $P = 0.08$) and had less NDF (65.1 vs. 66.9 ± 1.15% NDF; $P = 0.03$). Stalks from BMR were lower in DM content (15.5 vs. 18.3 ± 0.60% DM; $P = 0.02$) and greater CP (7.3 vs. 6.1 ± 0.23% CP; $P = 0.01$) than FLY. Stalks from BMR had lower NDF than FLY (65.2 vs. 70.5 ± 0.67% DM; $P < 0.0001$). Stalks from FLY tended to have a greater ash content than CON (7.42 vs. 7.04 ± 0.17% ash; $P = 0.08$). Cobs from BMR had lower DM content (29.8 vs. 31.0 ± 0.49% DM; $P = 0.01$) and greater CP (19.3 vs. 17.7 ± 0.40% CP; $P < 0.0001$) than FLY. Flag leaves from BMR were lower in DM content (28.2 vs 32.5 ± 2.07% DM; $P = 0.001$) and lower in CP (13.9 vs. 16.4 ± 0.25% CP; $P < 0.0001$) than FLY. In conclusion, BMR
corn had greater stalk and cob CP than FLY and FUNG treated corn tended to have greater ash content than CON.

**Key Words:** corn plant, foliar fungicide


The objective of this study was to evaluate the effect of plant population, hybrid relative maturity, and cutting height on yield, nutrient composition and ruminal in vitro starch (ivSD; 7 h incubations on dried and 4 mm ground samples) and NDF (ivNDFD; 30 h incubations on dried and 1 mm ground samples) digestibility in whole-plant corn forage. Eight hybrids (4 early [ER; 94–99 d] and 4 late [LT; 105–108 d] maturity) were each planted at 4 different plant populations (64,000 [64K], 79,000 [79K], 94,000 [94K] or 109,000 [109K] plants/ha), in triplicate, and harvested at 2 cutting heights (15 [LO] or 60 [HI] cm). Data were analyzed as a split-plot design using Proc Mixed of SAS with the fixed effects of maturity, plant population, cutting height and their 2- and 3-way interactions. Hybrid and plant population × maturity × hybrid interaction were random effects. Except for greatest CP content for 64K and lowest for 109K (P = 0.001) or a trend (P = 0.09) for 1.3%-units greater ivNDFD for 94K than 64K, no effects of plant population on nutritive value were observed (P > 0.10). Yield of DM (t/ha) was (P = 0.001) greatest for 109K and 94K, and lowest for 64K. Milk production was reduced (P = 0.001) by 2248 kg/ha, on average, for 64K than other treatments. Hybrid maturity did not affect (P > 0.10) any parameter. Reduced (P = 0.001) ADF (21.4 vs. 24.6%), NDF (33.8 vs. 37.7%) lignin (3.2 vs. 3.7%) and undigested NDF after 240 h of incubation (10.5 vs. 12.4%) concentrations and corresponding increased ivNDFD (52.7 vs. 49.6% of NDF) were observed for HI. Furthermore, HI had greater starch content (P = 0.001; 41.7 vs. 37.5%) but not digestibility (P = 0.13). A hybrid maturity × cutting height interaction was observed (P < 0.05) for DM yield/ha and milk production/ha. Although HI had lower DM yield than LO for both maturities, the magnitude of the difference was exacerbated in LT hybrids (2.56 vs. 1.54 t of DM/ha). This led to a reduction of 1203 kg of milk/ha for HI in LT but not ER hybrids. Plant population affected yield but not quality of whole-plant corn forage. In addition, cutting height increased nutritive value but at the expense of reduced yield.

**Key Words:** corn silage, plant population, cutting height

80 Forage herbage mass and quality of two different cover cropping systems for grazing organic dairy steers. B. J. Heins*1, H. Phillips2, K. Delate2, and R. Turnbull2, 1University of Minnesota, St. Paul, MN, 2Iowa State University, Ames, IA.

Integrating crops and livestock on a multi-function operation could have multiple benefits and the potential to improve the profitability of these kinds of operations. A long-term, organic, integrated crop-livestock rotation design was established at the West Central Outreach and Research Center organic dairy (Morris, MN) during 2015. The objective of this study was to compare forage quality and herbage mass of winter wheat and winter rye for grazing dairy animals. Winter wheat and winter rye forages were planted on Sept. 11, 2015, for grazing during spring 2016. During the spring, 12-mo old organic dairy steers were randomly assigned to replicated groups (winter wheat or winter rye), but balanced by breed group to reduce potential breed bias. Grazing of dairy steers was initiated on April 25, 2016 when forages were 20–30 cm tall and strip size was adjusted to leave 7–13 cm of refusals. Random samples of pasture forage were sampled every 3 d when a group of steers moved to a new paddock. Pasture clippings were randomly collected in a 0.76 m² square of pasture. Forage samples were sent to Rock River Laboratory, Inc., Watertown, WI and were analyzed with NIR spectrophotometry for DM, CP, and total-tract NDF digestibility (TTNDFD). Data were analyzed using the MIXED procedure of SAS. Independent variables for analyses were the fixed effects of forage (winter wheat or winter rye), date of clipping, and the interaction of date and forage, and replicate paddock was a random variable. Winter rye (2,944 kg DM/ha) had greater (P < 0.05) herbage mass compared with winter wheat (2,266 kg DM/ha). The DM was lower (P < 0.05) for winter rye (18.9%) compared with winter wheat (20.8%). The CP was 17.6% and 19.3% for winter rye and winter wheat, respectively (P < 0.01). The TTNDFD was 56.3% for both winter rye and winter wheat, respectively (P = 0.099). In summary, CP was greater for winter wheat compared with winter rye; however, TTNDFD did not differ between cover cropping systems. Winter rye and winter wheat may provide adequate forage for dairy cows during the spring grazing season.

**Key Words:** grazing, winter rye, cover crops
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81 A metabolomics approach to identify novel pathways involved in metabolic transition of periparturient dairy cows. A. Kenéz*1, S. Dänicke1, U. Rolle-Kampczyk1, M. von Bergen1, and K. Huber1, 1Institute of Animal Science, University of Hohenheim, Stuttgart, Germany, 2Institute of Animal Nutrition, Federal Research Institute for Animal Health, Braunschweig, Germany, 3Department of Molecular Systems Biology, Helmholtz Centre for Environmental Research, Leipzig, Germany.

High-yielding dairy cows face a stressed metabolic condition during the transition from late pregnancy to early lactation. To overcome this, complex adaptation processes are necessary in an orchestrated manner, affecting various physiological systems. To broaden our understanding of the biology underlying an efficient metabolic adaptation, this study aimed to characterize the changes of metabolic phenotype in healthy periparturient dairy cows in a targeted metabolomics approach. Twenty-six German Holstein cows were used to collect blood samples repeatedly during the transition period: 42 and 10 d before calving and 3, 21, and 100 d after calving. Blood serum samples were subjected to a targeted liquid chromatography-mass spectrometry (LC-MS) based metabolomics assay using the AbsoluteIDQ p180 Kit of Biocrates Life Science AG (Innsbruck, Austria). Processed metabolomics data were evaluated by principal component analysis (PCA) and by heatmap visualization to characterize metabolic patterns during the course of transition period. Further data analysis was applied to link these identified patterns with physiological and production performance data. A clear separation according to sampling days was revealed by the PCA, indicating a notable shift of the metabolic phenotype. According to the heatmap, acylcarnitine concentrations reflected patterns of high variation between individuals. Furthermore, the concentration of several glycerophospholipids and sphingolipids was remarkably decreased 10 d before and 3 d after calving than earlier and later in the transition period. Amino acids and biogenic amines showed a diffuse pattern with less variation over time. Metabolic pathways affecting specific lipid species, such as cell integrity, inflammation, and lipid trafficking, could be identified as targets for metabolic adaptation during transition from late pregnancy to early lactation. Analyzing longitudinal changes of the blood metabolome can help us improve our understanding of the multifaceted metabolic adaptation of transition cows, and can ultimately lead to defining and predicting healthy and unhealthy metabolic phenotypes in dairy cattle.

Key Words: metabolomics, transition period, dairy cow

82 Methionine and choline feeding during the periparturient period alter the liver metabolome to different extents. Z. Zhou*1, Z. Li1, X. Dong1, D. Luchini2, and J. Loor1, 1University of Illinois, Urbana, IL, 2Adisseo S.A.S, Alpharetta, GA.

Periparital dairy cows undergo a period of low methionine (MET) and choline (CHOL) supply, hence, rumen-protected MET or CHOL feeding may improve performance and cow health at least in part via alterations of the tissue metabolome. Objectives were to investigate the effect of feeding rumen protected MET (Smartamine M) or rumen protected CHOL (Reashure) during the peripartial period on liver metabolomics profile and identify the most-impacted metabolites using a high-resolution mass spectrometry-based untargeted metabolomics approach. Twenty-four multiparous Holstein cows were randomly assigned from −21 to +30 d relative to parturition to a basal control (CON) diet; CON plus rumen-protected methionine (MET) at a rate of 0.08% of DM; or CON plus rumen-protected choline (CHOL) at 60 g/d. Liver samples were harvested at −10, 7, and 20 d. Samples were analyzed using the Q-Exactive MS system after LC (Liquid chromatography) separation. Data analysis was performed using the MetaboAnalyst 3.0 program. A total of 3346 metabolites were identified. Multivariate statistical analysis (PLS-DA, and OPLS-DA) revealed clear separation of metabolite profile among CON, MET, and CHOL groups at −10, 7, and 20 d. Metabolites with “importance in projection (VIP)” scores >1.0 and P < 0.05 were considered significantly different among treatments. The top 15 metabolites with highest VIP score were identified by exact molecular weight (mass error ppm <5) for comparisons at −10 d, 7 d, and 20 d, respectively. Among significant metabolites at −10 d, dityrosine (an oxidative stress biomarker, VIP = 3.6) and nororphthalamic acid (a glutathione analog, VIP = 2.9) levels were greater in CHOL-fed cows but lower in MET-fed cows. In contrast, ophilthamic acid (another glutathione analog, VIP = 3.7) at 20 d was greater in MET-fed cows but lower in CHOL-fed cows compared with CON. At 7 d, hepatic dimethylglycine was greater (VIP = 2.6) in CHOL cows but lower in MET-fed cows compared with CON. These analyses indicate that peripartal MET or CHOL feeding can influence the liver metabolome to different extents. The biologic significance of these alterations remains to be elucidated.

Key Words: methionine, choline, metabolomics

83 Untargeted metabolomics of skeletal muscle in Holstein cows during the periparturient period in response to feeding rumen-protected methionine or choline. Z. Zhou*1, Z. Li1, X. Dong1, D. Luchini2, and J. Loor1, 1University of Illinois, Urbana, IL, 2Adisseo S.A.S, Alpharetta, GA.

Objectives were to investigate the effect of feeding a rumen-protected methionine (Smartamine M) or a rumen-protected choline (Reashure) source during the peripartial period on the skeletal muscle metabolome and identify the most-impacted metabolites using a high-resolution mass spectrometry-based untargeted metabolomics approach. Twenty-four multiparous Holstein cows were randomly assigned from −21 to +30 d relative to parturition to a basal control (CON) diet; CON plus rumen-protected methionine (MET) at a rate of 0.08% of DM; or CON plus rumen-protected choline (CHOL) at 60 g/d. Skeletal muscle samples from the hind-leg were harvested at −10, 7, and 20 d. Samples were analyzed using Q-Exactive MS system after LC (Liquid chromatography) separation. Data analysis was performed using the MetaboAnalyst 3.0 program. A total of 1504 metabolites were identified. Multivariate analysis (PLS-DA, and OPLS-DA) revealed clear separation of metabolomics profiles among CON, MET, and CHOL groups at −10, 7, and 20 d. Metabolites with ‘importance in projection (VIP)’ scores >1.0 and P < 0.05 were considered significantly different among treatments. The top 15 metabolites with highest VIP score were identified by molecular weight (mass error ppm <5) for comparisons among treatments at −10, 7, and 20 d, respectively. Compared with CON, lactic acid (VIP = 3.3) was lower in MET-fed cows but greater in CHOL-fed cows at −10 d. A greater skeletal muscle proline (VIP = 2.9) level also was observed in MET-fed cows but not in CHOL-fed cows compared with CON. As incomplete breakdown products of protein catabolism, although concentrations of dipeptides such as gamma-glutamyl-alanine (VIP = 2.6) and asparaginyl-valine (VIP = 2.5) were both greater in MET-fed cows compared with CON at 7 d, a lower level at 20 d of L-gamma-glutamyl-l-valine (proteolytic breakdown product of larger proteins) was observed for MET-fed cows. These preliminary analyses indicate
that peripartal MET or CHOL feeding influence the skeletal muscle metabolome to different extents. The biologic significance of these alterations remains to be elucidated.

**Key Words:** methionine, choline, metabolomics

### 84 Muscle-targeted metabolomics in dairy cows during the transition from late pregnancy to early lactation. Y. Yang², C. Prehn², J. Adamski², J. Rehage¹, S. Dänicke³, H. Sauerwein¹, and H. Sadrí¹, ¹Institute of Animal Science, Physiology & Hygiene Unit, University of Bonn, Bonn, North Rhine-Westphalia, Germany, ²Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Bavaria, Germany, ³Clinic for Cattle, University of Veterinary Medicine Hannover, Foundation, Hannover, Lower Saxony, Germany, ⁴Institute of Animal Nutrition, Friedrich-Loeffler-Institute, Braunschweig, Lower Saxony, Germany.

In the dairy cow, late gestation and early lactation are characterized by a complexity of metabolic processes required to maintain homeostasis. The metabolome, representing the terminal downstream product of the genome, transcriptome, and proteome provides a direct measure of physiological changes in a particular condition. The objective of this study was to characterize skeletal muscle metabolome in the context of metabolic changes occurring during the transition from late pregnancy to early lactation in dairy cows. Twenty-one German Holstein cows receiving 100 g/d conjugated linoleic acid (CLA; n = 11; Lutrell pure, BASF, Germany) or a control fat supplement (n = 10; Silafat, BASF) were studied. The metabolome was characterized in M. semitendinosus samples collected at d −21, 1, 21, and 70 relative to calving using a targeted quantitative metabolomics approach (AbsoluteIDQ p180 Kit; Biocrates Life Sciences AG, Austria). Multivariate analysis of the data (PCA and PLS-DA) did not reveal separation between CLA and control animals across and within time points, whereas a clear separation between different time points, regardless of the treatment, was evident. Furthermore, ANOVA-simultaneous component analysis on the metabolites showed only time effect (P = 0.04). Out of 188 metabolites, 42 were identified as the most relevant (variable importance of projection score > 1 and P < 0.05) for the observed separation. Metabolic pathway analysis identified 4 pathways that were significantly (false discovery rate < 0.10) impacted over time, based on significant annotated metabolites. Out of these 4, there were 3 AA metabolism pathways including Arg and Pro metabolism, Phe, Tyr, and Trp biosynthesis, and D-Glu and D-Glu metabolism significantly affected by time along with aminoacyl-tRNA biosynthesis, which prepares for translation. In addition, long-chain fatty acyl carnitine species (C16 and C18:1) were elevated around parturition, reflecting an enhanced but incomplete mitochondrial oxidation of fatty acids. These data contribute toward a better understanding of the skeletal muscle metabolic adaption in dairy cows during the transition period.

**Key Words:** metabolomics, skeletal muscle, dairy cow

### 86 Metabolomics profiling of the serum from dairy cow with different milk protein yield using gas chromatography–time of flight/mass spectrometry. X. H. Wu¹, H. Z. Sun¹, D. M. Wang³, M. Y. Xue¹, L. L. Guan², and J. X. Liu¹, ¹Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, China, ²Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Canada.

Milk protein yield (MPY) is a crucial milk performance of dairy cows, which is closely related to the profits of dairy farms. This study aimed to investigate the metabolic differences between the cows with higher and lower MPY, and clarify the latent roles of key metabolites. Forty multiparous Holstein dairy cows with day in milk of 160.0 (±29.1 SD) d, and parity of 3.3 (±1.5 SD) were selected from a group of 343 cows fed on a same diet with a ratio of forage-to-concentrate at 40:60. Twenty of them were higher in MPY (>1.11 kg/d, higher milk yield and higher milk protein content, HH) and 20 were lower in MPY (<0.87 kg/d, lower milk yield and lower milk protein content, LL). Serum were collected from all the cows to characterize the metabolites using the gas chromatography–time of flight/mass spectrometry method. Partial least squares discriminant analysis (PLS-DA) and the orthogonal PLS-DA demonstrated a clear disparity of metabolite profiles between the 2 groups. A total of 132 metabolites were identified with 18 metabolites notably different between HH and LL (VIP > 1 and P < 0.05). Pathway analysis and metabolite sets enrichment analysis revealed that the significantly different metabolites were mainly involved in fructose and mannose metabolism, glycerolipid metabolism, galactose metabo-
lism and arginine and proline metabolism. Among the 18 significantly different metabolites, 16 were higher in HH than in LL. Glucoheptonic acid (log2FC = 3.49, P < 0.01) relating to sugar metabolism, cancer and various diseases, and glutaric acid (log2FC = 1.71, P < 0.01) concerning about lysine degradation and fatty acid metabolism in the human were also probably vital for the milk protein synthesis. In sum, our results revealed that there were some metabolic discrepancies in dairy cows with different MPY, which were mainly related to sugar, lipid and amino acid metabolism and immunity.

Key Words: milk protein yield, metabolomics, dairy cow

87 The effects of α-linolenic acid supplementation on production, health, and fertility of dairy cows. U. Moalem*, H. Lehner, and L. Lifshitz, Department of Ruminant Science, ARO, Volcani Center, Rishon LeZion, Israel.

In recent years, several reports shown that specific long chain fatty acids as omega-3 had beneficial effects on fertility of dairy cows. Also, among the fatty acids, the omega-3 fatty acids possess the most potent immunomodulatory activities. The objectives were to test the effects of supplementation of α-linolenic acid (ALA) from flaxseed on yields, health and fertility of dairy cows. The experiment was conducted in a large commercial dairy farm. 516 cows were randomly divided into 2 groups 21 d before the expected calving. The cows in the treatment group (FLX; n = 276) were fed during the dry period a diet that contained 4% of the same supplement providing 220 kg/d; and postpartum a diet contained 5% of the same supplement providing 220 g ALA per cow/day. The control (CTL; n = 240) cows were fed a diet with similar content. Cows were routinely examined by a veterinarian 7 to 10 d after calving, treated according to the farm’s routine management and body condition score (BCS) was determined at that visit and at peak lactation. Production data were analyzed using the PROC MIXED procedure. Treatment was analyzed as a fixed effect whereas the cow and days in milk as random effects. Distributions were analyzed using the PROC FREQ procedure of SAS. Milk yields were 4.5% higher (P < 0.001), and fat and protein percentages were 3.2 and 3.69%, and 3.16 and 3.22%, in the FLX and CTL groups, respectively (P < 0.002). Ketosis incidence was lower in the FLX than in the CTL group (23.5 and 31.2% respectively; P < 0.05). Severe ketosis occurred less in the FLX cows (P < 0.03), and the incidence of severe metritis tended to be less in the FLX cows (P < 0.1). The mortality rate in the FLX was 0.7% compared with 4.6% in the CTL group (P < 0.005). The BCS and body weight were higher in the FLX than in the CTL cows (P < 0.006). No differences were observed in conception rates at 1st or 2nd inseminations, but waste and open days were 17 and 18 d, respectively, lower in the FLX cows (P < 0.07). In conclusion, supplementation of ALA to dairy cows increased milk yield, reduced fat and protein percentages, improved body condition, decreased the incidence of ketosis, severe metritis and mortality rate, and tended to decrease waste and open days.

Key Words: omega-3, fertility, health


Immunoaetivation via a lipopolysaccharide (LPS) bolus elicits well-characterized immune and metabolic responses. However, acute LPS injections likely do not mimic chronic on-farm immunoaetivation. Therefore, experimental objectives were to characterize metabolic responses to continuous LPS infusion in lactating Holstein cows (164 ± 22 DIM; 676 ± 16 kg BW; parity 3.1 ± 0.4). Following 3 d acclimation, cows were enrolled in 2 experimental periods (P). Period P1 (3 d) served as baseline for P2 (7 d) during which cows received 1 of 2 treatments: (1) saline-infused and pair-fed (CON-PF; i.v. 40 mL saline/h; n = 5) or (2) continuous LPS-infused and ad libitum-fed (LPS-AL; E. coli O55:B5; 0.017, 0.020, 0.026, 0.036, 0.055, 0.088, and 0.148 μg/kg BW/h for d 1–7, respectively; n = 6). Pair-feeding was utilized to eliminate confounding effects of dissimilar nutrient intake. Blood samples were collected on d 1 and 2 of P1 and d 1, 3, 5, and 7 of P2. LPS reduced DMI (28%) on d 1 but DMI gradually returned to pre-infusion levels by d 6. Overall milk yield was reduced similarly (17%) between treatments. There were no differences in circulating glucose (P = 0.42). Plasma insulin was increased 140% in LPS-AL relative to CON-PF cows (P = 0.01). Circulating nonesterified fatty acids were increased from d 1 to 3 and subsequently decreased from d 3 to 7 in both treatments (P < 0.01). Relative to P1, CON-PF cows had a 29% decrease in plasma β-hydroxybutyrate (BHB; P = 0.04) while BHB in LPS-AL did not change (P = 0.40), thus LPS-AL cows had overall increased (41%) BHB relative to CON-PF cows (P = 0.01). Blood urea nitrogen (BUN) gradually decreased in CON-PF cows and it increased in LPS-AL cows from d 1 to 5 (P = 0.04), resulting in an overall 25% increase in LPS-AL versus CON-PF cows (P = 0.02). In conclusion, continuous LPS infusion increased insulin, BHB, and BUN relative to CON-PF cows. Increased insulin is characteristic of both acute and chronic LPS models; however, chronic LPS infusion increased BUN and BHB; which is distinct from acute models and may indicate increased muscle proteolysis and altered carbohydrate/lipid metabolism.

Key Words: insulin, lipopolysaccharide

89 Effects of lactational stage and conjugated linoleic acid supplementation on glucose metabolism during hyperglycemic clamps. L. Grossen-Rösti1, E. Kessler1, A. Tröschler2, R. Bruckmaier*1, and J. Gross1, 1Veterinary Physiology, University of Bern, Bern, Switzerland, 2BASF SE, Lampertheim, Germany.

The metabolic transition from pregnancy to early lactation of dairy cows is characterized by significant increases in glucose demand for milk synthesis. Supplementing conjugated linoleic acid (CLA) may spare glucose due to the milk fat depressing effect of the trans-10, cis-12 CLA isomer, and allows repartitioning nutrients despite an energy deficiency in early lactation. We hypothesized that dairy cows supplemented with CLA would have a decreased HGC glucose infusion rate during early lactation. We conducted 3 consecutive hyperglycemic clamps (HGC) in wk −2, +2 and +4 relative to parturition in Holstein cows (ad lib. feeding of hay) supplemented daily either with 70 g of lipid-encapsulated CLA (6.8 g trans-10, cis-12 and 6.6 g of cis-9, trans-11 CLA isomer; CLA; n = 11) or 56 g of control fat (hydrogenated palm fat, CON; n = 11). From wk −3 up to wk +4 relative to parturition, milk yield and DMI were recorded daily, while BW and milk composition were obtained once weekly. Blood samples were taken once weekly and every 30 min during the HGC (before infusion, during 4 h of glucose infusion and 2 h thereafter). Plasma was analyzed for concentrations of glucose, fatty acids (FFA), β-hydroxybutyrate (BHB), insulin, triglycerides (TG), cholesterol, and RQUICKI was calculated as indicator for insulin sensitivity. CLA supplementation did not affect performance and metabolism except higher BHB, cholesterol (P < 0.05), and TG
Furthermore, insulin concentrations and insulin sensitivity were higher by trend in CLA ($P < 0.10$). During the HGC in wk+2 and +4, insulin response was lower and decrease of FFA and BHB greater compared with the HGC in wk −2 though glucose target concentration achieved during the steady state period was similar for all 3 HGC. Our findings suggest that body reserves are preserved through CLA feeding without restraining the animal’s performance. Furthermore, CLA effects on cholesterol and TG concentrations indicated beneficial effects on hepatic lipid export contributing to an improved efficiency of prevailing metabolites in circulation.

**Key Words:** glucose turnover, conjugated linoleic acid, dairy cow

90 Transcriptional changes in the gut of neonatal dairy calves undergoing a mild diarrhea revealed by a non-invasive technique. F. Rosa*1,4, S. Busato1,2, F. C. Avaroma1,2, E. Trevisi3, M. Bionaz1, and J. S. Osorio4, 1Oregon State University, Corvallis, OR, 2Escuela Agrícola Panamericana El Zamorano, El Zamorano, Francisco Morazán, Honduras, 3Università Cattolica del Sacro Cuore, Piacenza, Italy, 4South Dakota State University, Brookings, SD.

Diarrhea is the leading cause of mortality in neonatal dairy calves in US during the first 4 weeks of life. Therefore, studying physiological adaptations of the gastrointestinal (GI) tract epithelial cells to diarrhea is of great importance to the dairy industry. This study aimed to isolate RNA from exfoliated cells present in fresh fecal samples from neonatal dairy calves and evaluate the GI tract physiological changes in response to mild diarrhea via transcriptomic profiling. Newborn Jersey calves (n = 8) were housed in individual pens from birth to 5 wk of age at Oregon State University Dairy Unit. Calves had ad-libitum access to water and starter grain and were fed 2× daily a total of 5.6 L whole milk. Blood samples were collected weekly for metabolic and inflammatory profiling during the experiment. Fecal score (FS), body weight (BW) and starter intake were recorded throughout the experiment. RNA isolated from fresh fecal samples collected weekly was used for RT-qPCR analysis. Data were analyzed using the PROC MIXED procedure of SAS. Orthogonal contrasts were used when a time effect ($P < 0.05$) was observed to evaluate linear or quadratic effects over time. Starter intake and BW increased ($P < 0.01$) over time. FS was greatest (2.6 ± 0.3; $P < 0.01$) at 2 wk. Blood glucose and BHBA had a linear ($P ≤ 0.04$) effect over time. Biomarkers of liver function, cholesterol, albumin, and paraoxonase had a linear response ($P < 0.01$) over time. In blood, reactive oxygen metabolites reached a peak at wk 2, which was explained by a positive quadratic effect ($P < 0.01$). Similarly to FS, blood inflammatory biomarkers, IL-6, ceruloplasmin, and haptoglobin had a positive quadratic effect ($P ≤ 0.04$). The inflammation-related genes $TLR4$, $TNFα$, $IL8$, and $IL1B$ in fecal RNA had a positive quadratic effect ($P = 0.05$). A negative quadratic effect ($P ≤ 0.05$) was observed on cell membrane transporters genes, $AQP3$ and $SLC5A1$. Overall, our findings support the use of fecal RNA as a non-invasive tool to evaluate transcriptomic alterations during diarrhea in neonatal dairy calves. Future research could use such method to observe physiological alterations in response to dietary treatments.

**Key Words:** dairy calf, gut health, gene expression
Evaluation of an ear tag based behavior and temperature monitor (Cow Manager) during a heat stress induction trial using electric heat blankets (EHB). M. Al-Qaisi*, L. Timms, and L. Baumgardn, Iowa State University, Ames, IA.

Objectives were to evaluate behavior monitors to assess behaviors and ear temperature during an induced heat stress study using electric heat blankets (EHB). Eartags (Cow Manager SensOor, Agis Automatisering, Netherlands) were placed in left ears of trial cows (n = 8) before trial barn entry (P0). Cows were moved into individual stalls (curtain sided barn with radiant heaters). Cows acclimated to trial barn for 1 week (P1). Cows were then fitted with an EHB (Thermotex Therapy Systems Ltd. Calgary, AB, Canada) that was on for 1 week (P2). Data was summarized for the last 4 d of P 0 – 2 (3 d acclimation). Following EHB removal, cows returned to their respective barn and monitored for 7 d (P 4 = 1–3 d post trial and P5 = 4–7 d). Surface ear temperatures (T) and accelerometer movements were recorded and transcribed through proprietary algorithm into 5 behaviors (hourly or daily %): ruminating (R); eating (E); Not active or moving (NA); active (A); and very active. Twenty-four hr. continuous time lapse video was taken on cows during trial barn time. Freestall barn T before moving was 1–10°C, with ear T 1.6–20°C, reflecting changes due to daily and diurnal temperatures differences. Upon entry into trial barn (15–21°C), ear T immediately increased to 29–32°C, indicating increased blood flow and potential heat dissipation. Ear T remained between 29 and 35°C during trial barn period (w/out and with EHB). Daily average behavioral (DAB) % (SEM) for P0 were: R 40% (1.1); E 28% (1.45); NA 25% (1.02); A 4% (0.34); VA 3% (0.44). DAB % for P1 (slightly warmer trial barn acclimation) were: R 49%; E 17%; NA 24%; 5%: 5%. (E decreased 36% (P < 0.01) and R increased 23% (P < 0.05) compared with P0. Video showed decreased eating times and altered meal patterns but panting transcribed as a rumination movement. DAB% for P2 (EHB) were: R 63%; E 10%; NA 19%; 6%; 3%. (41 and 17% decrease in E and NA; 29% increase in R (panting) compared with P1 (P < 0.05. DAB for P 3–4 (return to barns) were: R 39.44; E 22.25; NA 31.24; A 3.3; and VA 4.3 (normal behaviors within 1–7d). EHB was successful and monitors can be an excellent tool to quantify behavioral changes in early and severe heat stress.

Key Words: behavior monitor, electric heat blanket, heat stress


Heat stress (HS) is an annual environmental issue which negatively affects a variety of production parameters including milk yield and composition, growth, and reproduction. However, precisely studying HS requires expensive climate-controlled facilities; infrastructure inaccessible to most researchers. Alternative low cost methods to study HS in dairy cows would benefit HS research. Thus, study objectives were to explore the efficacy of an electric heat blanket (EHB) as an alternative method to model HS and to determine whether EHB-induced hyperthermia affects production parameters similarly to natural HS. Lactating Holstein cows (n = 8) were housed in individual box-stalls and allowed to acclimate (3 d). The trial consisted of 2 experimental periods (P). During P1 (3 d), cows were fed ad libitum and housed in thermonutral conditions for baseline data collection. During P2 (7 d) cows were fitted with an EHB consisting of 12 infrared heating pads as a heat source (Thermotex Therapy Systems Ltd. Calgary, AB, Canada). Cows were milked twice daily (0600 and 1800 h). Rectal temperature (Tr) and respiration rate (RR) were obtained twice daily (0600 and 1800 h) during both P1 and P2. Overall, there was an increase compared with the baseline in Tr and RR at both 0600 h and 1800 h (1.0°C and 25 bpm, 1.2°C and 29 bpm, respectively P < 0.01). The EHB decreased DMI (25%; P < 0.05) by the end of P2. Similarly, milk yield was decreased (21%; P < 0.05) by d 7 of P2. Milk protein tended to decrease (4.4%; P = 0.07), while milk urea nitrogen increased during P2 (33%; P < 0.01) relative to P1. No other differences were observed in milk composition (P > 0.10). No differences in circulating glucose levels were observed during P2 (P > 0.10) when compared with P1. However, nonesterified fatty acids tended to be slightly increased in P2 compared with P1 (55%; P = 0.09). In summary, results indicate that employing the EHB affects production parameters similarly to natural HS and thus is an effective and inexpensive research tool to evaluate the biological consequences of HS in lactating dairy cows.

Key Words: dairy cow, heat stress, electric heat blanket

91 Evaluation of an ear tag based behavior and temperature monitor (Cow Manager) during a heat stress induction trial using electric heat blankets (EHB). M. Al-Qaisi*, L. Timms, and L. Baumgardn, Iowa State University, Ames, IA.


Heat stress is an annual environmental issue which negatively affects a variety of production parameters including milk yield and composition, growth, and reproduction. However, precisely studying HS requires expensive climate-controlled facilities; infrastructure inaccessible to most researchers. Alternative low cost methods to study HS in dairy cows would benefit HS research. Thus, study objectives were to explore the efficacy of an electric heat blanket (EHB) as an alternative method to model HS and to determine whether EHB-induced hyperthermia affects production parameters similarly to natural HS. Lactating Holstein cows (n = 8) were housed in individual box-stalls and allowed to acclimate (3 d). The trial consisted of 2 experimental periods (P). During P1 (3 d), cows were fed ad libitum and housed in thermonutral conditions for baseline data collection. During P2 (7 d) cows were fitted with an EHB consisting of 12 infrared heating pads as a heat source (Thermotex Therapy Systems Ltd. Calgary, AB, Canada). Cows were milked twice daily (0600 and 1800 h). Rectal temperature (Tr) and respiration rate (RR) were obtained twice daily (0600 and 1800 h) during both P1 and P2. Overall, there was an increase compared with the baseline Tr and RR at both 0600 h and 1800 h (1.0°C and 25 bpm, 1.2°C and 29 bpm, respectively P < 0.01). The EHB decreased DMI (25%; P < 0.05) by the end of P2. Similarly, milk yield was decreased (21%; P < 0.05) by d 7 of P2. Milk protein tended to decrease (4.4%; P = 0.07), while milk urea nitrogen increased during P2 (33%; P < 0.01) relative to P1. No other differences were observed in milk composition (P > 0.10). No differences in circulating glucose levels were observed during P2 (P > 0.10) when compared with P1. However, nonesterified fatty acids tended to be slightly increased in P2 compared with P1 (55%; P = 0.09). In summary, results indicate that employing the EHB affects production parameters similarly to natural HS and thus is an effective and inexpensive research tool to evaluate the biological consequences of HS in lactating dairy cows.

Key Words: dairy cow, heat stress, electric heat blanket

93 1H Nuclear magnetic resonance-based metabolomics of urine in heat-stressed dairy goats. A. Contreras-Jodar*, N. Nayan1,2, A. A. K. Salama1, S. Hamzaoui1,3, and G. Caja1, 1University Autonoma of Barcelona, Bellaterra, Barcelona, Spain, 2Wageningen University, Wageningen, the Netherlands, 3University of Bouira, Bouira, Algeria.

With the aim of completing a previous study on whole blood transcriptomics of heat stressed dairy goats (Contreras-Jodar et al., 2016; EAAP Annual Meeting, Belfast, UK, p. 126), urine biomarkers were investigated by 1H nuclear magnetic resonance (1H-NMR) spectroscopy in the same goats. Eight adult Murciano-Granadina dairy does in mid-lactation (42.8 ± 1.3 kg BW, 1.73 ± 0.15 L/d) were placed in metabolic cages and milked once-a-day. Does were fed a TMR and exposed to 2 climatic treatments according to a crossover design with periods of 19 d. Treatments and temperature-humidity index (THI; NRC, 1971) were: 1) thermal neutral (TN: 15 to 20°C, 40 to 45% HR; THI = 59 to 65), and 2) heat stress (HS: 37°C-40% HR day; 30°C-40% HR night; THI = 86 and 77, respectively). Day-night was 12–12 h. Urine samples were collected at d 19 and analyzed by 1H-NMR spectroscopy (Bruker Avance-III; 600.13 MHz and 298°K). Multivariante data analyses included PCA (principal component analysis) and PLS-DA (partial least square–discriminant analysis) assessment with cross validation to identify HS biomarkers. The metabolites were assigned using the Human Metabolome Data Base (www.hmdb.ca). PLS-DA revealed 2 separated clusters corresponding to TN and HS goats (R2 = 0.45; Q2 = 0.43). Metabolites that most discriminate between TN and HS were phenylalanine metabolic derivative compounds, such as hydroxyphenylacetate (7.27 ppm), hydroxyphenylacetylglycine (7.20 ppm) and phenylglyoxylic acid (7.62 ppm), which were increased in HS does (P < 0.001). A greater excretion of these compounds in urine together with the previously observed most downregulated gene in blood cells, HGD (homogenitase 1,2-dioxygenase; FC = -3.66), suggest that HS decreased the capability of goats to synthesize tyrosine from its precursor phenylalanine. Consequently, HS impaired milk production may be a consequence of the reduced synthesis of catecholamines (i.e., L-DOPA, epinephrine, norepinephrine) and thyroid hormones. In conclusion, heat stress caused significant changes in the urine metabolomic profile.
of dairy goats. These changes may be related to the synthesis of neurotransmitters and to the hormonal regulation of nutrient metabolism.

Key Words: heat stress, urine metabolomics, dairy goats

94 Effect of heat stress, dietary zinc source and intramammary lipopolysaccharide challenge on metabolic responses of lactating Holstein cows. T. N. Marin,*1, R. M. Orellana1, X. Weng1, A. P. A. Monteiro1, J. Guo1, J. K. Bernard1, D. J. Tomlinson2, J. M. DeFraın2, and S. Tao1, 1University of Georgia, Tifton, GA, 2Zinpro Corporation, Eden Prairie, MN.

The objective of this study was to evaluate cows’ metabolic responses when fed 2 dietary Zn sources under 2 environmental conditions and subjected to an intramammary lipopolysaccharide challenge (IM-LPS). Multiparous lactating Holstein cows (n = 72) were randomly assigned to 4 treatments with a 2 × 2 factorial arrangement. Treatments included 2 different environments (E): cooled (CL) using fans and misters or non-cooled (NC), and 2 dietary Zn sources: 75 ppm Zn hydroxychloride (IZ) or 35 ppm Zn hydroxychloride + 40 ppm Zn-Met complex (ZC). All cows were cooled and fed respective dietary treatments during first 84 d of the trial (baseline phase [BP]). During the following 84 d (environmental challenge [EC]), NC cows were deprived of cooling. Air temperature and relative humidity were recorded and temperature-humidity index was calculated, which averaged 73 and 78 during BP and EC. Plasma was collected at d −73, −45, −31, −17, −3, 1, 3, 5, 12, 26, 41, 54, 68, and 81 relative to EC. At d 34 of EC, a subset of cows (n = 16) were subjected to IM-LPS, with plasma collected at 0, 3, 6, 12, 24, 48, 72, 96, 120, and 144 h after infusion. During BP, ZC cows tended (P = 0.11) to have higher plasma triglyceride than IZ (5.8 vs. 5.5 mg/dL). After EC, a tendency (P = 0.10) for E × diet interaction for plasma glucose was observed, as IZCL had highest. Interactions of E × d (P ≤ 0.06) were observed for β-hydroxybutyrate, triglyceride, nonesterified fatty acids (NEFA) and insulin due to lower metabolites but higher insulin for NC than CL 3 to 5 d after EC. After IM-LPS, ZCNC cows had the lowest NEFA (E × diet, P = 0.05). Relative to IZ, ZC cows tended (P = 0.08) to have higher β-hydroxybutyrate (5.1 vs. 6.0 mg/dL) and had higher (P = 0.04) insulin (0.5 vs. 0.8 µg/L). NC cows had lower (P = 0.05) plasma glucose and higher insulin at 3 and 12 h after IM-LPS (E × h, P < 0.01) than CL. Relative to CL, NC cows had higher plasma NEFA at 3 h but lower at 24, 48 and 120 h of IM-LPS (E × h, P = 0.01). In conclusion, heat stress and dietary Zn source altered lactating cows’ metabolic responses.

Key Words: heat stress, volatile fatty acid, rumen

96 Using calf jackets to minimize cold stress in Jersey calves. X. Wen,* A. Adams Progar, D. A. Moore, J. H. Harrison, and J. Schafer, Washington State University, Pullman, WA.

When environmental temperatures are lower than a calf’s thermoneutral zone (TNZ), cold stress ensues. Jersey calves are especially vulnerable to cold weather conditions because of their small frame. This study investigated the effectiveness of using calf jackets to minimize the effects of cold stress to improve health and growth performance in Jersey calves. Twenty Jersey heifer calves were randomly assigned to 1 of 2 treatments: (1) provided jacket from 1 – 14 d of age (J); or (2) not provided a jacket (NJ). During the first 2 wk after birth, calf behavior was recorded at 5-min intervals (during daylight hours) using a time-lapse video camera and calf body temperatures were recorded at 1 h intervals using ThermoChron iButtons attached to the underside of each calf’s tail. Calf BW were recorded every 7 d until weaning, and health treatment records were collected over the course of the study. Environmental conditions (ambient temperature and relative humidity) were recorded at 1 h intervals using HOBO® data loggers and used to calculate the temperature-humidity index (THI). Data were analyzed as a randomized complete block design with repeated measures using SAS. Average ambient temperature was 3.65°C (average high: 19.44°C/average low: −18.85°C). No significant differences were detected between the 2 treatment groups for calf lying behavior (P = 0.15), initial BW (26.12 ± 3.51 kg; P = 0.43), final BW (J: 57.83 ± 3.97 kg; NJ: 59.52 ± 4.30 kg; P = 0.79), average daily gain (J: 1.08 ± 0.16 kg/day, NJ: 1.16 ± 0.13 kg/day; P = 0.42), number of health treatments recorded (all P ≥ 0.44), or body temperature (P = 0.71). As ambient temperatures decreased, THI within the hutch decreased (P < 0.0001) and calf body temperatures increased (P < 0.0001). More lying behavior was observed in calves as temperatures within the hutch decreased (P = 0.004) and the number of health treatments recorded decreased as the calves aged (P = 0.002).
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**97**  Technical and economic performance of Holstein crossbred versus pure Holstein herds using a stochastic simulation model. M. López-Suárez†, L. Castillejos1, P. Miera, J. M. Loste and S. Calsamiglia. 1Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, 2Centre Veterinari Tona, Tona, Barcelona, Spain, 3Alhakide, Irurtzun, Navarra, Spain.

Crossbreeding can be used as an approach to counteract the negative effects of inbreeding within Holstein herds and to improve functional traits such as fertility, calving ease, health and survival of cows. The objective of this study was to compare the productive and economic performances of pure Holstein (H) with Holstein crossbred (C) herds using a stochastic dairy farms simulation model (www.dairyfarm.es). Data from 3 farms with a subpopulation of pure Holstein and a subpopulation under a crossbreeding program, with the same feeding and management systems, were used for the simulation. Thus, using performance data of each subpopulation, within each real farm, 2 subherds were generated in the simulator: an H herd and a C herd. The same average economic conditions were used in the 3 farms. Results were obtained from simulations of a 4-year period. The C herds had higher fertility (7 to 10 percentage units), fewer days open (24 to 32 d), more calves born and lower milk production (1.4 to 2.3 L/d/cow) compared with H herds. Moreover, the C herds had lower feed and reproduction costs, lower milk sales revenues and lower income over feed costs compared with H herds in the 3 farms. The gross margin adjusted for the animal inventory was higher in H than in C herds in 2 of the 3 farms studied (+€136 and +€264/cow/year) due to the penalty for higher milk production and revenues. In the third farm, the difference in gross margin was slightly higher in C than in H herd (+€12/cow/year), due to the penalty for the high SCC in H (average of 310 and 524,000 cells/mL for C and H, respectively), which decreased the milk price, and the smaller difference in milk production between H and C herds (1.4 L/d/cow) compared with the other 2 farms, where the difference in milk production was larger (2 and 2.3 L/d/cow). Results suggest that the improvement in reproductive performance of crossbreeding does not compensate for the lower milk production of crossbred herds.

**Key Words:** crossbreeding, milk production, economics

**98**  Herd contextual effect modulates the relationship between cow milk yield and reproductive performance. R. Rearte1,2, S. LeBlanc3, 2, R. de la Sota4, S. Corva5, I. Lacau-Mengido6,3, and M. Giuliodori. 1, 2Cátedra de Higiene, Epidemiología y Salud Pública, Facultad de Ciencias Veterinarias – Universidad Nacional de La Plata (FCV–UNLP), La Plata, Argentina, 2Cátedra y Servicio de Reproducción Animal, FCV–UNLP, La Plata, Argentina, 3Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina, 4Instituto de Biología y Medicina Experimental–CONICET, Buenos Aires, Argentina, 5Department of Population Medicine, University of Guelph, Guelph, ON, Canada, 6Cátedra de Fisiología, FCV–UNLP, La Plata, Argentina.

Some studies have evaluated the relationship between milk yield and reproductive performance but without considering the multilevel nature of the data. So, our objective was to evaluate the effect of milk production at cow and herd levels and their interaction on the odds of pregnancy at the cow level; that is, to test for a herd contextual effect modulating the cow’s probability of pregnancy. Data from 657,968 lactations started from 2001 to 2012 from 677 Argentinean dairy herds were included in the study. A mixed logistic regression model was fit to assess the odds of pregnancy by 100 DIM (P100) with fixed effects of cow milk yield to 80 DIM (CM80), quartiles of herd mean CM80 per herd-year (HM80) and their cross-level interaction. Herd-year was considered as a random effect. CM80 had a significant negative effect on P100 (OR = 0.95). Thus a cow belonging to a top quartile milk yield herd with CM80 1 SD (400 kg) over their HM80 had 5% lower odds of P100. The same increment in milk yield in cows from the lowest quartile of herd yield was predicted to have 1% lower odds of being pregnant by 100 DIM. An average producing cow in a top quartile yield herd had 5% greater odds of P100 than an average producer in a bottom quartile herd (Table 1). The herd contextual effect explained 4% of the observed variation in P100. The relationship between cow milk yield and reproductive performance is statistically significant but of small magnitude, and varies among herds: the association is negative in high-producing herds and positive in low-producing herds.

**Table 1 (abstract 98).** Logistic regression model of the effect of milk yield to 80 DIM at cow and herd levels on odds of pregnancy by 100 DIM

<table>
<thead>
<tr>
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<th>Odds ratio</th>
<th>95%CI</th>
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<tbody>
<tr>
<td>CM801</td>
<td>0.95</td>
<td>0.95–0.95*</td>
</tr>
<tr>
<td>HM802</td>
<td>Top</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Upper Middle</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Lower Middle</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Bottom</td>
<td>0.95</td>
</tr>
<tr>
<td>CM80×Top</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CM80×Upper Middle</td>
<td>1.00</td>
<td>0.94–1.06</td>
</tr>
<tr>
<td>CM80×Lower Middle</td>
<td>1.02</td>
<td>1.01–1.03*</td>
</tr>
<tr>
<td>CM80×Bottom</td>
<td>1.04</td>
<td>1.03–1.05*</td>
</tr>
</tbody>
</table>

1CM80 = overall milk yield per cow by 80 DIM centered on HM80 (SD= 400 kg).
2HM80 = mean CM80 per herd-year divided into quartiles.
*P < 0.05.

**Key Words:** contextual effect, reproduction

**99**  Precision dairy herd management—A quantile regression approach. J. Richard* and T. Mark, University of Kentucky, Lexington, KY.

With new information, it is evident that there is a need for new methodologies for precision dairy (PD) data analysis. Dairy producers have a variety of PD technologies available to them, which creates the need for investment analyses. This study serves as early work in data valuation of new information streams that these technologies collect. Academics need experience with these new data sets and potential methodologies to contribute to producer-targeted recommendations. The objective of this case study is to investigate the complexities of these data intensive herd records. This initial work has provided early evidence that new PD technology can inform herd management decisions at the individual cow level. This data starts in June of 2014 and extends through July 2015, where daily and hourly data were collected from wearable technology on the herd at Coldstream, the University of Kentucky research farm. A quantile regression technique was applied to the unbalanced panel data set, where the functional form was: Daily Milk Yield = β0 + β1 Days in Milk + β2 Body Weight + β3 SCC + β4 Eating Time + β5 Steps + β6...
Resting Bouts + βTHI This analysis separates the herd into 3 quantiles: the 25th, 50th, and 75th percentiles. Sorting the data by lactation stage and lactation number isolates the effects of the production variables for each category. The results indicate that production variables have different effects on high performing cows as compared with the same variables’ effects on low performing cows. Body weight for example, is 0.02 and 0.04 lbs higher than the 25th percentile in the 50th and 75th quantile respectively, at the 1% significance level. Days in milk findings reveal that cows in the 75th percentile have coefficient magnitudes 0.03 and 0.05 lbs/day higher than cows in the 25th percentile. This small but significant (all at the 5% significance level) finding reiterates what the literature has documented about days in milk, but reveals marginal effects in more specific cow groups. The coefficient sign (+) of variables such as steps taken, resting bouts, and THI, were also found to change across stage of lactation. The successful application of this technique will allow finer detail to be taken with the model specification, and thus better herd management based off this precision data.

**Key Words:** precision dairy, quantile regression, quantiles
**Ruminant Nutrition Symposium: Metabolomics Applications in Dairy Cow Metabolism**

**100 Discovering isomeric milk metabolites with liquid chromatography drift tube-ion mobility mass spectrometry.** T. Shen, I. Blaženović, and O. Fiehn*, West Coast Metabolomics Center, University of California-Davis, Davis, CA. An overview is given on mass spectrometry-based metabolomics: past, current, and future developments. Understanding the metabolome is challenging due to the enormous diversity of metabolite structures. Metabolomics uses a range of tools, established as service at the NIH West Coast Metabolomics Center at UC Davis. We here explore the use of ion mobility spectrometry (IMS) in combination with liquid chromatography (LC) and high resolution mass spectrometry (MS) for milk analysis by increasing total peak capacity, specifically to distinguish isobaric and isomeric species that are not resolved by LC-MS alone. As an example, we used LC-IM-QTOF MS for unraveling the complex lipidome compositions in human, goat and cow milk. Measurements were performed on an Agilent 1290 Infinity II LC with 6560 drift tube-ion mobility-QTOF MS system using a Waters Acquity CSH C18 column with a 15 min-gradient of isopropanol/acetonitrile (90:10, B) varied from 15% to 99% and acetonitrile/water (60:40, A), ammonium formate/formic acid buffered. Twenty µL of milk samples were extracted with MTBE and methanol. Dried extracts were reconstituted in methanol before LC-IM-MS analysis. Measured ion mobility drift times were corrected with external calibration for time spent outside the drift tube for precise determination of collision cross sections. We optimized the control over ion trapping in the drift cell. We analyzed lipid extract from 3 types of milk samples with untargeted LC-IM-MS. IM mode provided 6x more molecular features compared with regular LC-MS analysis without ion mobility. Using Agilent Mass Profiler to report IM peak feature, we developed a specific command line tool in R to characterize co-eluting isobaric species, followed by downstream identification by MS-DIAL. Our R command screened the IM features that exhibited distinct IM drift time (e.g., ΔDT > 0.4 msec) by approximate m/z and LC retention time. We reduced the false discovery rate by evaluating DT intensities and m/z-specific DT after fitting a power-law regression. We discovered 73 isobaric species in milk lipidomic extracts that required ion mobility separation in addition to LC-QTOF MS.

**Key Words:** metabolomics, lipidomics, mass spectrometry

**102 NMR metabolomic analysis of dairy cows reveals milk glycerophosphocholine to phosphocholine ratio as prognostic biomarker for risk of ketosis.** M. S. Klein1, N. Krattenmacher2, S. Wiedemann2, W. Junge2, G. Thaller2, P. J. Oefner1, and W. Gronwald*1, 1Institute of Functional Genomics, University of Regensburg, Regensburg, Bavaria, Germany, 2Institute of Animal Breeding and Husbandry, Christian-Albrechts-University, Kiel, Schleswig-Holstein, Germany.

In this contribution we show that nuclear magnetic resonance (NMR)-based analysis of milk allows identifying prognostic biomarkers for risk of ketosis in dairy cows. Diagnostic markers for ketosis such as acetone and β-hydroxybutyric acid (BHBA) are known, but disease prediction remains an unsolved challenge. Milk is a steadily available biofluid and routinely collected on a daily basis. This high availability makes milk superior to blood or urine samples for diagnostic purposes. We demonstrate that high milk glycerophosphocholine (GPC) levels and high ratios of GPC to phosphocholine (PC) allow for the reliable prediction of ketosis incidence. During the first month of lactation, molar GPC/PC ratios equal or greater than 2.5 indicate a very low risk for developing ketosis. This threshold was validated for different breeds (Holstein-Friesian, Brown Swiss, and Simmental Fleckvieh) and for animals in different lactations, with observed odds ratios between 1.5 and 2.38. In contrast to acetone and BHBA, these measures are independent of the acute disease status. A possible explanation for the predictive effect is that GPC and PC are measures for the ability to break down phospholipids as a fatty acid source to meet the enhanced energy requirements of early lactation.

**Key Words:** ketosis, metabolomics, nuclear magnetic resonance (NMR)

**101 Lipidomic studies can inform on the effects of low-fat or full-fat dairy foods on cardiometabolic health: Potential benefit of full-fat dairy products.** P. J. Meikle*, Baker Heart and Diabetes Institute, Melbourne, Australia.

The metabolic syndrome incorporating obesity, hypertension, dyslipidemia and elevated plasma glucose has reached epidemic proportions leading to an increase in type 2 diabetes (T2D) and cardiovascular disease (CVD). First line treatment for metabolic syndrome is lifestyle intervention (diet and exercise). However, we do not fully understand the effects of diet or exercise on the disease process. Dyslipidemia (raised plasma triglycerides and LDL-cholesterol, and decreased HDL-cholesterol) is an independent risk factor for T2D and CVD. However, these measures do not provide a complete view of the alterations to lipid metabolism associated with these diseases, nor how interventions may influence these processes. Lipidomics presents a new set of tools to address these issues. We have developed a targeted lipidomics platform using liquid chromatography electrospray ionization-tandem mass spectrometry to profile 300–400 lipid species from 10 µL plasma. We have applied this technology to multiple clinical and population based cohorts to define the plasma lipidomic profiles associated with T2D and CVD and to evaluate the potential application of these profiles to assess disease risk. We have performed dietary intervention studies with low fat and full fat dairy foods to characterize the relationship between dairy consumption, lipid metabolism and the risk of cardiometabolic disease. In acute studies differential responses to dairy and soy meals derived through lipidomic analysis of phospholipids (plasmalogens with antioxidant capacity) suggest differences in the metabolism of soybean oil and dairy fat. In a long-term studies, lysophosphatidylcholine, lyso-platelet-activating factor, and several phospholipid fatty acids correlated with full-fat dairy consumption. Lysophosphatidylcholine and lyso-platelet-activating factor were also directly associated with insulin sensitivity and inversely with insulin resistance. These results suggest that both the composition and metabolism of dairy fat may be important factors in cardiometabolic health and disease.

**Key Words:** full-fat dairy, lipidomics, lipid metabolism

**103 Characterization of the bovine lipidome: Discovery of the sphingolipid ceramide as a biomarker of insulin resistance in dairy cattle.** J. W. McFadden*, West Virginia University, Morgantown, WV.
The development of insulin resistance represents a homeorhetic adaptation to support lactation in dairy cattle; however, impaired insulin action can be a detriment to metabolic health during the peripartum. To delineate the mechanisms of insulin resistance, we have focused on the sphingolipid ceramide which is an antagonist of insulin action in diabetic monogastrics. A lipidomics workflow that includes the application of targeted and untargeted mass spectrometry has been employed to investigate the relationship between ceramide metabolism and insulin sensitivity in dairy cattle. Studies have established that the peripartal progression of hyperlipidemia, hepatic lipid accumulation, and insulin resistance is accompanied by hepatic and circulating ceramide accumulation. Although these changes develop in all cows, enhanced adiposity predisposes cows to heightened ceramide accrual which may explain their observed impairment in lipolytic responsiveness to glucose. Hepatic de novo ceramide synthesis and export can respond to increased fatty acid delivery and altered mitochondrial fatty acid processing. Specifically, the induction of hyperlipidemia by intravenous triacylglycerol infusion or by nutrient-restriction can increase de novo ceramide synthesis in dairy cattle. However, ceramide pools may also rise in response to inflammation-provoked sphingomyelin hydrolysis. Importantly, the ability of ceramide to antagonize insulin action is influenced by the acyl moiety and glycosylation status. Our series of lipidomic studies have revealed circulating lignoceroyl sphingosine (C24:0-ceramide) as an insulin resistance biomarker. Feeding regimens utilized on-farm can influence C24:0-ceramide supply. For example, palmitic acid feeding increases C24:0-ceramide supply in lactating dairy cows, relative to stearic acid or no fat supplementation. Also, micronutrients nicotinic acid and choline can influence ceramide levels. On-going research is investigating novel pharmacological and nutritional approaches to modify ceramide synthesis and insulin sensitivity as a means of improving dairy cow health and performance.

Key Words: ceramide, insulin resistance, lipidomics

104 Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in dairy cow diets: Application of MetaboAnalyst. F. Saleem*1,3, Q. Zebeli2, B. N. Ametaj2,3, N. Psychogios3, M. J. Lewis3, S. M. Dunn2, J. Xia3, and D. S. Wishart3, 1University of Agriculture Faisalabad, Faisalabad, Pakistan, 2Department of Agricultural, Food and Nutritional Science, Edmonton, AB, Canada, 3Departments of Computing Science and Biological Sciences, University of Alberta, Edmonton, AB, Canada.

Early lactation is a very critical phase in the nutrition and management of dairy cows. To alleviate any negative energy balance during early lactation cows are fed high proportion of cereal grains. Transitioning from a diet of grass and hay to one consisting primarily of grains cause the incidence of many metabolic disorders (ketosis, fatty liver and rumen acidosis). However, the precise mechanism(s) of how grain feeding alters the rumen environment and causes metabolic disorders is not clear. Because metabolomics is such a powerful approach for studying the chemical changes in biological systems, we undertook a comprehensive, quantitative metabolomics analysis of rumen fluid samples taken from 8 dairy cows fed graded amount of barley grain (i.e., 0, 15, 30, and 45% of diet dry matter). By using improved metabolite detection techniques, including proton NMR, GC-MS, and DFI-MS, we identified and quantified 93 metabolites in 4 different ruminal fluid samples. Data collected by these advanced metabolomics techniques, was analyzed by MetaboAnalyst (an online tool for metabolomic data analysis). Multivariate analysis showed that when cows fed with high-grain diets (>30%) resulted in increased rumen fluid concentrations of several toxic, inflammatory, and unnatural compounds including putrescine, methylamines, ethanol, glucose, urea, ethanolamine, and short-chain fatty acids. Perturbations in several amino acids (phenylalanine, ornithine, lysine, leucine, arginine, valine, and phenylacetylglycine) were also observed. By using ANOVA, it was also revealed a drop in ruminal pH and a decreased concentration of 3-phenylpropionate in cows fed greater amounts of cereal grain. These results certainly underline the importance of gaining a better understanding of the biochemical function of rumen as a whole ecosystem. Deeper understanding of how diet influences rumen health, as well as improved methods for monitoring these changes should enable us to maintain the fine balance between high milk productivity and good herd health.

Key Words: MetaboAnalyst, metabolomics


Supplementation of live yeast (LY) in the diet is an interesting practice to limit the negative effects of SARA. Measurement of ruminal redox potential (Eh) has been shown to be a tool to understand the mode of action of LY in rumen. The objective of this study was to quantify the effect of LY (5 g/d of Saccharomyces cerevisiae, 1010 cfu/g DM, CNMC I-4407, Phileo Animal Care, France) on ruminal Eh, live yeast, ruminal redox and the decrease of ammonia content response (Eh).

Yeast products main- determined for BCS and body weights (P < 0.01). No statistical significance was reached for BCS and body weights among treatments. Feed efficiencies, calculated as energy corrected milkDMI, were similar among treatments (1.51, 1.36, 1.51; SEM = 0.15), but there was a treatment by week interaction (P < 0.01). A treatment effect for PUN was detected (16.86, 14.10, 16.15; SEM = 0.444; P < 0.01). No statistical significance was determined for BCS and body weights (P > 0.05). Yeast products maintained performance, rather than improving production as hypothesized.

Key Words: yeast supplement, lactation performance, dairy cow

107 Effects of Saccharomyces cerevisiae fermentation products and subacut ruminal acidosis (SARA) on apparent digestibility of dry matter, NDF, and phosphorus in lactating dairy cows. V. P. Senaratne1,1, H. Khalouei1, K. Fehr1, J. Guo1, I. Yoon2, E. Khafipour1, and J. C. Plaizier1, 1Department of Animal Science, University of Manitoba, Winnipeg, Canada, 2Diamond V, Cedar Rapids, IA.

The effects of Saccharomyces cerevisiae fermentation products (SCFP) on the apparent digestibilities of dry matter (DM), neutral detergent fiber (NDF) and phosphorus (P) in lactating cows during control feeding and during grain-based subacut ruminal acidosis (SARA) challenges were investigated. Thirty-two Holstein lactating dairy cows were assigned to 4 treatments, i.e., control, and 3 different SCFP supplementations. Cows in the 3 SCFP treatment groups received 14 g/d Diamond V Original XPC (XPC), 19 g/d NutriTek (NTL), or 38 g/d NutriTek (NTH) mixed with 126, 121, and 102 g/d ground corn, respectively, while the cows in the Control group received 140 g/d ground corn only. Supplements were top dressed once daily immediately after feed delivery from 4 wk pre-calving to 11 wk post-calving. At wk 5 and 8 after calving one-week grain-based SARA challenges were conducted by switching from a lower to a higher concentrate diet (50% to 70% concentrate, DM basis). Diet samples were collected weekly and fecal samples of individual cows were collected twice weekly. Samples were pooled for wk 1–4 after calving (preSARA), wk 5 after calving (first SARA challenge), and wk 8 after calving (second SARA challenge). Samples were analyzed for DM, acid insoluble ash (AlA), NDF and P (% DM basis). Apparent total-tract digestibilities for DM, NDF and P were calculated using AlA as an internal marker. The apparent total-tract digestibility of DM and P were not affected by the SARA challenges and SCFP, and averaged 68.9 and 52.6%, respectively, across treatments and weeks. The SARA challenges reduced the apparent total-tract digestibility of NDF from 61.1 to 49.0% (P < 0.01), but the NTH supplementation increased NDF digestibility from 52.7 to 61.8% (P < 0.02). Our results show that SCFP can increase fiber digestion, which is particularly important during high grain feeding.

Key Words: dairy cow, SARA, Saccharomyces cerevisiae fermentation product

108 Effects of Saccharomyces cerevisiae fermentation products on endotoxins and acute phase proteins in lactating dairy cows. J. Guo1, H. Khalouei1, K. Fehr1, V. Senaratne1, Z. Zhang1, H.
Derakhshani1, M. Scott2, G. Crow1, I. Yoon*2, E. Khaifipour1, and J. C. Plaizier1, 1University of Manitoba, Winnipeg, Canada, 2Diamond V, Cedar Rapids, IA.

The objectives were to determine the effects of Saccharomyces cerevisiae fermentation products (SCFP) on free lipopolysaccharide (LPS) in rumen fluid and feces, and on acute phase protein in blood plasma during normal feeding and SARA challenges in lactating dairy cows. Thirty-two Holstein lactating dairy cows fixed with rumen canulas were assigned to 4 treatments, including control and 3 SCFP treatments: Diamond V Original XPC (XPC, 14 g/d), NutriTek low concentration (NTL), and NutriTek high concentration (NTH) in a randomised complete block design. Treatments were administered between 4 wk before until 11 wk after calving. During lactation, cows received a mixed ration containing 50% concentrate, with the exception that during wk 5 and 7 grain-based SARA challenges were conducted by ingestion of the toxin. Milk samples were collected twice daily from d 26 to 33 and plasma was sampled for liver enzymes on d 25 and 30 before the morning feeding. Data were analyzed with the GLIMMIX procedure of SAS. Transfer of ingested AFB1 into milk aflatoxin M1 (AFM1) was greater in T than CL or CL+SCFP (1.65 vs. 1.01 and 0.94%, respectively, P < 0.0001) on d 26 to 30. The CL and CL+SCFP treatments reduced milk AFM1 concentration compared with T (0.45 and 0.40 vs. 0.75 µg/kg, respectively; P < 0.001) and unlike T, they resulted in lower AFM1 concentrations than the FDA action level (0.5 µg/kg). Milk yield tended to be greater during the dosing period in cows fed CL+SCFP instead of T (39.7 vs. 37.7 kg/d, P = 0.08). Plasma glutamic oxaloacetic transaminase concentration, indicative of aflatoxicosis and liver damage, was numerically increased by T (87.9 vs. 95.2 U/L, P = 0.11) but reduced by CL compared with T (85.9 vs. 95.2 U/L, P = 0.03), adding CL+SCFP resulted in similar level as Control (87.9 vs. 88.7 U/L, P = 0.99). Adding CL or CL+SCFP to the diet reduced AFB1 transfer to milk and AFM1 excretion but only CL+SCFP prevented the decrease in milk yield caused by ingestion of the toxin.

Key Words: aflatoxin, milk, clay

109 Effect of sequestering agents based on a Saccharomyces cerevisiae fermentation product and clay on the performance of lactating dairy cows challenged with dietary aflatoxin B1. Y. Jiang1, D. H. Kim1, I. M. Ogunade1, X. Li2, A. A. Pech-Chevantes1, A. S. Oliveira2, K. G. Arriola1, J. P. Driver1, C. R. Staples1, D. Vyas1, and A. T. Adesogan1, 1Department of Animal Sciences, University of Florida, Gainesville, FL, 2Department of Animal Sciences, China Agricultural University, Beijing, China, 3Institute of Agriculture and Environmental Sciences, Federal University of Mato Grosso, Sinop, MT, Brazil.

The objective was to examine the effect of supplementing bentonite clay with or without a Saccharomyces cerevisiae fermentation product (SCFP; 19 g Diamond V NutriTek + 16 g MetaShield) on the performance and liver function enzymes of dairy cows challenged with aflatoxin B1 (AFB1). Twenty-four Holstein cows (64 ± 11 DIM) were stratified by parity and milk production and randomly assigned to 1 of 4 treatment sequences. The experiment had a balanced 4 × 4 Latin square design with 6 replicate squares, 433-d periods and a 5-d washout interval between periods. Cows were fed a TMR containing 36.1% corn silage, 8.3% alfalfa hay and 55.6% concentrate (DM basis). Treatments were: 1) Control (no additives); 2) Toxin (1,725 µg/head/d of AFB1; T); 3) T + Clay (200 g/hd/d; top-dressed; CL); and 4) CL + SCFP (25 g/hd/d; top-dressed; CL+SCFP). Cows were adapted to diets from d 1 to 25 (pre-dosing period) and AFB1 was orally dosed from d 26 to 30 (dosing period) and withdrawn from d 31 to 33 (withdrawal period). Data were analyzed with the GLIMMIX procedure of SAS. Plasma excretion but only CL+SCFP prevented the decrease in milk yield caused by ingestion of the toxin.

Key Words: aflatoxin, milk, clay

110 Effects of phytonutrients or ionophore on productivity, blood cells, and fat mobilization in lactating dairy cows. J. Oh*1, M. Harper1, E. Wall2, and A. Hristov1, 1The Pennsylvania State University, University Park, PA, 2Pancosma, Geneva, Switzerland.

Phytonutrients exhibit both ruminal and post-ruminal effects in ruminants and there may be additive effects on performance when phytonutrients are combined with other rumen modifiers such as ionophores. The objective of this experiment was to investigate the effects of phytonutrients alone or in combination with monensin on productivity, blood cells, and fat mobilization in lactating dairy cows. Thirty-six Holstein cows (average days in milk, 120 ± 23.1 d; average body weight, 676 ± 75.8 kg) were used in a 9-wk randomized complete block design study. Cows were blocked in blocks of 3 based on days in milk, milk yield, and parity following a 2-wk covariate period. Cows within a block were assigned to 4 treatment sequences. The experiment had a balanced 4 × 4 Latin square design with 6 replicate squares, 433-d periods and a 5-d washout interval between periods. Cows were fed a TMR containing 36.1% corn silage, 8.3% alfalfa hay and 55.6% concentrate (DM basis). Treatments were: 1) Control (no additives); 2) Toxin (1,725 µg/head/d of AFB1; T); 3) T + Clay (200 g/hd/d; top-dressed; CL); and 4) CL + SCFP (25 g/hd/d; top-dressed; CL+SCFP). Cows were adapted to diets from d 1 to 25 (pre-dosing period) and AFB1 was orally dosed from d 26 to 30 (dosing period) and withdrawn from d 31 to 33 (withdrawal period). Data were analyzed with the GLIMMIX procedure of SAS. Transfer of ingested AFB1 into milk aflatoxin M1 (AFM1) was greater in T than CL or CL+SCFP (1.65 vs. 1.01 and 0.94%, respectively, P < 0.0001) on d 26 to 30. The CL and CL+SCFP treatments reduced milk AFM1 concentration compared with T (0.45 and 0.40 vs. 0.75 µg/kg, respectively; P < 0.001) and unlike T, they resulted in lower AFM1 concentrations than the FDA action level (0.5 µg/kg). Milk yield tended to be greater during the dosing period in cows fed CL+SCFP instead of T (39.7 vs. 37.7 kg/d, P = 0.08). Plasma glutamic oxaloacetic transaminase concentration, indicative of aflatoxicosis and liver damage, was numerically increased by T (87.9 vs. 95.2 U/L, P = 0.11) but reduced by CL compared with T (85.9 vs. 95.2 U/L, P = 0.03), adding CL+SCFP resulted in similar level as Control (87.9 vs. 88.7 U/L, P = 0.99). Adding CL or CL+SCFP to the diet reduced AFB1 transfer to milk and AFM1 excretion but only CL+SCFP prevented the decrease in milk yield caused by ingestion of the toxin.
cinnamaldehyde, eugenol, and capsicum (XT), and 250 mg/cow/d of a product containing capsicum oleoresin in addition to MO (MOCAP). Cows were housed in a free-stall barn equipped with Calan Feeding System for monitoring individual feed intake and fed ad libitum once daily. Treatments were top-dressed at the time of feeding. Dry matter intake and milk yield were not affected \((P \geq 0.18)\) by treatments (average 31.0 and 47.4 kg/d; SEM = 0.52 and 0.81, respectively). Compared with MO, XT increased \((P = 0.04)\) feed efficiency (1.48 and 1.58 kg/kg, respectively). Concentrations of milk fat, true protein, and lactose were similar \((P \geq 0.46)\) among treatments. The expression of hormone-sensitive lipase in adipose tissues tended to increase \((P = 0.09)\) for MOCAP compared with MO. However, blood nonesterified fatty acids were not affected \((P = 0.26)\) by MOCAP. Treatments had no effect \((P \geq 0.15)\) on blood urea nitrogen, red blood cells, and white blood cells except a slight decrease \((P = 0.10)\) in monocyte counts for XT. Relative to monensin, phytomutrients had no or subtle effect on feed intake, milk yield and composition, blood cells, and fat mobilization in dairy cows. However, a mixture of cinnamaldehyde, eugenol, and capsicum increased feed efficiency compared with monensin.

**Key Words:** milk fatty acids, immune function, tea saponin

### 111 Effect of tea saponins on milk performance, milk fatty acids, and immune function in dairy cows. B. Wang*1,2, Y. Tu3, J. X. Liu1, B. H. Xiong4, and L. S. Jiang2, 1Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China, 2Beijing Key Laboratory for Dairy Cow Nutrition, College of Animal Science and Technology, Beijing University of Agriculture, Beijing, China, 3Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China, 4State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

This study investigated the effects of tea saponins (TSP) on milk performance, milk fatty acids, and blood immune function in dairy cows. A total of 20 early-lactation Holstein cows (day in milk = 66.4 ± 16.8 d; parity = 1.75 ± 0.91; and milk yield = 36.3 ± 7.32 kg/d; mean ± SD) were randomly divided into 4 homogeneous groups, with TSP added at the levels of 0 (control), 20, 30, and 40 g/d per head, respectively. All cows had 2 weeks of adaptation and 6 weeks of treatments. Feed, milk, and blood were sampled and analyzed weekly. The data were analyzed using PROC MIXED program of SAS with a randomized complete block design with repeated measures. The yield of milk, milk protein, lactose, and total solids were lower in the cows fed 40 g/d TSP compared with other groups \((P < 0.01)\). Energy-corrected milk of cows fed 30 g/d TSP was greater than the control \((P < 0.01)\). The milk fat content of cows fed 40 g/d TSP was greater than the control and the 20 g/d TSP group. The proportion of milk unsaturated fatty acid such as cis-9 C16:1, cis-9 C18:1, and C20:4n-6 in the 40 g/d TSP group was greater than in the control \((P < 0.05)\). Plasma malondialdehyde concentration decreased as the supplemental value of TSP increased (quadratic \(P < 0.01\)), whereas, the concentration of superoxide dismutase increased as the supplemental value of TSP increased (linear \(P < 0.01\)). The plasma concentration of tumor necrosis factor-\(\alpha\) and interferon-\(\gamma\) increased as the supplemental value of TSP increased (linear \(P < 0.01\)). This study showed that the supplementation of 40 g/d TSP to dairy cows could decrease the dietary dry matter intake, resulting in a lower milk yield. The supplementation of TSP could reduce oxidative stress in the cows and might improve their immunoreaction. The increased milk fat content and milk unsaturated fatty acids proportion were achieved in cows fed 40 g/d TSP, which may indicate the TSP played a part in lipometabolism of dairy cows.

**Key Words:** milk fatty acids, immune function, tea saponin

### 112 Improving the long-chain fatty acid profile of milk and cheese in dairy cows by supplementation with microalgae. B. E. Tilly*1, J. A. Huntington1, J. Taylor-Pickard2, and L. A. Sinclair1, 1Harper Adams University, Shropshire, UK, 2Alltech Biotechnology Institute, Dunboyne, Ireland.

The benefits of long-chain fatty acids (FA) on human health have long been recognized, in particular the long-chain n-3 polyunsaturated FA (LC n-3 PUFA) such as docosahexaenoic acid (DHA). Microalgae (ALG) is the primary producer of LC n-3 PUFA in the food chain, and supplementing the diet of dairy cows with ALG has been shown to increase the content of DHA in milk, although little work has been conducted on the effect of rate of inclusion on milk or cheese composition. The objective of the current study was to determine the effect of rate of inclusion of ALG on milk and cheese FA profile. Twenty cows yielding approximately 40 kg/d at the beginning of the study were randomly allocated to 1 of 4 diets, in a 4 x 4 Latin square design, with 4 periods each of 28 d duration. The basal ration contained corn and grass silages and concentrate feeds, and was supplemented with one of 4 levels of ALG: 0 (Control; C); 50 (Low; LA); 100 (Medium; MA) or 150 (High; HA) g/cow per day. Milk samples were taken on 2 consecutive milkings during the final week of each period, the milk fat extracted and methylated before FA analysis by gas chromatography. Milk was also collected for making into a cheddar cheese. Data were analyzed using Genstat (v17). Supplementation with ALG had no effect on milk yield \((P > 0.005)\) which averaged 38.5 kg/d, but milk fat content decreased linearly with increasing inclusion rate \((P = 0.01)\), with mean values of 39.6, 38.4, 37.1 and 35.9 g/kg for C, LA, MA and HA respectively. Supplementation with ALG reduced the saturated FA content of milk from 68.7 to 66.7 g/100g in HA, and in the cheese from 67.9 to 66.2 g/100g. The PUFAs and cis-9,trans-11 conjugated linoleic acid content of milk and cheese increased linearly \((P < 0.001)\) with the addition of ALG in the diet. Milk fat content of DHA increased linearly from 0.08 in C to 0.37 g/100g in HA, and cheese DHA content increased from 0.06 in C to 0.35 g/100g in HA. There was no effect \((P > 0.05)\) of treatment on cheese yield which averaged 0.26 kg/kg. In conclusion ALG can be added to the diet of dairy cows to improve the milk and cheese content of DHA without affecting cheese yield.

**Key Words:** DHA, microalgae, fatty acids

### 113 The effect of calcareous marine algae, with or without marine magnesium oxide, and sodium bicarbonate on milk production in mid-lactation dairy cows. E. W. Neville*1, A. G. Fahey2, B. P. Molloy3, S. J. Taylor1, and F. J. Mulligan1, 1School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland, 2School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland, 3Celtic Sea Minerals, Carrigaline, Cork, Ireland.

The production of high yielding dairy cows is often impacted by low rumen pH. The objective of this experiment was to evaluate different dietary buffers and their influence on dairy cow performance. The supplements included were: calcareous marine algae (Lithothamnion calcareum), with or without marine magnesium oxide (precipitated magnesia derived from seawater), and sodium bicarbonate. Fifty-two multiparous and 4 primiparous cows (62.7 ± 3.4 d in milk) were assigned...
to 4 experimental treatments based on parity, calving body condition score (3.1 ± 0.03), pre-experimental milk yield (34.7 ± 0.79) kg/d and previous 305 d milk yield (7073 ± 198). The diets were based on a forage: concentrate ratio of 46:54. Dietary treatments consisted of the control (283 g starch and sugar, and 230 g neutral detergent fiber (NDF) from forage per kg dry matter (DM) including no dietary buffer (CON); the control plus 3.5 g/kg DM calcareous marine algae (CMA); the control plus 3.5 g/kg DM calcareous marine algae and 0.9 g/kg DM marine magnesium oxide (CMA+MM); the control plus 7 g/kg DM sodium bicarbonate (SB). The experiment lasted for 80 d, including 7 d acclimatisation and 73 d of data collection. The CMA treatment increased the production of milk solids (fat and protein kg/d) compared with CON (+ 0.16 kg, P < 0.01), CMA+MM (+ 0.09 kg, P < 0.01) and SB (+ 0.10 kg, P < 0.01). Both CMA and CMA+MM increased milk fat yield (P < 0.05) compared with CON but were not different to each other or SB. All 3 buffer treatments increased milk fat concentration (P < 0.01) compared with the control but did not differ from each other. The SB treatment reduced milk protein concentration (P < 0.01) compared with all other treatments. The SB treatment reduced the efficiency of milk production, energy-corrected milk (ECM) per kg of DMI, (P < 0.01) compared with all other treatments. Results indicate that the addition of rumen buffering products can benefit milk solids production when included in lactating dairy cow diets. The use of calcareous marine algae when compared with sodium bicarbonate, in such diets, can increase milk production efficiency.

Key Words: calcareous marine algae, lactating dairy cow, rumen buffer


This study evaluated if hydrolysis by exogenous fibrolytic enzymes (EFE) can be synergistically improved by recombinant bacterial expansin (BsEXLX1) and examined if the expansin alone can expand plant cell walls. In Experiment 1 (E-1), the objective was to examine if EFE (2.3 mg/g) and BsEXLX1 (162 µg/g) synergistically hydrolyze filter paper (FP; 6 mg) and xylan (20 mg). The substrates were incubated in quadruplicate in 3 runs for 12h at pH 6 and 39°C and reducing sugars were assayed using Control, EFE, BsEXLX1 and EFE + BsEXLX1. In Experiment 2 (E-2), the objective was to verify disruptive activity of BsEXLX1 (162 µg/g) and control (Buffer only) on cotton fibers (20 mg) incubated in triplicate for 1 h at pH 4 and 50°C. Cell wall extension was measured and immunofluorescence was used to localize BsEXLX1. Experiment 3 (E-3) examined the existence of synergistic effects of EFE (2.3 mg/g) and BsEXLX1 (162 µg/g) on in vitro ruminal degradability and preingestive hydrolysis of bermudagrass silage (0.5 g). Dried ground (1 mm) silage samples were incubated with nothing, BsEXLX1, EFE or EFE + BsEXLX1 in quadruplicate for 24 h in each of 3 runs. Data were analyzed using the NMLE package of R and a model that included fixed effects of EFE, BsEXLX1, run and interactions (E1) or random effect of cow nested within run (E3). In E-1, the amount of sugars released from FP was 12% greater (P < 0.01) with EFE+BsEXLX1 compared with EFE (25.9 vs 23.2 mg/g FP). However, adding BsEXLX1 did not improve (P=0.7) xylan hydrolysis by EFE (130.6 vs 129.2 mg/g xylan). In E-2, on average, BsEXLX1 expanded the diameter of cotton fiber cell walls by 30% (P < 0.01) compared with the Control and immunofluorescence confirmed the expansion was due to BsEXLX1. In E-3, EFE improved DM degradability in buffered rumen fluid by 4% (47.5 vs 45.7%, P < 0.01) and preingestive hydrolysis by 11% (20.6 vs 18.5%, P < 0.01); however synergism with BsEXLX1 was only detected for the latter (21.2 vs 20.1%, P = 0.02). Therefore, BsEXLX1 can expand plant cell walls and synergistically improve the hydrolysis of EFE in a substrate-dependent manner.

Key Words: enzymes, expansins, immunofluorescence

116 Effect of a recombinant bacterial expansin (BsEXLX1) and fibrolytic enzymes on in vitro digestibility and preingestive hydrolysis of bermudagrass silage. A. A. Pech-Cervantes*1, I. M. Ogunade1, D. H. Kim1, F. X. Amaro1, Y. Jiang1, K. G. Arriola1, C. F. Gonzalez2, D. Vyas1, and A. T. Adesogan1, 1Department of Animal Sciences, University of Florida, Gainesville, FL, 2Department of Microbiology and Cell Science, University of Florida, Gainesville, FL.

The objective was to evaluate if synergistic effects exist between an exogenous fibrolytic enzyme mixture (EFE) and a recombinant bacterial expansin-like protein (BsEXLX1) on ruminal in vitro neutral detergent fiber digestibility (NDFD) and preingestive hydrolysis of bermudagrass silage. In Experiment 1 (E-1), the effects of 2 levels of EFE (0, 2.33 mg/g) and 4 levels of BsEXLX1 (0, 136, 272, 408 µg/g) were tested on bermudagrass silage in a 4 × 2 factorial arrangement of treatments. Samples were preincubated with EFE and BsEXLX1 using sodium phosphate buffer (pH 6) for 1 h at room temperature in triplicate and further incubated in rumen fluid media for 24 h at 39°C. The experiment was...
repeated 3 times. In Experiment 2 (E-2), synergistic effects of the same levels of EFE and BsEXLX1 on preingestive hydrolysis were examined in quadruplicate by incubating ground bermudagrass silage samples (0.5 g) for 24 h at 25°C and measuring DM loss and NDF hydrolysis. Data were analyzed using the NMLE package of R and the model for E-1 included the fixed effects of EFE, BsEXLX1, run, interactions and the random effect of cow nested within run. The model for E-2 included the fixed effects EFE, BsEXLX1, run, and interactions. In E-1, DMD and NDFD were increased by 7% (46.9 vs 50.3%, $P < 0.01$) and 3% (35.4 vs 36.4%, $P < 0.01$) by EFE, respectively. However, no interactions or synergistic effects on DMD (49.6, 50.4, 50.6, and 50.4%, $P = 0.8$) and NDFD (36.0, 36.8, 36.6 and 36.0%, $P = 0.2$) were observed when BsEXLX1 was added with EFE. Total VFA was increased by 9% with EFE compared with control (83.7 vs 91.1 mM, $P < 0.01$) and reduced by 5% (87.4 vs 92.3, 86.7 and 83.3 mM, $P < 0.01$) by the highest dose of BsEXLX. In E-2, compared with the Control, EFE increased DM loss and NDF loss by 25% (21.4 vs 26.9%, $P < 0.01$) and 15% (20.9 vs 24.1%, $P < 0.01$), respectively but the expansin had no effect. In conclusion, the EFE increased DM and NDF digestibility of bermudagrass silage but the BsEXLX1 did not. The BsEXLX1 dose did not affect DM loss and NDF hydrolysis. The efficacy of the EFE was not improved when various doses of BsEXLX1 were added.

**Key Words:** enzymes, expansin, bermudagrass
Current market trends of sheep and goat milk, farm structures and production costs. G. Pulina1, M. J. Milán Sendra2, M. P. Lavin3, A. Theodoridis4, E. Morin5, and J. Capote6, 1University of Sassari, Sassari, Italy, 2University Autonoma of Barcelona, Barcelona, Spain, 3Consejo Superior de Investigaciones Científicas, León, Spain, 4Aristotle University of Thessaloniki, Thessaloniki, Greece, 5Institut de l’Élevage, Paris, France, 6Canary Islands Institute of Agricultural Research, Canary Islands, Spain.

Produced sheep (10.2 Mt) and goat milk (17.8 Mt) are only 1.3 and 2.3%, respectively, of world total milk. Sheep dairy breeds and farms are well specialized and it is expected an increase of 2.5 Mt milk for 2025. The leader in sheep cheese export is IT, followed by FR, BG, GR and ES, whereas the largest importers are the US and DE. Protected Designation of Origin cheeses play a major role in the international trade (Pecorino Romano, Roquefort, Feta and Manchego) and have a growing market. In contrast, only few goat breeds are dairy specialized and most goats are double purpose. Asia and Africa contribute to about 95% the heads and approximately 82% the milk, whereas Europe accounts for 14% goat milk. World goat’s milk increased by 30% in the last 20 yr, and it is expected to increase by 4.1 Mt for 2025. In Italy, dairy sheep are raised in semi-extensive systems, where grazing natural pasture represents the main feeding source, whereas goats are intensive or extensive. Their production costs represent 80 to 120% of the gross income. In France, dairy sheep farms are mainly located in mountainous, in semi-extensive systems, based on local breeds and natural or cultivated grasslands. Milk is processed into PDO cheeses and productions costs represent 80 to 120% of the gross income. In Spain, traditional semi-extensive farms (based on local breeds) and modern farms (based on foreign or improved local breeds), coexist. A large variability in profitability has been reported, but the rise of milk prices during the last decade (19% for sheep and 37% for goat), supported the Spanish sheep and goat industry during the economic crisis. Greek dairy sheep sector is characterized by the greatest diversity of systems, goat being more extensive than sheep. Production costs are lower than in other countries, but milk share (46% incomes) indicates a low specialization. Increase of production costs, decrease of milk prices and financial stress, severely affected the sector and to intensive farms. On the contrary, the extensive farms are less vulnerable to economic conditions due to low capital endowments and family labor.

Key Words: dairy sheep, dairy goat, production system

Update on lactation biology and milking strategies of small ruminants. M. Rovai1, G. Caja2, A. Argüello3, C. Peris4, X. Such5, and P.-G. Marnet6, 1South Dakota State University, Brookings, SD, 2University Autonoma of Barcelona, Bellaterra, Barcelona, Spain, 3University of Las Palmas de Gran Canaria, Gran Canaria, Spain, 4Polytechnic University of Valencia, Valencia, Spain, 5Agrocampus-Ouest, Rennes, France.

Sheep and goats markedly differ in mammary gland structure and milk secretion processes, which modifies the characteristics of their milk and dairy products. Whereas ewe’s milk is mainly secreted in the MEC by a merocrine process, the goat’s is apocrine with high shedding of cytoplasmic particles and SCC in milk. Presence and dynamics of stem and progenitor cells in the mammary glands of ewes and does, also help explain their differences in plasticity during lactation. Prolactin is essential for milk secretion and modifies milk yield by regulating MEC functioning, proliferation and exfoliation, all being suppressed by dopamine agonists. Serotonin plays a paracrine-autocrine role inhibiting lactation in ewes and does, but with differences in receptors by species. Udder cistern compartment is larger in goat (70–80%) than in sheep (40–60%) depending on breeds, which conditions milking machine settings, routines and schedules. Milk ejection and component yields are influenced by the presence of offspring and milking-suckling strategy used. Milking from parturition increases the amount of milk sold, especially in sheep, although results vary according to breed and conditions (e.g., udder morphology). Consequently, a short sucking period (2 to 4 wk) and sale of milk-fed lambs are widely used. Today, machine milking is widespread, resulting in better milk hygiene and higher quality of dairy products. Intensification increased the size of the flocks/herds and milking time is a major constraint for farmers. Simplification of
milking routines and extension of intervals are usual ways to solve the problem. Both ewes and does are able of being milked once-a-day for the entire lactation, with cost-efficient milk losses (5 to 15%), no effects on udder health, small variations of milk composition and inconsistent effects on dairy products. Automation of milking routines (e.g., cluster removal) and automated milk recording (i.e., electronic identification and milk flowmeters) are now generalized trends, with benefits in milking throughput and genetic improvement. As a result, a greater dairy specialization and easy milkability are expected at the mid-term in dairy small ruminants.

Key Words: small ruminant, dairy sheep, dairy goat


Industries using specialized dairy breeds of sheep and goats for commercial milk production are well established in Europe and the Middle East but much more recent in North America. Dairy goats were first imported into North America in the early 1900s, but the commercial industry is only about 40 years old. The dairy sheep industry in North America is younger with the first importation of dairy sheep breeds in the early 1990s. Dairy goat breeds of the greatest importance in North America are the breeds of Alpine, LaMancha, Nubian, Saanen, and Toggenburg. Production of genetic evaluations for dairy goats in the US is a cooperative effort by the American Dairy Goat Association, regional dairy records processing centers, and the Council on Dairy Cattle Breeding. There were 589 herds with 17,381 does in this milk-recording program in 2016. Canadian dairy goat genetic evaluations are produced by goatgenetics.ca through cooperation of the Canadian Goat Society, Canadian Livestock Records Corporation (CLRC), Canadian DHI, and Holstein Canada. The primary dairy sheep breeds for commercial production in North America are East Friesian and Lacaune with smaller numbers of British Milk Sheep and Awassi. In Canada, genetic evaluations for dairy sheep have started through GenOvis in cooperation with CLRC. There are no organizations in the US for maintaining pedigree records or calculation of genetic evaluations for dairy sheep. Several successful programs for dairy small ruminants are in operation in Europe for genetic improvement of both production and functional traits (e.g., sheep: French Lacaune and Manech, Spanish Manchega and Latxa, and Italian Sarda; and goats: Spanish Murciano-Granadina, Malaguèña, Florida, and Payoya and French Alpine and Saanen). Some of these programs use genomic data as well as traditional pedigree and performance information. These European programs can serve as a source of imported genetics to improve the production efficiency of North American dairy small ruminants and also as examples of the types of improvement programs that could be implemented in North America for continual improvement of local populations of dairy small ruminants.

Key Words: dairy sheep, dairy goat, breed

121 Intake prediction and energy requirements for lactating dairy small ruminants: Comparison of models. A. Cannas*1, F. Bocquier2,3, P. Hassoun1, S. Giger-Reverdin4, D. Sauvant4, L. O. Tedeschi5, and G. Caja6, 1University of Sassari, Sassari, Italy, 2INRA-Montpellier SupAgro, Montpellier, France, 3INRA, Montpellier, France, 4INRA-AgroParisTech-Université Paris-Saclay, Paris, France, 5Texas A&M University, College Station, TX, 6University Autonoma of Barcelona, Bellaterra, Barcelona, Spain.

Feeding systems use similar feed evaluation approaches for all dairy ruminants, whereas feed intake prediction and requirement assessment are species-specific. This review compares the most recent models currently used to predict dry matter intake (DMI) and energy requirements in lactating dairy small ruminants (DSR), i.e., the Small Ruminant Nutrition System (SRNS, 2004 and 2010, updated in the Ruminant Nutrition System, 2016), the NRC(2007), and the new INRA-Systal (2017) systems, to highlight differences in approach, inputs required and flexibility used. Because DSR are normally group fed, accurate prediction of feed intake is necessary to adjust diet composition according to their requirements. However, when predicting DMI for ewes and goats, the approaches used, variables considered and predicted values vary considerably. All the models used are empirical and species-specific, and use body weight and milk yield as main predictors. Variables associated with diet composition and/or type of feeds are also considered, but vary among models, while dietary particle size is not considered. The SRNS and NRC do not have specific models to predict intake on pasture, while INRA uses the Fill Unit system (i.e., based on a forage of reference) to predict DMI considering both housing and grazing conditions. For energy requirements, the SRNS uses the same model for both species, while NRC and INRA use species-specific models. The INRA (2017) largely updated these predictions for all productive functions. For maintenance energy requirements, besides some variability in the basal metabolic rate and energy units used (i.e., ME or NEL), most differences regard the additional factors affecting these requirements (e.g., age, gender, body condition, growth, previous nutrition, movement, cold and heat stress, dietary nitrogen unbalance). Differently, energy requirements for milk production and pregnancy are estimated using similar approaches and values. Overall, the feeding systems for dairy sheep and goats have markedly evolved in the last decade, by improving their ability to account for the great diversity of production systems in which they are raised worldwide.

Key Words: intake, energy requirement, lactating small ruminant

122 ADSA*-EAAP Speaker Exchange Presentation: Animal–environment interactions in dairy small ruminants: Cause-and-effect relationships and strategies of alleviation. A. A. K. Salama*1, D. R. Yañez-Ruiz2, C. Fernandez2, N. Koluman4, M. Ramon2, N. Silanikove6, A. Goetsch7, and G. Caja1, 1Group of Ruminant Research (GRR), Universitat Autonoma de Barcelona, Bellaterra, Spain, 2Estación Experimental del Zaidín (CSIC), Armilla, Granada, Spain, 3Research Centre ACUMA, Animal Science Department, Polytechnic University of Valencia, Valencia, Spain, 4Cukurova University, Agricultural Fac., Department of Animal Science, Adana, Turkey, 5Centro Regional de Selección y Reproducción Animal (CERESTRA-IRIAF), Valdepeñas, Spain, 6Institute of Animal Science, Agricultural Research Organization, Volcancit Center; Bet Dagan, Israel, 7American Institute for Goat Research, Langston University, Langston, OK.

Carbon footprinting can be useful to assess how activities such as different types of livestock production impact climate change. Sheep and goats represent approximately 55% of ruminants in the world, although they produce just 12 to 14% of greenhouse gases emitted, with CH4 from enteric fermentation contributing over 60% of total emissions. Concentrate feedstuffs often used to meet nutrient requirements decrease CH4 emission but can increase diet cost. Alternatively, the partial replacement of grains with agricultural byproducts in combination with sources of...
lipids or essential oils is a cost-effective mitigation strategy to reduce ruminal methanogenesis. Furthermore, maintaining good animal health and longevity will reduce CH$_4$ emission per kg of milk produced. As a result of climate change, dairy small ruminants are expected to be more subject to periods of thermal stress, both heat and cold conditions. Heat stress (HS) frequently decreases feed intake, impairs fertility, causes discomfort, and alters behavior. Concomitantly, milk yield is depressed and cheese-making properties deteriorate. The HS decreases sensitivity of adipose tissue to lipolytic signals and less insulin is released by the pancreas when glucose is administered. Transcriptomics and metabolomics of blood and milk indicate alteration of immune cell function and inhibition of mammary cell synthetic capacity. Exposing the goat fetus in utero to HS not only modifies postnatal response to HS but also behavior reactions to other stresses such as isolation in an unfamiliar environment. Moreover, cold stress decreases milk yield by dairy ewes and goats as a result of adverse effects on many different physiological conditions. Sensor systems allow collection of information relating to rumen characteristics and behavior in thermal-stressed sheep and goats that will be valuable in production system of the future. Conventional and modern strategies of alleviation include ventilation, sprinkling, dietary supplementation with vegetable oils, coat shearing, and genomic selection to increase thermo-tolerance.

**Key Words:** carbon footprinting, thermal stress, omics
123 Mentoring undergraduate students in dairy science. L. Berning*, California Polytechnic State University, San Luis Obispo, CA.

Shifting demographics in American higher education has created opportunities and challenges relative to mentoring undergraduate students. The changing background of the greater student population is reflected in dairy science as well. Increasing diversity in the student population has created an environment where cultural factors impact the professor-student relationship. In addition, a shift of population away from the rural lifestyle means that fewer students are coming into the university with dairy/agricultural experience. The current makeup of the student population and their prior knowledge base has created the need for different approaches to student mentoring. In the current environment, what is the essence of an effective mentoring relationship? To gain insight into this issue from the student perspective, dairy science and various agriculture majors were asked to think about people they considered their most significant higher education mentors. They were then asked to summarize what qualities they valued most highly in a mentoring relationship. This presentation will highlight the traits that characterize an effective mentor and the ingredients of a positive mentoring environment for undergraduate students.

Key Words: advising, mentoring


Dairy and Animal Sciences together are popular majors in the agricultural sciences in the US. Currently, minorities make up a small but growing student population in higher education. Today’s student population represents our future leaders in this field. With the complex challenge of providing food security for 10 billion people by the year 2020 on current or less natural resources in the future, it is imperative that an educated and diverse workforce is prepared. Our land grant institutions have recognized the need to resolve this challenge. To ensure interested students from all populations matriculate through our dairy and animal sciences’ programs, mentoring is believed to be one area to support student success, especially among minorities. One such organization was undertaken at North Carolina A&T State University. An 1890 land grant institution in Greensboro that also is the largest HBCU in the country and has the largest enrollment among the 1890s in the College of Agriculture. The organization was called “Men-on-the-Move (MOTM).” Focusing on mentorship, leadership and engagement by minorities, especially African American males, an effort was taken to see what impact MOTM would have on retention (RR) and graduation rate (GR). Started by open enrollment in the Department of Animal Sciences with 15 students, the organization has grown to over 35 members in 10 department and 5 college/school campus wide. Leadership training has impacted elected executive officers among 5 organizations which has grown from 1 to 5 AA males. Peak mean values tended to grow, but not significant (P > 0.05) in RR (86% vs. 80%) and GR (35% vs. 45%) between AA males in MOTM when compared with those in the Department of Animal Sciences. Mentorship, leadership and peer support played a vital role in successful engagement and achievement of the AA males at North Carolina A&T State University. The organization has been recognized for its achievement in the region, and student success continues to improve.

Key Words: minorities, mentorship, dairy/animal sciences

125 Mentoring graduate students as a young faculty: Challenges and opportunities. A. Faciola*, University of Nevada, Reno, NV.

The objective of this presentation is to highlight challenges and opportunities associated with effective mentoring as a young faculty. Effective mentoring, at any stage of one’s career, is challenging. However, effective mentoring while on the tenure-track is crucial for the success of the mentor and the mentee. The current academic environment is fast-paced, highly competitive, and expectations are higher than ever before. Young faculty are expected to excel in teaching, research, and service. High quality teaching and service are very important, but time consuming and often undervalued by administration. High quality research, evidenced by publications and extramural funding, is generally the most important criterion for tenure and promotion. This environment leaves little room for mentoring, and young faculty may feel the need to choose between mentoring or writing papers and grant proposals. Effective mentoring is widely recognized as important for student success; however, I would argue that it is also important for the success of young faculty members: whether one receives tenure is highly dependent on the productivity and success (or lack thereof) of his/her graduate students. Considering this, I have developed a mentor-mentee agreement that aims to (1) provide students with clear information on what resources and support the lab can offer them, (2) explain what the lab will expect from them, and (3) give students an opportunity to share their previous experiences and future goals, so that a tailored mentoring plan can be made. The goal of this agreement is to achieve the best lab environment possible – one that fosters high productivity and student satisfaction by providing a healthy work environment where everyone feels valued and committed to the success of the group. The current academic environment may seem counteractive to effective mentoring; however, allowing time to clearly lay out expectations, tailoring development plans for each student, and following up on a regular basis can increase the likelihood of student success, which will contribute to young faculty success.

Key Words: individual development plan, student success, tenure

126 Mentoring and empowering women in dairy science. K. M. Schoenberg*, Elanco Animal Health, Greenfield, IN.

Women in dairy science face similar challenges as women in fields such as engineering and technology where women are under-represented. While 80% of veterinary students in the US (77% in the UK) are female, they are grossly under-represented as practice owners, association leaders, and managers. In the United States, 54% of all practicing veterinarians are female, and 19% of food animal veterinarians are female. Research shows that these women are judged and assessed differently due to unconscious biases, are provided different opportunities, and face additional pressures outside of their career. While generations before us fought for equal access to these careers, women now face differing, often confounding, challenges which result in them having dissimilar experiences from their male counterparts. As more women continue to
enter dairy science fields in academia and industry, the need for support in the form of effective mentoring will grow. With added awareness of the challenges and mitigation strategies we can all minimize the potential negative impact on careers for women in dairy science. In particular, for young females starting their career in dairy science, there may be a dramatic shift in their awareness of challenges they may face. Mentors can provide tools to navigate these transitions. First, for women at all levels, mentorship and coaching in the areas of confidence, a thriving inner mentor, and tips to navigate times of change and uncertainty are key. Second, the type of support women in dairy science will require will change as their career progresses. Third, throughout these changes, a thriving network will help women identify personal and professional contacts to help them navigate. Finally, a diverse set of experiences will allow women to find their true areas of passion and where they can thrive. Above all, it is up to all of us to build awareness of the unconscious biases that exist and may impact these talented professionals and do our part to influence people and systems to interrupt the biases and even the playing field. Everyone benefits from the greater diversity of thought that is brought to the table when women in dairy science are empowered to be the best that they can be.

Key Words: mentoring, bias, gender

127  Mentoring postdocs in an increasingly competitive environment. P. Clifford*, University of Illinois at Chicago, Chicago, IL.

The National Institutes of Health and National Science Foundation define a postdoctoral fellow as someone “who is engaged in a temporary and defined period of advanced training to enhance the professional skills and research independence needed to pursue his or her chosen career path.” How do PhD scientists decide on their chosen career path? And what are the professional skills that are needed for success? This session will discuss the value of creating an individual development plan (myIDP.sciencecareers.org) to facilitate career progression.

Key Words: mentoring, career development, professional development

128  Mentoring young faculty to succeed in teaching and research. K. A. Weigel*, Department of Dairy Science, University of Wisconsin, Madison, WI.

New faculty members represent an investment of roughly $1.5 million for salary, benefits, and start-up package during the probationary period, and more importantly they determine the department’s climate, productivity, impact, and reputation for the next 3 decades. The rewards of successfully hiring and mentoring an exceptional candidate are great, whereas the penalties of failure are severe. Young assistant professors are full of energy and enthusiasm, which are powerful indicators of success when coupled with intellectual prowess and strong graduate and postdoctoral training. They are also under tremendous pressure, particularly at a top research university, where achieving promotion and tenure can feel like becoming a partner at a top law firm. Balancing work and home life is a challenge, especially when plans for starting a family have been postponed during the candidate’s graduate and postdoctoral studies. Faculty colleagues play a key role in the success of a new assistant professor, as their informal daily interactions are critical to helping the individual understand departmental, college, and campus policies, procedures, and priorities. An effective mentor committee, which typically includes tenured faculty members from within and outside the department, is also critical. The department chair or head must find the right balance between protecting the young faculty member and fostering his or her independence. Too much responsibility too quickly, in terms of teaching load, research expectations, or service requests, can lead to frustration and burn-out. Conversely, too much protection for too long can lead to isolation of the faculty member, as well as resistance or disenchantment several years later, when he or she is confronted with a heavier teaching load, significant service activities, and the expectation of a self-sufficient research program. We have tended toward the former – full and quick integration of the young faculty member into all aspects of departmental life, while trying to monitor the workload and commitments. Excellent time management and organizational skills are therefore essential, particularly as the individual transitions from a relatively unknown assistant professor to a highly sought-after speaker and collaborator.

Key Words: mentoring, faculty, tenure
The role of flavored milk in school nutrition.  B. Bowman*, D. Winston, and K. Daniels, Virginia Tech, Blacksburg, VA.

American school children face a health crisis as obesity and its accompanying complications rise; nutrition policies are changing to address the situation. Calorie reductions in school lunches are promising; however, recommendations to decrease calories could reduce consumption of nutrient-dense foods, such as milk. Three studies were evaluated: the role of flavored milk in student milk consumption (Yon and Johnson, 2014); student acceptance of reduced calorie flavored milk (Quann and Adams, 2013); and the influence of sugar-sweetened beverages (SSBs) as competition for flavored milk (Lasater et al., 2011). When flavored milk was no longer offered, school milk sales declined 26.0%, and the amount of milk discarded increased by 11.4%. Thus, overall school milk consumption decreased 37.4%. When calories were reduced in flavored milk, participation in the National School Lunch Program (NSLP) declined 2.6% but then stabilized after 4–6 mo, indicating student acceptance. Between 1989 and 2008, calories from sugar-sweetened beverages increased from 130 kcal/day to 209 kcal/day, while calories from milk consumption decreased from 218 to 170 kcal/day, indicating there is competition for fluid milk consumption.

Key Words: flavored milk, school milk consumption

Exploring the market for goat milk products. L. Scott*, Clemson University, Clemson, SC.

The goat milk product market is growing in some parts of the world but others remain at a standoff due to unfamiliarity with goat products. The market potential of goat milk products is high but only as people become more educated and acquire the taste for these products. A systematic literature research was conducted to further investigate this topic. Databases used included Google Scholar, Science Direct, and Pubmed. Keywords included goat milk, benefits, and market. There are many benefits in consuming goat milk, yogurt, or specialty cheeses. Many reports show that these products have a tendency to reduce allergies and asthma to people in all stages of life: sick, elderly, or young. Babies and toddlers have the potential to gain the most benefit from goat milk when compared with cow and human milk. Goat milk soaps have been known to reduce sunburns significantly. Research shows many differences in goat milk composition in terms of vitamins, minerals, amino acids, and fatty acids but this information can only be viewed through trends due to the lack of research and consistency between breeding goat breeds and herds among farms. Another market that has been weakly explored is that of third world countries who stand to gain the most by using goats. Goats are easily kept in areas with little shrubbery and produce more efficiently than other dairy animals. A few milking goats are enough to provide a family enough nutrition to survive and make profit off the dairy products. Goat milk products have many known advantages but the surface has just barely been scratched. Based on this review, there is an opportunity in this market for much more concrete research but unless there is an increase of education and interest in this market then the products may never reach their greatest potential.

Key Words: goat milk, benefits, market

The health benefits of donkey milk. N. P. Uzee* and C. C. Williams, Louisiana State University, Baton Rouge, LA.

Milk is regarded as nature’s perfect food. However, for children with allergies to proteins present in dairy cow’s milk, who make up around 3% of the population, consuming this indispensable drink is impossible. To continue reaping the benefits of milk consumption, substitutions must be found. One such substitution is donkey milk, which is naturally hypoallergenic. When considering various milks’ compositions, those of human and donkey milk are very similar in amount of total protein, casein, and lactose, with stark differences from that of cow’s milk. In addition, the proteins present in donkey milk are most similar to those found in human breast milk, which makes them much easier to digest. Recent studies have indicated that in children with a cow’s milk protein allergy (CMPA), donkey milk has a high rate of tolerability. Donkey milk also has a light, sweet taste that makes it much more acceptable than milk replacers for children with allergies. The health benefits of donkey milk do not stop with cases of allergies; donkey milk has been regarded as a way to preserve youth since the days of Cleopatra, and its uses in cosmetology still exist today. With the unsaturated fats and several vitamins present, donkey milk is applied to the skin for its anti-aging properties. It also contains more anti-inflammatory omega-3 fatty acids than cow’s milk, has also been shown to enhance immunity, and may help prevent atherosclerosis. Donkey milk is a versatile and healthy product that offers many benefits for human consumption.

Key Words: donkey milk, health benefits

Health benefits of whole milk in comparison with other milk fat options. S. E. Mac*, C. M. Truman, and J. M. Bewley, University of Kentucky, Lexington, KY.

Whole milk is not considered to be a healthy option due to the stigma and misconceptions consumers have. Through the media, whole milk has been associated with health issues including diabetes, heart disease, and obesity. Impressions of negative health risks result in consumers purchasing other milk-fat alternatives such as reduced fat (2% fat) milk, low-fat (1% fat) milk, and skim milk. As research progresses, evidence of health benefits with drinking whole milk increase. Milk protein is associated with decreasing systolic blood pressure by 3.7 mmHg (He et al., 2011). Protein content does not vary widely among milk fat options. Calcium and vitamin D have been linked to osteoarthritis relief and reduced risk of colon cancer (Lee et al., 2009 and Lu et al., 2014). Drinking 7 or more glasses of milk is correlated with osteoarthritis relief (Lu et al., 2014). Milk has been found to reduce the risk of colon cancer by 22% (Lee et al., 2009). After milk is fortified, the calcium and vitamin D levels in whole milk, reduced fat milk, low-fat milk, and skim milk are relatively the same. The primary component varying among fluid milk options is milk fat. Results found that dairy products containing higher fat reduced the risk of obesity by 8% (Rautianen et al., 2016). Those who consumed any dairy in their diets had a lower risk of colon cancer than those without dairy in their diets. Whole milk contains the highest fat content, 3.25%, in comparison to the other types of dairy milk resulting in stronger obesity prevention, although any milk consumption provides more colon cancer than no milk consumption. Whole milk contains all of the essential nutrients associated with the health benefits of lower blood pressure, osteoarthritis relief, colon cancer prevention, and prevention of obesity.

Key Words: whole milk, health benefits of whole milk
133  **Whey management options in Greek yogurt production.**
Z. Curtis* and D. Olver, *Pennsylvania State University, University Park, PA.*

Greek yogurt sales have exploded over the past decade. These products are typically higher in protein and are thicker than regular yogurts because they are more heavily strained to remove liquid whey and lactose. However, the byproduct left behind from Greek yogurt production poses a challenge to manufacturers who must dispose of increasing amounts of acid whey. Acid whey, named from the high concentration of lactic acid present in the liquid, is too acidic to be released into waterways without treatment. Although originally regarded as a waste product, recent advances in filtration techniques and biodigester utilization have led to several options for the management of acid whey. Reverse osmosis and ultrafiltration allow Greek yogurt producers to separate whey into acids, sugars, and minerals and then divert the remaining water to treatment plants. Acid whey is a good source of sodium, potassium, calcium, and phosphorus. Lactose in the acid whey can be converted by enzymes into galacto-oligosaccharides, soluble fibers often included in cereals and snack foods to enhance digestive health. Additionally, acid whey is a viable source of fuel to create methane in a biodigester system. The large quantities of acid whey generated in Greek yogurt production make it imperative that treatment options be further developed and harnessed to allow for the sustainability of this industry.

**Key Words:** Greek yogurt, acid whey

134  **The potential impact of a novel canned latte on the North American dairy products market.**
K. Alward* and J. Bohlen, *University of Georgia, Athens, GA.*

More than 80% of the US adult population enjoys coffee in the morning or a variation of it. From iced coffee to frappuccinos to lattes, there’s something for everyone. LaColombe revolutionized the typical café when they started putting their lattes on draft. Serving lattes out of a tap has been a huge hit with customers who are intrigued by the unique process. Pasteurized milk is combined with cold-pressed expresso and emitted from a tap, which creates a consistency less like a coffee drink and more akin to a frothy beer. The company then took their novel creation one step further by being the first company in North America to sell their draft lattes in a can. Unlike competitors who sell similar products, LaColombe invented a way to create the froth that lattes are known for when the can is opened, something that has never been done before. This patented technique infuses nitrous oxide into the bottom of the can via a grommet during the production process before selling. Then, when the customer pops the top on the can, the valve stretches, allowing a pin to enter and add the nitrous oxide. This new process features the only FDA approved valve that has been found suitable to interact with food. This valve keeps the latte from being exposed to air until the moment it is opened, which triggers the nitrous oxide influx, and thus creates the froth. Besides this, the can also has a unique plastic attachment to the top of the can, which protects the lip of the can and the consumer’s mouth from coming into contact with a contaminated surface. Upon entry onto the market, this product has seen great success due to the increasing demand for coffee, the decreasing demand for other caffeinated alternatives, and the fact that lattes are the #1 ordered coffee beverage in cafes. Not only this, but it will allow another product onto the market that utilizes fluid milk, the consumption of which has been steadily declining. This patented new technique will revolutionize the coffee industry and allow the dairy industry yet another unique product onto the market with the hopes of increasing dairy consumption in the North America.

**Key Words:** novel food technology, canned latte, dairy product consumption
Vision on dairy cattle nutrition towards 2030. M. D. Hangan* and R. R. White, Virginia Tech, Blacksburg, VA.

The growing global population, changing climate, and decreasing resource availability create a need to improve efficiency and durability of food production. Within the current US food production system, dairy products play an important role in providing high quality protein from human inedible inputs. The environmental impact of dairy products is lower than many other animal products and several non-animal derived foods; however, greenhouse gas emissions and N and P runoff from dairy operations should be minimized wherever possible. Enhancing energy and N use efficiency of dairy cattle production will reduce these environmental impacts and potentially improve farm economic viability. In current systems, gross energy and N are converted to milk energy and N with approximately 20% and 25% efficiency, respectively. Phosphorus capture in milk is slightly greater at 40%. Gains in energy efficiency occur with increasing production through dilution of maintenance costs; however, there are opportunities to improve energy efficiency through enhanced digestive action or improved metabolic efficiency. These will reduce methane emissions. Experimental evidence suggests ample opportunity to enhance N use efficiency. The rumen can be operated above 100% apparent efficiency by feeding low N diets and forcing greater reliance on blood urea yielding a potential improvement of 5% units in efficiency. Postabsorptive N efficiency is approximately 35% and the upper limit is probably as high as 70%, which suggests potential of improving by an additional 10 to 20% units. The latter requires enhanced knowledge of amino acid metabolism. Gains in the efficiency of P utilization will likely require significant improvements in our basic understanding of the regulation of P metabolism. The digestive tract generates excess quantities of absorbable inorganic P suggesting the current limit is not one of digestibility. There are additional opportunities associated with the use of feeding technologies and facility designs that can tailor diets for individual cows based on current and projected production and their genomic profiles, sense and respond to variation in feed inputs, and alter rations to mitigate environmental stress and variation.

Key Words: precision nutrition, dairy production, environmental impact

Vision on dairy cattle physiology and limits of milk production growth towards 2030. R. M. Bruckmaier*1, J. J. Gross1, and H. Sauerwein*2, 1Veterinary Physiology Vetsuisse Faculty, University of Bern, Bern, Switzerland, 2University of Bonn, Institute for Animal Science, Bonn, Germany.

The increased incidence of health disturbances and the decrease in fertility observed in modern dairy cows selected for high milk yield remains largely unimproved. The one-sided selection for high milk yields increased the gap between the output of nutrients with milk and the input via voluntary feed intake. It also exerts negative effects on immune competence, reproduction, and thus lifetime performance. The endeavor for adapting housing, feeding and management to the demands of such cows has not resulted in a noticeable reversal of the trend, nor has the additional consideration of functional “fitness” traits besides milk yield in breeding. Due to limitations in availability and quality of forage, feeding became highly dependent of imported protein-rich feed and also went into competition for crops suitable for human nutrition. Beside these problems, the use of antibiotics, fate of male calves, as well as the detrimental ecological and economic effects represent relevant issues in the public perception and also reduce the production efficiency of dairying. The present contribution will elucidate open potentials and their sustainability, but also restrictions of alternative production strategies under consideration of existing physiological limitations. Such strategies might include for instance improving the rearing practices for dairy calves, use of dual purpose breeds, shortening the dry-period length, extending lactation by delaying insemination, and individualized feeding. Feed additives may also have potential to alleviate deficiencies, at least in short-term. Breaking the peak of the lactation curve by either omitting the dry period or manipulating the endocrine regulation of early galactopoiesis imply economic losses due to only partial use of the available genetic potential, but reduce the metabolic load, risk of health disorders and culling. Within each individual local production system efficiency must be maximized and intensification in pasture-based milk production systems with suitable genotypes may result in higher methane emission, but allow welfare orientated animal husbandry and efficient use of nitrogen and grassland sources without competition for human food and costly concentrate supplementation.

Key Words: dairy cow, physiology, limits

ADSA®-EAAP Speaker Exchange Presentation: Dutch vision on environmental and system aspects of dairy farming towards 2030. K. de Koning*1, P. Galama2, and A. Kuipers2, 1Wageningen Livestock Research–Dairy Campus, Leeuwarden, the Netherlands, 2Wageningen Livestock Research, Wageningen, the Netherlands.

As a case study, the vision of the “Dutch Sustainable Dairy Chain” toward 2020 is presented: a sector in which we work safely and with pleasure; with fair income and high quality food; we handle our animals and environment respectfully; a sector which is appreciated by society. Objectives: Climate neutral development (20% reduction in GHG versus 1990; 16% sustainable energy; energy-efficiency +2%/year); maintain grazing (81.2% of farms should apply grazing); continuous improvement of animal welfare and health (70% reduction antibiotic use; longevity + 6 mo vs 2011; improve score welfare index, in operation by 2017); maintain biodiversity and environment (100% use of responsible soya; national kg phosphate at level 2002; ammonia emission - 5 kton vs 2011; biodiversity tool in 2017). Goals are annually monitored by Wageningen Economic Institute. For each theme a sector team is active to realize goals. In 2017 new goals will be formulated toward 2030, intended to be in line with the ever increasing societal influence, which may be in the short run more dominant in Europe than in the USA. Current examples of societal participation are: existence of the “Animal party” in the parliament; phosphate plafond; discussions about obligatory grazing and calf with cow rearing in region; dispute about no. of animals/farm; less meat consumption in relation to climate change; negative interpretations of word “industry,” as in “dairy industry.” The authors will outline the development directions for the dairy sector: high tech farming (incl. milk robot of which 1st prototype was at our station) versus low cost; resource efficiency including labor; specialization versus diversification and climate neutral dairy chain. Specific practices to be explained: cow transition period/pregnancy gymnastics; smart grazing techniques; sensor technology in- and outside barn; mobile milking and feeding.
systems (also in combination with cows as nomads in nature areas); innovative housing concepts, such as cow-garden (slatted floors and cubicles under scrutiny). Future images about energy saving, manure handling and soil quality, and the circular economy will be addressed, as well as opportunities of new communication techniques.

**Key Words:** dairy industry, future vision, sustainability


The future of animal care and technology in the dairy industry is tightly connected to larger geopolitical and social trends. As countries develop and incomes rise, consumption of high quality dairy products and by-products also increases. While rapid growth of dairy farming is occurring in several highly populated emerging regions, it is unlikely that these regions will be able to meet increased domestic demand. Thus, export driven growth will likely continue in countries that already have well-developed dairy industries. Growth and globalization will greatly influence management practices on dairy farms. The ability to participate in export markets is based on production of high quality milk and milk by-products that meet rigorous quality standards that sometimes serve as non-tariff trade barriers. Global quality standards will continue to get more rigorous and more uniform and will likely be market driven and enforced primarily by milk purchasers. New technologies for testing (both on-farm and at the processing plant) for an increasing number of potential residues (chemical metabolites, antibiotics, microbes etc.) will be developed and deployed. Management practices on farms will need to change to ensure continued market access. Historically, dairy farm workers have been drawn from rural inhabitants but are increasingly immigrants from less developed regions. Recent geopolitical trends are uncertain but seem to be reducing opportunities for immigrants to freely move to regions that need dairy farm workers. These trends indicate that farm workers will likely be less experienced and less available, thus driving increased adoption of automation in every aspect of animal care and milk harvesting. Increased automation requires more technically skilled managers and also access to highly skilled support people. Social pressure to reduce antimicrobial usage in agriculture will continue to increase. Use of antimicrobials and hormones will be increasingly restricted and testing methods for these compounds will be increasingly sensitive. These changes will drive tremendous changes in husbandry and management practices and require innovation to continue to reduce infectious disease in all age groups of dairy cattle.

**Key Words:** milk quality, husbandry, dairy


An adequate supply of good quality food is essential for human health and well-being and milk and dairy products have been recognized as important human food sources from as early as 4000 BC. The important contributions of dairy products in meeting requirements for energy, high quality protein, and several key minerals and vitamins are well documented, although the nutritional importance of dairy fats is often less well understood. Dietary guidelines around the world recommend daily consumption of dairy products for the overall health of the population. The dairy sector has made continuous advancement over the years and today we have a wide variety of dairy products available to the consumer. There is growing consumer recognition of the link between diet and health and this awareness will continue to impact food choices. New research and re-evaluation of previous research questions long-held dogma on the relationship between milk fat and human health; whether we need to alter milk fatty acid composition to improve human health will be challenged in the future. There will be increased consumer acceptance of full-fat dairy products and a growing recognition among the nutrition community that consumption of dairy products is associated with chronic health benefits including reduced risk of cardio-vascular disease and type-2 diabetes. Emerging evidence will continue to show that several minor components in milk also have health benefits. The nutritional value of non-dairy beverages (milks) and how they compare with dairy products will be challenged. Potential effects of different agricultural production practices on product quality and health outcomes will be of importance. An increased awareness of the concept of nutritional quality of food products in relation to environmental sustainability will further highlight an important role for dairy products in sustainable diets. With the projected growth in world population and the increased demand for animal-derived food products as living standards improve, dairy products will continue to be an important dietary source of essential nutrients and bioactive components. We emphasize that all dairy products are an excellent source of nutrients for health maintenance and disease prevention.

**Key Words:** human health, dairy product, milk fat
ADSA Graduate Student (PhD) Production Oral Competition

140 Effects of camelina cake supplementation at two dietary fat levels on ruminal fermentation and nutrient flow in a dual-flow continuous culture system. V. L.-N. Brandao*, X. Dai, L. G. Silva, E.M. Paula, T. Shenkoro, and A. Faciola, University of Nevada, Reno, NV.

This study aimed to assess whether camelina cake (CC) could partially replace canola meal at 2 dietary fat levels and its effects on ruminal fermentation and nutrient flow in a dual-flow continuous culture system. Diets were randomly assigned to 8 fermenters in a double 4x4 Latin square design with four 10-d experimental periods consisted of 7 d for diet adaptation and 3 d for sample collection. Treatments were as follows: CC5: 7.7% CC inclusion at 5% ether extract (EE); NCC5: no CC at 5% EE; CC8: 17.7% CC at 8% EE; and NCC8: no CC at 8% EE. Diets contained 55% of forage, and fermenters were fed 72 g of DM/d twice daily. During sampling days, effluent samples were collected for analyses of digestibility, VFA, NH₃, N-balance, microbial growth, and amino acids (AA) and fatty acids (FA) flow. Statistical analysis was performed using the MIXED procedure of SAS. Partial data are presented in Table 1. Ruminal pH, total VFA, bacterial-N, RUP and RDP were not affected by treatments. Diet NCC8 had the greatest molar proportion of acetate while CC5 and CC8 had greatest molar proportion of propionate. Ruminal digestibility of OM and CP were not affected, but CC8 diet had the lowest NDF and ADF digestibility. Concentrations of iso-C18:1, C18:2, and C18:3 were affected by treatments, overall CC diets had greater ruminal outflow of iso-C18:1 and C18:3. Inclusion of CC decreased biohydrogenation. The increased outflow of important FA and reduction in biohydrogenation observed on CC5 and CC8 diets may be nutritional advantageous; however, NCC8 diet had deleterious effect on AA flow.

Table 1 (abstract 140)

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Key Words: amino acids, canola meal, fatty acids

141 Temporal changes of milk odd- and branched-chain fatty acids in response to acidoegenic diets fed to dairy cows. E. Baumann*, P.Y. Chouinard, AR Alfonso-Avila, and R. Gervais, Université Laval, Québec, QC, Canada.

The milk profile of odd- and branched-chain fatty acids (OBCFA) is generating interest as a non-invasive method for evaluating rumen function. Synthesis by rumen microorganisms is the primary source of milk OBCFA and different groups of rumen bacteria synthesize distinct categories of OBCFA. Sub-acute ruminal acidosis (SARA) is a highly prevalent and costly disease that engenders modifications in rumen bacterial populations, which could be identified by tracking changes in milk OBCFA and how they relate to rumen bacterial groups. The increase of DCAD may alleviate the effects of acidoegenic diets, whereas the supplementation of soybean oil (SBO) in the diet may exacerbate them. This trial aimed to determine the length of time required for milk OBCFA to respond to the consumption of an acidoegenic diet and to evaluate the effects of DCAD, adjusted using dietary K₂CO₃, and SBO supplementation on the evolution of the milk OBCFA profile. Twenty-eight early lactation Holstein dairy cows (39 ± 9 DIM) were used in a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Cows were fed a high-risk SARA diet of 40% forage (58% corn silage) and 60% concentrate, with 0 or 1.5% K₂CO₃ (DM basis), and 0 or 2% SBO. Collection period lasted 3 weeks, and samples were taken on d 1, 4, 7, 10, 14, and 17. Effects of time (repeated measures), K₂CO₃, SBO, and their interactions were evaluated and contrasts declared significant at P < 0.05. Milk iso 13:0 and iso 17:0 concentrations increased linearly after cows began receiving the acidoegenic diets. Similarly, milk concentrations of 5:0, 7:0, 9:0, 11:0, 13:0, and 15:0 increased linearly over time. A 3-way interaction was also significant for 15:0. In contrast, concentrations of iso 14:0, iso 16:0, and iso 18:0 in milk decreased linearly over time. No time or treatment effects were observed for anteiso fatty acids. The common temporal pattern of changes within groups of OBCFA, and the differences between groups indicate distinct origins of OBCFA. Overall, the results show that milk OBCFA respond to the consumption of an acidoegenic diet within 3 weeks.

Key Words: rumen acidosis, diagnostic tool, milk fat


The objective of this longitudinal cohort study was to discover whether the milk microbiome of healthy bovine mammary glands differs from that of inflamed mammary glands during the first 150 d in milk (DIM). Aseptic milk samples were collected from each mammary quarter (n = 649) of cows in the UW-Madison dairy herd immediately before dryoff and twice within the first 14 DIM. Samples were cultured for bacterial growth and SCC was measured. Mammary quarters (n = 107) were enrolled to 4 cohorts based on microbiological status and somatic cell count (SCC) at dryoff and early lactation milk samples: LowNeg quarters (n = 81) had a SCC <100,000 cells/mL and no bacterial growth at all 3 samples; HighNegPre quarters (n = 17) had a SCC ≥150,000 cells/mL and no bacterial growth at the dryoff and first calving samples with a variable second calving sample; HighNegPost quarters (n = 6) had a SCC <100,000 cells/mL at the dryoff sample with a SCC ≥150,000 cells/mL at both calving samples and no bacterial growth at any of the 3 samples; HighPos quarters (n = 3) had bacterial growth and a SCC ≥150,000 cells/mL at all 3 samples. Milk samples were collected from all enrolled quarters weekly for SCC and an aseptic milk sample was collected every 28 d until 150 DIM for microbiological analysis. DNA was extracted from aseptic milk samples and PCR was performed with barcoded primers to the V4 region of the 16S rRNA gene; amplified PCR
products were run on a gel, extracted, and sequenced on an Illumina MiSeq platform. Overall bacterial DNA load in our milk samples was low, as evidenced by only approximately 50% of milk samples that had visible DNA bands after PCR. Sequence data were analyzed in mothur. In samples that could be sequenced, *Halomonas* spp. were the most common operational taxonomic unit (OTU) at dryoff, calving, and through the first 150 DIM. Bacterial richness was similar among cohorts across time while Shannon diversity was greatest in LowNeg samples (mean = 2.93; \( P = 0.02 \)). Differing microbial diversity in inflamed and uninflamed mammary quarters suggests that the milk microbiome may be associated with health status and change during lactation.

**Key Words:** microbiome, mammary inflammation

143 An on-farm algorithm to guide selective dry-cow therapy. A. K. Vasquez1, C. Foditsch1, M. Wieland1, R. A. Lynch2, P. D. Virkler1, S. Eicker3, and D. V. Nydam1, 1Cornell University College of Veterinary Medicine, Ithaca, NY, 2Department of Animal Science, Cornell University, Ithaca, NY, 3Valley Ag. Software, Tulare, CA.

A selective-dry-cow therapy algorithm was evaluated for microbiological cure risk, new infection risk, culling and occurrence of clinical mastitis before 30 DIM, and 1st-test milk yield and linear score (LS) in a randomized on-farm clinical trial including 612 dairy cows. An algorithm using DC305 and test-day data was used to identify cows as “low risk” (cows that likely will not benefit from dry cow antibiotics) or “high risk” (cows that will benefit). Low risk cows were those that had all of: < 200k SCC at last test, an average SCC <200k on the last 3 tests, no signs of mastitis at dry-off, and have not had more than 1 clinical mastitis event in the current lactation. Low risk cows were randomly assigned to receive either intramammary antibiotics and external teat sealant (DCT) or teat sealant only (TS). Quarter milk samples were obtained from cows at dry-off and 1–7 DIM to determine cure and new infection at the quarter level. Samples from high risk cows were used to determine positive and negative predictive values (PPV, NPV) of the algorithm. Mastitis events, milk production, LS, and culling data were retrieved from DC305. Data analysis was performed in SAS 9.4: categorical outcomes were analyzed using Fisher’s exact tests while continuous outcomes were compared with \( t \)-tests. PPV and NPV were each 70%. Of cultures eligible for cure analysis (\( n = 157 \)), 91% of DCT cured, while 83% of TS did (RR of non-cure TS:DCT = 1.9; 95%CI: 0.8–4.6). Positive cultures for coagulase negative staphylococcus (CNS) at dry-off accounted for 95% of the non-cures (\( n = 19 \)). Risk ratio for new infection was 1.4 for TS:DCT (95%CI: 1.0–2.0). CNS accounted for 50% of new infections (\( n = 86/135 \)). There were no statistical effects of treatment group on culling (DCT \( n = 14 \); TS \( n = 18 \)), clinical mastitis (DCT \( n = 5 \); TS \( n = 3 \)), milk (kg) (DCT = 38.9; TS = 39.8), or LS (DCT = 2.4; TS = 2.5). The impact of CNS to increased new infection risk and decreased bacteriological cure needs to be further investigated. These results suggest that the employed algorithm decreased dry cow antibiotic use by 64% without adversely impacting production outcomes.

**Key Words:** selective dry-cow therapy

144 Advancement of Dairying in Austria (ADDA): Conventional dairy farm management with respect to mastitis prevention and detection. C. L. Firth1, C. Schleicher2, A. Käsböhrer1, and W. Obritzhauser1, 1University of Veterinary Medicine, Institute of Veterinary Public Health, Vienna, Austria, 2Austrian Agency for Health and Food Safety (AGES), Integrated Risk Assessment, Data and Statistics, Graz, Styria, Austria.

The ADDA project is based on a partnership between academia, farmers’ associations, veterinary organizations and the Austrian government. This was an observational study and farmers were invited to participate by their local veterinarian (respondent-driven sampling). It was hypothesized that a variety of farm management practices would influence mastitis incidence. A total of 251 Austrian dairy farmers enrolled in the study and 211 responded to the farm management survey. Of these, 165 (78%) farmers farmed conventionally. National milk recorders provided details of milking technique via a separate survey. Descriptive statistics determined that these conventional herds were small, with a mean size of 29 cows (range 8–94). The majority (55%) of farms used milking parlors, with 34% having vacuum milk-line systems in the barn, 9% operating a bucket milking unit and 7% using automated milking systems. Teats were dry-wiped on 38% of farms before milking and 60% of milkers wore gloves. Postmilking teat dipping was carried out on 65% of farms, with 11% spraying teats and 24% reporting not using dip. The California Mastitis Test (CMT) was regularly used to check all cows for high somatic cell counts on just 17% of farms, while its use was more common for symptomatic animals (79%). In animals observed to have symptoms of mastitis, a bacteriological culture was routinely ordered on 56% of farms. Discard milk was frequently (64%) fed to calves. Preliminary analysis determined a significant association in the study population between different types of milking machine and milkers wearing gloves (\( \chi^2 = 12.73, P < 0.001 \)), however, this correlation was less significant for teat dipping (\( \chi^2 = 5.41, P = 0.02 \)). No significant correlation was found between either milking machine or barn type, with respect to premilking teat cleaning method. As a multifactorial disease, mastitis requires a whole-farm solution. A preliminary analysis of management factors has determined a small number of significant correlations and it is hoped that the GLMM analysis of farm management factors and their influence on mastitis incidence rates will shed new light on methods to prevent udder disease.

**Key Words:** mastitis, bacteriology, farm management

145 Effects of oral administration of acetylsalicylic acid after parturition on milk yield and milk components in lactating dairy cows under certified organic management. A. A. Barragan1, L. M. Bauman2, L. da Costa1, J. Velez3, J. D. Rozo Gonzalez3, G. M. Schuenemann1, and S. Bas1, 1Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 2Department of Animal Sciences, The Ohio State University, Columbus, OH, 3Aurora Organic Farms, Boulder, CO.

A proper transition from gestation to lactation is critical to optimize health and milk production in the subsequent lactation. While some studies have suggested that the administration of nonsteroidal anti-inflammatory drugs after parturition resulted in increased milk production of lactating dairy cows in conventional dairy herds, others failed to show an effect. The objective of this study was to assess the effects of oral administration of acetylsalicylic acid after calving on milk yield, milk protein, milk fat and somatic cell count (SCC) in lactating dairy cows under organic management. This study was performed in 3 organic dairy herds. Cows were block by parity and calving ease (eutocia; dystocia) at parturition, and were randomly allocated to 2 treatment groups: 1) ASP (\( n = 278 \)): within 12 h after parturition cows received 4 consecutive treatments every 12 h with acetylsalicylic acid (100 mg/kg; 2 boluses); or 2) placebo (PLC, \( n = 285 \)): within 12 h after parturition cows received 4 treatments every 12 h with gelatin capsules (2 capsules) filled with water. Data from the first 5 DHIA tests after parturition were recorded. In addition, daily milk yields were collected for the first 30 DIM from a subset of cows (ASP = 110; PLA = 122). The data were analyzed
using ANOVA by the MIXED procedure of SAS as a randomized block design with repeated measures. Cows in the ASP group had higher (P = 0.048) daily milk yield (26 ± 0.88 kg/d) when compared with cows in the PLC group (24 ± 0.79 kg/d) during the first 30 DIM. Similarly, cows in the ASP group had higher milk production on the first 5 DHIA tests compared with PLC cows (P < 0.05; ASP = 31 ± 0.43 kg/d; PLC = 30 ± 0.43 kg/d). Additionally, ASP cows had lower SCC (P < 0.05; ASP = 153 ± 241 × 10^3 cells/mL; PLC = 207 ± 324 × 10^3 cells/mL) during the first 5 DHIA test compared with PLC cows. No difference was found in the percentage of milk fat and protein between groups. The results from this study suggest that treatment with ASP after calving may improve milk yields and decrease SCC during the first 5 DHIA tests. Further research aimed to evaluate strategies to improve the transition from gestation to lactation are required.

Key Words: parturition, acetylsalicylic acid, milk yield

146 Energy intake and balance of cows in the early postpartum period is affected by diet starch content and fermentability. R. Albornoz* and M. Allen, Michigan State University, Lansing, MI.

Our objective was to evaluate effects of diet starch content and fermentability on energy intake and balance during the early postpartum (PP) period. Fifty-two multiparous Holstein cows were used in a randomized block design experiment with a 2x2 factorial arrangement of treatments. Treatment rations were formulated to 22% (LS) or 28% (HS) starch content with dry ground corn (DGC) or high moisture corn (HMC) as the primary starch source. Rations were formulated for 22% forage NDF and 17% CP and fed from 1 to 23 d PP. Starch content was adjusted by substitution of corn grain for soy hulls. DMI and milk yield were measured daily, and milk components, BCS, and BW were measured weekly. Feeds and refusals, and fecal samples were collected once a week for 3 weeks. Data were analyzed using a mixed model with block and cow as random effects and day or week PP as continuous measures. DGC increased net energy (NEI) intake compared with HMC (2.4 Mcal/d; P = 0.01) by increasing DMI (2.2 kg/d; P < 0.01), with a greater effect on NEI intake for HS than LS (4.2 vs. 0.6 Mcal/d, interaction P = 0.06). In addition, HS decreased total-tract NDF digestibility compared with LS (P < 0.01), with a greater reduction (18%) for HMC compared with DGC (7.1%, interaction P = 0.09). DGC increased milk NEI compared with HMC (31 vs. 28.2 Mcal/d; P = 0.02) by increasing yields of milk (2.6 kg/d; P = 0.08), milk fat (16.5 g/d; P = 0.03) and protein (165 g/d; P = 0.01). Compared with HMC, DGC reduced BW loss (−33.4 vs. −64.7 kg; P < 0.01) and tended to reduce BCS loss (−0.23 vs. −0.34; P = 0.08). HS tended to improve energy balance compared with LS (−15.3 vs. −17.1 Mcal/d; P = 0.07), which was greater for DGC than for HMC treatments during the first week PP, but lower thereafter (interaction P = 0.08). Efficiency of utilization of digestible energy intake for milk production was highest for LS-DGC (67.8%), followed by LS-HMC (67.1%), HS-HMC (61.1%) and HS-DGC (59.8%; interaction P = 0.09). Feeding DGC improved energy intake and milk yield but had little effect on energy balance during the early PP period.

Key Words: starch fermentability, energy balance, early lactation

147 Feeding increasing amounts of ruminally-protected choline (RPC) increasingly reduced fatty liver of Holstein cows. M. G. Zenobi1, A. M. Lopez2, J. E. Zuniga1, M. B. Poindexter1, T. L. Scheffler1, S. R. Campagna2, B. A. Barton3, J. E. P. Santos1, and C. R. Staples1. 1University of Florida, Gainesville, FL, 2University of Tennessee, Knoxville, TN, 3Balchem Corp., New Hampton, NY.

Objective was to determine the optimal feeding amount of choline to reduce the triacylglycerol (TAG) concentration of liver and to increase absorption of TAG from the small intestine of dairy cows. Pregnant, nonlactating multiparous Holstein cows (n = 77) were enrolled at 64 ± 10 d before calculated calving date. Dietary treatments were 0, 30, 60, 90, and 120 g/d of ReaShure (RPC; Balchem Corp., New Hampton, NY). Experimental periods were 14 d during which cows ate ad libitum (AL) amounts of feed for 5 d and were restricted (R) to consume 36% of their energy/protein requirement for pregnancy and maintenance for 9 d to simulate lactating cows in negative energy balance. Methionine intake was similar during the AL and R periods due to methionine supplementation. Liver was biopsied on d 5 and 14. A saturated fatty acid (454 g of Energy Booster Mag, Milk Specialties, Eden Prairie, MN) was fed after biopsy on d 14 and blood plasma measured for TAG for 19 h post feeding. Data were analyzed using Proc Mixed procedures of SAS. Birth weight of the calves and number of days before calving at the time of enrollment were used as covariates. Significance was declared at P < 0.05. Dry matter intake and energy balance decreased from 9.5 to 3.3 kg/d and from 3.6 to −8.2 Mcal NEm/d during the AL and feed R periods, respectively. Concentration of liver TAG was not affected by RPC during the period of AL. Feed intake (2.3% of liver DM) but decreased linearly with increasing intake of RPC from 17.5 to 11.1% during the period of feed R. With increasing intake of RPC, concentration of liver glycogen increased linearly during the period of AL. Feed intake and tended to increase quadratically during the period of R feed intake, being greater in liver of cows fed RPC compared with that of control cows. Mean plasma concentration of TAG (0, 5, 7, 9, 11, 13, 15, 17, and 19 h after fat consumption) increased quadratically with increasing intake of RPC (13.6, 17.4, 16.9, 15.9, and 15.7 mg/100 mL). Supplementing RPC from 0 to 120 g/d improved liver status and fat absorption of feed-restricted pregnant, nonlactating multiparous Holstein cows.

Key Words: choline, fatty liver

148 Ethyl-cellulose rumen-protected methionine supply during late gestation enhances nutrient transporter expression in bovine placenta and calf birth weight. F. Batistel*1, A. S. M. Alharrith1, B. Saremii2, C. Parsy2, and J. J. Loor1. 1University of Illinois at Urbana-Champaign, Urbana, IL, 2Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.

Essential AA can activate mTOR signaling and elicit effects on cellular mechanisms controlling protein and lipid metabolism. During gestation the fetus relies almost exclusively on nutrients from the dam, hence, we hypothesized that increasing Met supply to the cow during late-pregnancy alters expression of nutrient transporters and mTOR signaling genes in the placenta leading to differences in calf birth weight. Multiparous cows were fed during the last 28 d of pregnancy with a control or Met-supplemented (Mepron, Evonik Nutrition & Care GmbH, Germany) diet. Mepron was supplied at a rate of 0.09% of DM to ensure a ratio of Lys to Met close to 2.8:1 in the metabolizable protein. Placenta samples were collected from 15 cows per treatment and mRNA expression of 41 genes analyzed using qPCR. Calves from Met-supplemented cows had greater body weight at birth (P = 0.04). MTOR and RPS6KB1 (ribosomal protein S6 kinase β-1) were upregulated (P ≤ 0.07) in Met-fed cows. Met-fed cows also had upregulated 5 neutral AA transporters (P ≤ 0.09; SLC34A2, SLC7A5, SLC38A1, SLC38A2, and SLC38A4) and 14 neutral AA transporters, Met upregulated (P ≤ 0.07) the expression of SLC2A1, SLC2A3, and SLC2A4, and down-regulated SLC2A8 (P = 0.07). In Met-supplemented cows the long-chain

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fatty acid transporter SLC27A1 and the betaine transporter SLC6A12 were downregulated (P = 0.06), while the multivitamin cotransporter SLC3A6 was upregulated (P = 0.09). Other genes analyzed related to transport of AA (SLC1A1, SLC1A5, SLC6A6, SLC7A8, SLC3A4, SLC3A8, SLC2A5, SLC2A6, SLC2A9, SLC2A10, SLC2A11, SLC2A12, SLC2A13, SLC5A11), fatty acid (SLC27A2, SLC27A3), vitamin (SLC19A2, SLC19A3, SLC44A1, SLC44A3), and mTOR signaling (AKT1, EIF4EBP1, EIF4EBP2, EEF1A1, ELF2, IRS1) were not affected by Met supply. Overall, our findings indicate that Met supply during late-gestation could enhance utero-placental transport of essential and non-essential AA, glucose and vitamins. As such, differences in body weight at birth in calves from Met-supplemented cows is, at least in part, a result of upregulation of nutrient transporters some of which are controlled by mTOR signaling.

Key Words: amino acid, mTOR, transporter


The ability of diet to affect rumen morphological development in calves has been known for many years. Underlying mechanisms remain unknown but are linked to ruminal VFA production and absorption. VFA production from a given diet is greatly influenced by diet composition, which may lead to differences in development of the rumen. The objective of this pilot-scale study was to determine the effect of diet composition on: calf BW and stature, rumen pH, VFA, lactate, and 3 measurements of rumen morphology. Eight neonatal Holstein bull calves were randomly assigned to one of 2 dietary treatments: high starch:low NDF pelleted starter (HS; n = 4; 19.7% CP, 26.7% starch, 23.6% NDF DM basis) or high NDF:low starch pelleted starter (HN; n = 4; 20.9% CP, 9.5% starch, 36.5% NDF DM basis). Starter grain and water were offered freechoice from birth and milk replacer (22.0% CP, 20.0% fat as-fed basis; 566 g DM/d) was fed twice daily at 13% solids. Calves were individually housed; feed intake was recorded daily. Calf BW, stature measurements, and orosophageally-obtained rumen content samples were collected from each calf weekly. All calves were euthanized at 7 wk of age to collect rumen morphology measurements. Data were analyzed by ANOVA using the MIXED procedure in SAS. There were no differences in BW, stature, or DMI measurements between treatments. Mean rumen pH throughout the 7 wk trial was lower for HS compared with HN calves (P = 0.006; 5.37 ± 0.08 and 5.81 ± 0.07, respectively). Mean acetate, propionate, butyrate, L-lactate, and D-lactate throughout the 7 wk trial were not affected by treatment (59.60 ± 3.42 mM, 37.15 ± 3.04 mM, 14.48 ± 3.97 mM, 6.02 ± 1.22 mM, and 5.27 ± 1.00, respectively). Treatment did not affect full or empty rumen mass (6.20 ± 0.93 kg and 1.12 ± 0.13 kg, respectively), ratio of absorptive surface to muscle (2.68 ± 0.39), or 2-dimensional surface area of papillae (4.56 ± 0.70 mm²). Feeding calf starters of 2 drastically different compositions did not affect overall calf or rumen growth. These findings support the idea that composition of diet may not influence the necessary changes in rumen fermentation that would alter rumen growth in calves.

Key Words: growth, nutrition, dairy

150 Rumen pH in Holstein dairy bull calves from pre-weaning to post-weaning. J. K. van Niekerk*, M. Middeldorp, and M. A. Steele, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada.

The goal of this research was to characterize ruminal pH changes that occur pre- and post weaning (until wk 12 of life) in calves fed an elevated plane of nutrition pre- and post-weaning. Six Holstein dairy bull calves (45 ± 1.5 kg birth weight) were fed milk replacer (MR; 150 g/L) at 15% of body weight in 2 equal volumes per day. Milk volumes were adjusted weekly according to body weight. Calves were weaned using a 1 step-down weaning method; therefore, at the end of wk 5 MR was decreased to 50% and at the end of wk 6 calves were weaned. All 6 calves were fitted with ruminal cannulas in the second week of life. Calf starter, chopped straw and water were offered ad libitum and recorded daily while body weight was recorded weekly. Ruminal pH was measured continuously every 5 min from wk 5 to 12 with a ruminal pH bolus. Data were analyzed using MIXED procedure of SAS. The model included week as a fixed effect and calf as a random effect. Body weight increased from 45 ± 1.5 kg at birth to 109 ± 3.0 kg in 12 weeks, while body weight was maintained at 79 ± 1.5 kg and 77 ± 2.7 kg during step-down (wk 6) and the week after weaning (wk 7) respectively. During step-down, starter intake increased significantly (P < 0.01) from 130 ± 26.7 g/d (wk 5) to 385 ± 41.8 g/d (wk 6) and kept increasing as calves aged (2824 ± 66.9 g/d; wk 12). Pre-weaning the average daily pH was low (5.6 ± 0.09), implying ruminal acidosis. The pH reached its lowest levels during the week after weaning (wk 7) with a mean of 5.5 ± 0.11 and did not increase before wk 11. Furthermore, ruminal pH was below 5.5 and 5.2 for approximately 745 ± 187 and 220 ± 78 min daily during wk 7 and 8, respectively. The pH increased significantly in wk 11 and 12 (P < 0.01), with a mean of 6.1 ± 0.12. In conclusion, our results show that even before step-down weaning, ruminal pH is depressed although starter intake is low. Furthermore, it demonstrates that dietary rumen adaptation post-weaning can take several weeks because of prolonged ruminal pH depression in calves fed an elevated plane of nutrient post-weaning. The prolonged depressed ruminal pH might impact gut development as well as gut health.

Key Words: cannulated calves, elevated plane of nutrition, ruminal pH

151 Alteration of intrinsic molecular structure by steam flaking process improved gastrointestinal digestion of carbohydrate in dairy cows. N. Xu1,2, J. Liu1, and P. Yu2, 1Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, China, 2Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada.

This study was aimed to investigate effects of steam flaking on rumen and intestinal digestibility of corn carbohydrate (CHO) on a molecular basis. Duplicates of 2 corns (Corn1, Corn2) and the corresponding steam-flaked corns (SFCorn1, SFCorn2) were collected from 2 commercial plants. Attenuated total reflectance Fourier-transform vibrational molecular spectroscopy was used to collect CHO molecular spectra (ca. 1188–950 cm⁻¹) with 3 major spectral peaks: 1st (ca. 1188–1132 cm⁻¹), 2nd (ca. 1132–1066 cm⁻¹) and 3rd Peaks (ca. 1066–950 cm⁻¹). Starch rumen and intestinal digestion characteristics were evaluated by conventional in situ procedure. PROC MIXED program of SAS 9.4 was used to analyze molecular spectral peak intensity and starch digestibility based on 2 factory design; and multivariate analyses (Statistica 8.0) were applied to CHO spectral absorption intensity data to distinguish CHO intrinsic molecular difference between raw and steam flaked types of corn. Truly digestible non-fiber carbohydrate was greater in steam flaked corn than their corresponding raw corn (P < 0.01). Rate constant of starch digestion increased (P < 0.01) after steam flaking. Rumen bypass starch of SFCorn1 was lower (P < 0.01) than that of Corn1, but...
no difference was found between SFCorn2 and Corn2. Steam flaking process increased effective rumen degradability of starch ($P < 0.01$), but had no effects on intestinally digestible bypass starch and total digestible starch ($P > 0.05$). Steam flaking process increased spectral peak area intensities of total CHO and CHO 3rd Peak ($P < 0.01$). For CHO 1st and 2nd Peaks, molecular spectral peak area intensities increased in SFCorn1 ($P < 0.01$) compared with Corn1, but this was not the case for Corn2. Molecular spectral peak height of CHO 2nd Peak was greater ($P < 0.01$) in SFCorn1 than in Corn1. Multivariate analysis demonstrated that steam flaked corn could be apparently distinguished from their raw corn in carbohydrate molecular spectral region. In summary, steam flaking process altered carbohydrate molecular spectral structure, and improved carbohydrate gastrointestinal digestion.

**Key Words:** steam flaking, carbohydrate molecular spectra, corn digestibility
The analysis found that dairy size, productivity, operator education level, and having off-farm income were not associated with BTSCC. Comparison of operating and no-longer-operating dairy farms suggests some underlying issues relating to financial motivations and consequences. “Increasing net farm income” as a goal for the dairy operation was assigned greater importance by operators of active dairies than closed dairies (F(1, 574) = 4.177, P = < 0.041). Also, operators of closed dairy farms assessed their ability to afford actions to decrease SCC more negatively than did operators of active dairies (F(1, 585) = 3.970, P = 0.047) and evaluated the adequacy of their milk quality premiums more negatively than did active dairies (F(1, 1569) = 4.834, P = 0.028).

The survey of open dairies shows a correlation between producers’ confidence in their knowledge of mastitis management procedures and producers’ assessment of their ability to afford to treat mastitis (r(607) = 0.379, P < 0.0005). This correlation bolsters the argument for Extension services and veterinarians to continue science-based training of dairy operators and dairy employees as a tool for supporting the sustainability of the dairy industry in the southeastern US.

Key Words: mastitis, attitudes, farm structure

153 Considerations for managing mastitis and milk quality on organic dairy farms. K. A. E. Mullen*, North Carolina State University, Raleigh, NC.

Organic dairy farms in the United States face a unique challenge when it comes to mastitis management, as synthetic antibiotics are not allowed for use in organic production. However, if a cow is sick and organic therapies do not work, antibiotics must be used to treat disease. Organic regulations in the United States mandate that any organic cow that receives antibiotic therapy forfeits her organic status. Many different mastitis mitigation strategies on organic dairy farms have been reported in the scientific literature but most studies were completed in the Midwest and Northeast. In the South, especially the Southeast, dairy farmers must contend with high heat and humidity for a longer period of the year and the consequent effects that heat stress has on dairy cattle. I will discuss the current scientific literature evaluating organic dairy farms in the South, from mastitis prevalence to breeding for a more mastitis resistant cow. Organic dairy farmers can follow most of the National Mastitis Council’s 10-point control plan; this and other considerations unique to organic dairy farms will be covered during this session.

Key Words: organic, mastitis, management

154 The role of housing facilities and management in improving milk quality. A. E. Stone* and P. D. Krawczel*, 1Mississippi State University, Starkville, MS, 2University of Tennessee, Knoxville, TN.

Milking production is forecasted to decline in the Southeastern United States in coming years whereas overall US production will increase. Additionally, the Southeastern US historically maintains a greater SCC than the rest of the nation. These factors increase the competitiveness of the southeastern dairy industry. Southeastern dairy producers have different management challenges compared with the rest of the nation. Loss of infrastructure and poor housing environments create large...
areas for potential management deficiencies. The overall objective of this presentation will be to address the challenges and opportunities of housing and facility management in the southeastern United States and their potential effect on milk quality. Topics will include: a review of freestall design recommendations compared with what is common in the SE, how overstocking negatively affects herds, why heat stress abatement techniques are important, the pros and cons of pasture as a management style, how general welfare parameters (e.g., lameness and hygiene) hold up to industry recommendations, and the relationship among welfare parameters and housing management present in the SE. Commonly used milk quality measures like SCC and SPC will be used as a focal point on reasons to implement these management strategies, but the effects on animal welfare and behavior will also be discussed. Results of applied housing research studies will be presented, particularly those of the Southeast Quality Milk Initiative, a 6-state applied research and extension project aimed at helping producers find cost-effective and science-based ways to improve milk quality. This project was supported by Agriculture and Food Research Initiative Competitive Grant number 2013–68004–20424 from USDA National Institute of Food and Agriculture.

Key Words: dairy housing, milk quality, dairy management

The role of technology in quantifying mastitis-related decisions. J. M. Bewley*, University of Kentucky, Lexington, KY.

Given the economic importance of both clinical and subclinical mastitis, early detection of mastitis is one of the most exciting Precision Dairy Farming applications. Early detection of mastitis may increase the likelihood of bacteriological cure; thus, the economic losses associated with a case of mastitis may be reduced. Reductions in the duration of pain associated with mastitis may also improve animal well-being. Potential also exists to separate abnormal milk automatically. These tools may also help reduce the likelihood of transmission of mastitis between cows and prevent the infection from becoming chronic. Mastitis changes the concentration of anions and cations in milk. Adoption of electrical conductivity systems has been limited because of sensitivity and specificity limitations; however, new algorithms and quarter based measurement systems hold potential for improved results. Thermography and milk temperature have also been proposed; however, both tools are limited because not all causes of mastitis result in a temperature response. Color variation (red, blue, and green) sensors have been included in some automatic milking systems to detect blood in milk and color pattern changes in infected quarters. In-line sensors that essentially automate the California Mastitis Test or Wisconsin mastitis test have been commercialized. These systems could provide valuable herd management information for identifying cows contributing to changes in bulk tank SCC. Image analysis may potentially differentiate between changes in the physical content in milk (i.e., flakes and clots) and other foreign materials (i.e., bedding and manure particles (Brandt et al., 2010). Spectroscopy (visible, near-infrared, mid-infrared, or radio frequency) has been applied in commercial sensors systems to identify changes in milk constituents including SCC and mastitis-causing pathogens. Biosensors designed to detect a specific biological component of milk may also be used for mastitis detection. For example, the enzyme, L-Lactate dehydrogenase (LDH), is released because of the immune response and changes in cellular membrane chemistry and has recently been commercialized as a mastitis detection tool.

Key Words: mastitis, data
Most calf mortality is due to a serious problem called dystocia. Dystocia is when cows have difficulty birthing due to a large or awkwardly positioned fetus. Dystocia has been a direct link to the failure of the passive transfer of immunoglobulins. Barrier et al. (2013) investigated neonatal physiology, survival, health, and growth of 455 calves following the occurrence of dystocia. Calves delivered during dystocia suffered from higher stress, higher mortality, and reduced transfer of immunoglobulins.

In other animals, oxygen therapy is used to improve physiological function and reduce stress, therefore, some research has focused on using supplemental oxygen in dairy calves. Bleul et al. (2008) examined the effects of intranasal oxygen on the respiratory state of neonatal dairy calves. Before and after oxygen was administered, researchers evaluated the arterial partial pressure of oxygen and carbon dioxide and oxygen saturation. Results concluded intranasal oxygen significantly improved the survival rate of calves with respiratory distress. Providing calves with supplemental oxygen post-birth may be used to improve the level of passive transfer of immunoglobulins, save farmers time and money and, most importantly, reduce calf mortality.

Key Words: immunoglobulin, supplemental oxygen, calf

Grocery by-product waste and how dairy cattle can help.
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Feed is the single largest expense in dairy farm production. Thus, it is important for producers to consider alternatives to traditional feedstuffs that could decrease the cost of feeding the animals while maintaining optimum animal performance. Grocery store waste (GSW) is one option to investigate as an alternative for the use of feeding dairy cattle. This includes fruits, vegetables, and bread/pastry products. Ruminants have the ability to derive nutrients from sources humans and other non-ruminants cannot. Therefore, ruminants can play an important role in recycling discarded materials from grocery stores. A literature review was conducted using online databases, such as Science Direct, Pubmed and Agricola. The objective of this review was to determine the nutritional, economical, and environmental benefits of using GSW to feed dairy cattle by evaluating different processing alternatives available to improve preservation and quality of this product. Grocery store waste takes up space in landfills and also contributes to the production of greenhouse gases. One reason so much grocery store waste is produced is because of the high standards of consumers. Consumers will often not buy produce that is blemished, bruised, oddly shaped, or discolored. These products are still nutritionally valuable, yet they are discarded, and cattle can maximize nutrient utilization if these are incorporated into a balanced ration. Viable preservation options include silage and dehydration. Based on these results, GSW can be fed to dairy cattle, potentially reducing costs, and environmental impact with no detrimental effects on production. Further investigation is required to determine nutritional characteristics and to improve preservation methods.

Key Words: grocery store waste (GSW), preservation, drying

The effects of grain-induced subacute ruminal acidosis on rumen epithelial transporters and volatile fatty acid concentrations.
L. Beckett*, R. White, and D. Winston, Virginia Tech, Blacksburg, VA.

Subacute ruminal acidosis (SARA) accounts for a loss of $400 per cow per lactation across the United States. Often, SARA is defined as a decrease in ruminal pH below 5.5 for multiple hours per day. There are no physical signs of SARA, but typically is associated with decreased dry matter intake (DMI) and a correlated decrease in milk production. Oba, et al. (2014) measured DMI, milk production, milk components, and feed sorting behavior of SARA-tolerant or susceptible cows. Milk yield and DMI were not statistically different between tolerant and susceptible groups; however, the tolerant cows had higher milk urea nitrogen concentrations and sorted feed less. The shift in milk urea N suggests the susceptible animals may have impaired fermentation. To better understand the mechanisms behind SARA, it is beneficial to understand how the rumen environment changes during low pH. Markantonato et al. (2008) conducted an experiment that compared VFA production and absorption rates on a ration designed to simulate SARA.
conditions (high concentrate) and a ration high in forage. The authors were able to determine there is a slower turnover of VFA during SARA conditions compared with high forage rations. The SARA-type ration also had a larger amount of acetate present in the rumen. A potential explanation for why VFA accumulate in the rumen during SARA conditions is provided by Laarman, et al. (2016). The study by Laarman, et al. (2016) monitored rumen epithelial transporters during SARA or restricted feeding using Ussing chambers. Total, protein-mediated, and passive diffusion VFA fluxes and the abundance of specific rumen epithelial transporters were measured. The transporters, NHE1 and NHE3 were both positively correlated with protein-mediated flux, and only NHE3 was correlated with total acetate flux. These data suggest that shifts in the rumen epithelium may contribute to the sequestration of VFA that occurs during SARA. All of these studies help characterize animal responses to SARA and help us better understand the underlying biology that drives the economic losses associated with this condition.

Key Words: subacute ruminal acidosis, rumen epithelial transporter, volatile fatty acid concentration

160 Using genomic selection to improve dairy cattle heat tolerance. C. N. Folmar*, C. M. Truman, and J. M. Bewley, University of Kentucky, Lexington, KY.

Heat stress can be defined as the physiological stress that animals experience when the environmental temperature is above their thermoneutral zone. Dairy cattle begin to experience heat stress around 20°C. During heat stress the animal may have reduced milk yield, feed intake, and reproductive performance. However, when accessing heat stress external factors affect the threshold temperature such as radiant energy, relative humidity, and wind speed. To account for some of these variables, a metric called the Temperature-Humidity Index (THI) is often used. When an animal is more resistant to heat stress, it is considered to be more heat tolerant. Heat tolerance is important in dairy cattle because their bodies produce a large amount of internal heat. Dairy producers select for greater milk production, requiring cattle to increase DMI, indirectly increasing body heat production. When producers make breeding selections based on milk production, heat tolerance decreases. A negative correlation (R = −0.3) exists between milk production and heat tolerance (Ravagnolo and Misztal, 2000). It may be possible to improve both because there is a low correlation between milk production and heat tolerance traits. Milk yield data were collected from 366,000 cows and compared with weather data for an 11-year period. The cows were divided into heat tolerant and heat susceptible groups based on their decrease in milk production while experiencing heat stress. Genomic information was analyzed to predict genomic estimated breeding values for heat tolerance. Two groups of 24 cows that were genetically identified as the most heat susceptible or heat tolerant were exposed to high heat conditions for 4 d. Cows that were genetically typed as heat tolerant displayed a lower reduction in milk production and DMI (Garner, 2016). As genomics continue to improve and heat tolerance is better understood, selection for more heat tolerant dairy cattle may be possible in the future.

Key Words: heat tolerance, dairy cattle, genetic selection

161 Measuring fecal cortisol metabolites to assess the impact of management stressors on dairy cattle. Y. I. Ruiz* and J. M. Huzzey, California Polytechnic State University, San Luis Obispo, CA.

Cortisol is a hormone that helps animals respond to acute stress and facilitate survival by supporting energy metabolism and helping other hormones like epinephrine increase vascular tone, activate the immune system and increase vigilance. Chronically elevated cortisol concentrations, however, compromise the immune system and increase an animal’s risk for disease. Several management practices occur on a dairy that may cause stress to the dairy cow, such as regrouping and overcrowding. Little work has documented the physiological responses of these kinds of stressful management practices, but this work is important to understand the full impact on cow wellbeing. One challenge with interpreting plasma cortisol concentrations is that handling stress during the blood sample collection can increase cortisol concentrations within a matter of minutes. Researchers have determined that fecal cortisol metabolite concentrations [e.g., 11,17-dioxyandrostenediol (11,17-DOA)] are useful estimates of cortisol production in cattle. Using ACTH and dexamethasone tests it’s been discovered that the concentration of this metabolite parallels that of cortisol in blood with a delay time of 10 to 12 h. Plasma cortisol is also highly variable throughout the day due to its pulsatile release from the adrenal gland; this variation is attenuated in feces, making 11,17-DOA a better measure of overall basal cortisol concentrations when sampling the animal once per day. In a recent study, it was demonstrated that when nonlactating dairy cows are overstocked, 11,17-DOA concentration tends to be greater compared with when they are not overstocked, suggesting that cattle experience elevated levels of physiological stress when forced to compete for resources. The measurement of fecal cortisol metabolites rather than plasma cortisol may be a valuable tool researchers can use to evaluate the magnitude of physiological stress caused by dairy management practices. Identifying and mitigating these stressors is critical for maintaining healthy and productive dairy cows.

Key Words: overstocking, fecal cortisol metabolites, stress

162 The impacts of manure management in dairy production. B. Young*, West Virginia University, Morgantown, WV.

Manure management on dairy farms is an important aspect of milk production. The lactating dairy cow produces on average 34 kg of milk each day. In doing so, she will also excrete approximately 54.5 kg of manure daily that needs to be collected, stored, and then utilized in an environmental but effective way. Several factors can affect manure management, storage being one of the most crucial. Storage systems can directly influence the value of nutrients within the manure. In addition to having the necessary capacity for storage, farmers need sufficient land base for application of the nutrients, while adhering to environmental regulations. Most states have restrictions on when manure can be spread on cropland based upon weather. If land base is limited, storage volume needs to be increased to collect the manure for a longer length of time. The goal of manure management is to utilize the nutrients from the manure to maximize crop production. Understanding whole farm nutrient cycling and implementing conservative tillage practices can play a large role. These practices are enforced within the Chesapeake Bay watershed to reduce eutrophication, which has changed the operation of many producers. The same issues in the Chesapeake are starting to occur in the Gulf of Mexico. Thus, learning to manage nutrients within the manure can play a vital role in decreasing environmental issues and increasing production within the Mississippi River Watershed.

Key Words: eutrophication, dairy farms, nutrient management
Invisible impacts of mastitis: The long-term reproductive loss. E. Brenengen* and D. Olver, Pennsylvania State University, University Park, PA.

Mastitis is a disease that all dairies encounter. While the majority of financial losses from mastitis on dairy farms can be attributed to decreased milk production, the negative impact of mastitis on reproductive efficiency and function in dairy cattle is often overlooked. Multiple observational studies have examined the correlation between measures of fertility and the presence of clinical or subclinical mastitis. Reproductive efficiency measurements such as services per conception and days open are negatively influenced by mastitis. Large clinical studies have demonstrated that cows with clinical or subclinical mastitis are at a greater risk for aborting an existing pregnancy. This risk was greatest if infection occurred during the first 45 d of gestation. The effects of mastitis-causing pathogens on fertility are still an area of active investigation. Researchers have proposed various mechanisms to explain mastitis-induced infertility and have estimated economic losses to producers. Although economic impacts differ among dairies, it is clear that mastitis is an important contributor to poor fertility and represents a significant source of financial loss to dairy operations. As producers seek to limit these losses, measures to prevent mastitis and encourage good health in the transition period and during early lactation will become even more valuable.

Key Words: mastitis, reproduction, pregnancy

Evaluating the migration toward automated calf feeders on calf performance. M. Wright* and J. Bohlen, University of Georgia, Athens, GA.

The use of group housing systems with automated calf feeders are gaining in popularity in the United States. This is in stark contrast to the previous premise held that individual housing systems optimized calf health. Recent growth is based on several notable benefits cited for both producer and calf. These automated feeders allow farmers to receive more data on their calves while concurrently reducing labor costs. Another advantage to automated calf feeders is the ability to increase milk volume and number of feedings per day easily. Research regarding the advantage to feeding calves more and with more frequency, has provided mixed results. A portion of research trials suggest that calves allowed a higher volume of milk per day will have higher preweaning average daily gain, which correlates to higher milk yield in first lactation. Yet results from additional studies indicate that calves fed ad libitum milk have lowered rate of gain compared with calves fed a constant amount daily and that these consistently fed calves have higher feed efficiency, which could reduce calf-rearing costs. There is still other work that suggests many calves do not maximize their allotted daily milk, thus are able to optimize their milk utilization based on need. Growth and development of calves is also directly impacted by their health. Health in these group housed systems, research has identified, is a direct reflection of management techniques as is the case with individual housing systems. Finally, training to use calf feeders can require time for calf and producer. Calves should be worked with individually to get them using the feeders independently as quickly as possible. Producers need to give themselves time to adjust to this system of feeding calves and strategically pick management practices that work best for them and their calves. The ability to directly measure the impact of automated calf feeders on calf performance is often blurred by many producer related variables. Therefore, it is important to remember that with this system as with any calf rearing system, the results in calves is only going to be as good as the thoughtful management that went into them.

Key Words: automated calf feeder, group housing, calf rearing
165 The effects of body condition and dietary starch content on first ovulation postpartum in dairy cows. K. V. Murphy*, D. J. Ambrose1,2, and M. Obal1, 1Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, 2Livestock Research Section, Alberta Agriculture and Forestry, Edmonton, AB, Canada.

The objectives were to evaluate the effects of body condition and starch content of postpartum diets on days to first ovulation postpartum. Fifty-three pregnant Holstein cows (BCS at 28 d before the expected calving date: 3.23 ± 0.29; mean ± SD) were fed a common close-up diet with 13.2% starch content, and randomly assigned to either high (26.7%) or low (21.4%) starch diets immediately after calving. Then, all cows were fed the high starch diet from d 24 ± 3 to 42 ± 3 relative to calving. Ovulation was determined using transrectal ultrasonography performed twice weekly starting on d 7 ± 2 after calving until the first ovulation was confirmed. Days to first ovulation were 21.1 ± 2.5, and 22.1 ± 2.2 (LSM ± SEM), respectively for low and high starch diets, and did not differ between treatments (P = 0.78). Body condition score on d −28 relative to expected calving date was negatively correlated with days to first ovulation postpartum in primiparous cows (r = −0.45, P = 0.03), but not in multiparous cows (r = −0.001, P = 0.99; P = 0.01 for interaction). However, there was no difference in days to first ovulation between primiparous cows and multiparous cows (23.0 ± 2.5 vs. 20.6 ± 2.2; P = 0.46), and the interval from calving to first ovulation was not correlated to prepartum BCS change (d −28 to calving; r = 0.03) or postpartum BCS change (calving to d 24; r = 0.13, P = 0.35) regardless of parity. In addition, there was no relationship between β-hydroxybutyrate concentration in milk at the first milk test and days to first ovulation postpartum (r = −0.29, P = 0.12). In summary, starch content of postpartum diets did not affect the interval from calving to first ovulation, but higher BCS at d-28 relative to expected calving was associated with reduced days to first ovulation postpartum in primiparous cows.

Key Words: BCS, first ovulation, starch


Cows exposed to heat stress during late gestation produce less milk in the subsequent lactation. Moreover, there are long-term impacts on the fetus developing under these conditions; in-utero heat-stressed heifers produce less milk during their first lactation. We hypothesized that heat stress during late gestation affects lactation performance of the cow and the developing offspring by impairing mammary gland (MG) development. Cows were heat stressed (HT, only provided shade, n = 11) or cooled (CL, provided shade, fans, and soakers, n = 10) during late gestation (dry period ~46 d). After birth, heifer calves born to cows heat stressed during late gestation were managed as a group, housed under identical conditions, and followed to their first lactation (in-utero HT, n = 3; in-utero CL, n = 4). Milk yield was recorded daily from calving to 84 d in milk (DIM). Heifer MG was biopsied at 21 and 42 DIM and cow MG was biopsied at 14, 42, and 84 DIM of their respective lactations. Tissues were paraffin embedded, sectioned, and stained with hematoxylin and eosin. Images were taken at 10x and 20x magnification, respectively, using an EVOS microscope. The number of alveoli (at 10x) was counted and alveoli area (at 20x) was measured using Image J software. Data were analyzed by ANOVA, with DIM as a repeated measure. CL cows had greater milk yield relative to HT cows (40.0 vs. 34.3 ± 0.80 kg/d; P < 0.01). For heifers, milk yield was similar between treatments (31.0 and 30.5 ± 2.5 kg for in-utero CL and HT, respectively). CL cows had more alveoli than HT cows (197.7 vs. 167.3 ± 9.2; P = 0.04), but alveoli area was similar between treatments. In contrast, MG alveoli number of heifers was similar between treatments but alveoli area was lower for in-utero HT heifers relative to in-utero CL heifers (62.145 ± 0.008 vs. 69.12 µm²; P = 0.01). Our results indicate that exposure of pregnant cows to heat stress during late gestation (i.e., dry period) not only impairs normal development of the mammary gland during their subsequent lactation, but also affects the mammary development of the in-utero fetus.

Key Words: mammary alveoli, heat abatement, histology

167 Effects of trace mineral injections on liver and blood trace mineral concentrations in dairy cattle. K. Kelly*,1, E. Eckelkamp1, B. Kawonga1, D. Tracy2, C. Fendley1, and J. Bewley1, 1University of Kentucky, Lexington, KY, 2Multimin USA, Fort Collins, CO.

Trace minerals (TM) such as selenium (Se), manganese (Mn), copper (Cu), and zinc (Zn) are required in small amounts by the body and are vital for dairy cow reproduction, immunity, and production. The study objective was to determine if a TM injectable increased liver and blood concentrations of Se, Mn, Zn, and Cu following calving. Sixteen primiparous and 16 multiparous were divided evenly into 2 treatment groups and received either 5 cc of TM injection (MM90; Multimin 90, Multimin USA, Fort Collins, CO) or 5 cc of sterile saline (CONT). Blood samples and liver biopsies for TM concentrations were taken at 60 and 30 d peripartum, and 14, 30, and 60 d in milk (DIM) relative to expected calving date. The GLIMMIX procedure of SAS (Version 9.4, SAS Institute, Inc., Cary, NC) was used to assess the effects of day, treatment, parity, and all 2-way interactions for all models on liver and blood Se, Cu, Zn, and Mn concentrations. Stepwise backward elimination was used to remove non-significant interactions (P ≥ 0.05) with all main effects remaining in the models regardless of significance. LSMmeans of liver Se concentrations decreased on 30 d peripartum and 14 DIM but increased after 30 DIM compared with d 60 d peripartum (P < 0.01, respectively). Multimin 90 LSMmeans of liver Se increased 0.31 ng/g compared with CONT (P = 0.02). LSMmeans liver Cu increased significantly by 139.65 ng/g in multiparous compared with primiparous (P < 0.01). Liver Mn (P = 0.03) and liver Zn (P = 0.01) were significantly greater by over 39.32 ng/g and 0.97 ng/g on 14 DIM compared with peripartum days. No significant differences were found between the CONT and MM90 for liver Cu and Zn, blood Se, Cu, or Mn concentrations. Multiparous cows increased by 5.82 ng/mL in blood Se compared with primiparous (P = 0.01). Multimin 90 blood Mn levels increased by 1.22 ng/mL over CONT (P = 0.046). Trace mineral supplementation increased storage of Se and Cu, indicating MM90 may be a beneficial addition to dry cow management.

Key Words: trace mineral injectable, liver mineral concentration, blood concentration
Characterization of milk cellular response to intrammary phytoceutical administration in cows with clinical mastitis. C. M. Womble*1, K. A. E. Mullen1, S. P. Washburn1, and K. L. Anderson2. 1Department of Animal Science, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC. 2Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Due to restrictions on antibiotic use in US organic dairy production, some producers have adopted the use of plant-derived products, or phytoceuticals, to treat mastitis. The objective of this study was to evaluate how the administration of Phyto-Mast (PM), an intramammary (IMM) phytoceutical product, affected the number and types of leukocytes present in milk of cows with induced clinical mastitis. Milk leukocytes increase when foreign materials are introduced into the gland, and monitoring these cells can help to assess immune system stimulation and mammary irritation caused by this product. Milk cellular response to PM was previously evaluated in healthy cows: no significant differences in total leukocyte count (TLC) or distribution of cell types were observed. However, it was unknown if the same response occurred in cows with clinical mastitis. Eight Holstein, Jersey, and Holstein x Jersey cows >100 d in milk were selected based on initial somatic cell counts of <400,000 cells/mL and negative bacterial cultures from all quarters. After inoculation with Streptococcus uberis and subsequent evidence of clinical mastitis, 2 IMM doses of PM were administered 12 h apart in the infected quarter. Quarter milk samples were collected before dosing, 12 h after the first dose, and 12, 24, 48, 72, and 96 h after the second dose. Q Scout Milk Leukocyte Differential tests were used to measure TLC and neutrophil, macrophage, and lymphocyte levels. Least squares means and standard errors of the data were obtained using PROC MIXED in SAS 9.3 with cow as a repeated measure. The TLC in cells/mL was 10.6 ± 1.4 million at dosing, 8.8 ± 1.8 million at 12 h after the first dose, and 9.7 ± 1.7 million at 12 h after the second dose. Neutrophils represented 78 ± 2% of the TLC at dosing, 77 ± 2% at 12 h after the first dose, and 76 ± 1% at 12 h after the second dose. No significant cellular differences were observed among these values or macrophage and lymphocyte levels. In conclusion, infection status of the treated cow did not alter the milk cellular response to the IMM phytoceutical product tested in this study.

Key Words: phytoceutical, mastitis, leukocyte


Automatic milking systems (AMS) record a wide range of information each milking, including whether a cow kicks the teat cup off during milking. The objectives of this study were to determine the heritability of kicking behavior and to determine the genetic correlation between 305d milk yield and kicking behavior. Ancestry, kicking behavior, and milk yield were collected from a dairy farm with 8 AMS. Kicking behavior was collected from 452 cows across 170,368 individual milkings, whereas 305d milk yield was available for 6532 total lactations from 2472 cows. Kickoffs (1 = teat cup kicked off at least once; 0 = otherwise) were determined for each quarter during each milking and across all 4 quarters. The frequency of kickoffs for each quarter was 2.1%, 2.6%, 5.3%, and 5.2% for the left front, right front, left rear and right rear quarters, respectively. The frequency across all quarters was 10.7%. A threshold animal model was used to evaluate kickoffs in ASReml, and multiple trait linear animal models were used to determine genetic correlations among quarters and between kickoffs and milk yield. The kickoff models included significant (P < 0.05) fixed effects of lactation (1, 2, and ≥ 3), number of times a cow was milked that day, hour of day milked, and month. Random effects included animal, permanent environment, the interaction of robot and date, the interaction of days in milk and lactation group, and error. First lactation cows were more likely to have kickoffs, and morning was associated with fewer kickoffs than evening. The heritability of kickoffs across quarters was 0.07 ± 0.05 and the repeatability was 0.37 ± 0.02. Across the individual quarters, the heritability of kickoffs ranged between 0.00 and 0.071. The genetic correlation of kickoffs among quarters ranged from 0.19 to 1.00. The heritability of milk yield was 0.29 ± 0.04 and the genetic correlation between milk yield and kickoff was estimated to be 0.20 ± 0.24. This study suggests that there is likely sufficient genetic variation to select for cows that are less likely to kick during milking in an AMS and that kicking behavior is likely to have strong genetic correlations across quarters.

Key Words: automatic milking system, heritability

Relationship between lying behavior and subclinical ketosis in Holstein and Jersey dairy cows. O. Duner*, K. Kutina, Y. Ruiz, E. Whisler, and J. Huzee, California Polytechnic State University, San Luis Obispo, CA.

Dairy cattle are at risk for ketosis (negative energy balance) after calving due to the sudden increase in energy demands for milk production during a time when feed intake is low. Previous work has shown that cows diagnosed with ketosis had longer standing times both before calving and on the day of calving compared with non-ketotic cows. All work in this area has focused on Holstein dairy cattle. Jersey cattle differ from Holstein cattle both in behavior and physiology and therefore may also differ in their relationship between lying behavior and ketosis status. The objective of this study was to describe the lying behavior of Holstein and Jersey cattle with and without subclinical ketosis (SCK) before and after calving. Lying behavior was monitored from 2 wk before calving to 2 wk after calving using data loggers (HOBO Pendant G Acceleration Data Logger, Onset, Bourne, MA) strapped to the hind leg of 35 Holstein cows and 45 Jersey cows. At 7 d after calving a Precision Xtra Cowside Test (Abbott Laboratories) was taken to measure b-Hydroxybutyrate (BHBA) in the blood. BHBA ≥1.2 mmol/L indicated a state of SCK. Lying times and lying bouts were analyzed using a mixed model, which included the fixed affects of parity, postpartum body condition score, ketosis status, week and the ketosis status by week and parity by ketosis status interaction. Data for the behavior on the day of calving was compared between groups independently. A week by SCK status interaction was detected (P < 0.001); cows with SCK had lower lying times before calving compared with non SCK cows (e.g., wk −1: 648 ± 32.7 vs 744 ± 15.7 min/day respectively). In the week following calving, SCK cows spent more time lying than non SCK cow (680 ± 32.7 vs 603 ± 15.7 min/day, respectively). Across all periods there was a tendency for SCK cows to have fewer lying bouts than cows without SCK (11 ± 1.7 vs 14 ± 0.8 bouts/day respectively; P = 0.075). Improved detection of ketosis could improve efficiency of treatment which is beneficial to the welfare of the cow and the profit of the dairy farm.

Key Words: subclinical ketosis, lying behavior

The effect of electric disbudding on the reaction of phytohemagglutinin-P in Holstein calves. A. Taylor* and S. I. Kehoe, University of Wisconsin-River Falls, River Falls, WI.

Young dairy calves encounter many immune challenges and it would benefit a farmer to recognize an immune challenge before clinical symptoms. An injection of phytohemagglutinin-P (PHA-P) causes a cell-
mediated immune response and may be used to evaluate the response of the immune system. Electric disbudding creates a stress that may show differences in inflammation at the injection site of the PHA-P to identify the existence of an immune response. Twelve Holstein heifer calves were used from the University of Wisconsin – River Falls Lab Farm and grouped into a disbudded treatment (D) and a sham treatment (S). All calves were clipped, sanitized, and injected with 0.1 mL of 1.5 mg of PHA per mL as referenced by Ballou et al. (2008) intradermally at both the scapula and ear 2 days before D and S, and immediately following D and S. Blood samples and caliper readings were recorded at −48 h, −42 h, −36 h, 0 (at time of D or S), 6 h, 12 h, and 48 h. Blood was analyzed for cortisol, neutrophils, and lymphocytes. Caliper readings measured the amount of swelling at the injection sites. The mixed procedure of SAS (2012) was used with time as a repeated measure and calf as a random statement. There were no significant differences between treatments for caliper readings or blood measurements. Least squares means of caliper readings for treatment D were 7.57 mm and 6.77 mm (P = 0.16) and treatment S were 6.71 mm and 7.87 mm (P = 0.01), in the ear and scapula, respectively. Results show that using PHA-P does not indicate an immune response in dairy calves when they are disbudded. However, more sensitive calipers may provide different results.

Key Words: calif, immunity, stress

172 Flaxseed containing lipid supplement increases omega-3 content in milk by protecting dietary omega-3 from ruminal biohydrogenation. R. Wilson*1, S. Akers1, K. Swanson1, M. Keller1, L. Goddick1, G. Cherian1, R. Day2, and G. Bobe1, 1Oregon State University, Corvallis, OR, 2N3Feed LLC, Tualatin, OR.

Lipid supplements rich in PUFAs such as flaxseed are susceptible to biohydrogenation in the rumen. To prevent ruminal biohydrogenation, various methods have been tested with limited success. In this study, a novel method to “rumen-protect” flaxseed is proposed, which encapsulates flaxseed using a proprietary method (12BT40; N3Feed LLC; Tualatin, OR). To determine whether 12BT40 decreases rumen biohydrogenation of dietary PUFAs, we used a double 3 × 3 Latin square design; 6 mid- to late-lactation, pregnant Holstein cows (1 block each for primaparous and multiparous cows) were fed 0 kg/d (Negative Control), 3 kg/d of 12BT40 (Treatment), and 3 kg/d of the unprocessed ingredients of 12BT40 (Treatment Control) as top-dressing for 2-week periods each. Milk and serum samples were collected at the end of each 2-week treatment period and analyzed for their fatty acid profile and metabolic indicators, respectively. Individual feed intake and milk yield and components were measured to evaluate the effect of 12BT40 on production parameters. Data were analyzed using PROC MIXED in SAS version 9.4. Fixed effects were treatment, period, and parity. Repeated measures within cows were modeled with the random statement. Compared with Treatment Control, 12BT40 increased the proportion of omega-3 in milk FAs from 1.49 ± 0.23 wt% to 2.32 ± 0.23% (P = 0.005) (Negative control: 1.07 ± 0.23%). 12BT40 attenuated the flaxseed-associated increase in trans MUFAs (wt%) from 8.23 ± 0.78% to 5.51 ± 0.78% (P = 0.005) (Negative control: 2.52 ± 0.78%). For production characteristics and metabolic serum indicators, 12BT40 and its unprocessed ingredients did not significantly differ, with the exception that 12BT40 attenuated the flaxseed-associated increase in DMI from 23.3 ± 0.7 to 22.0 ± 0.7 kg/d (P = 0.04) (Negative control: 20.6 ± 0.2 kg/d) and increased milk lactose content from 4.84 ± 0.06% to 4.91 ± 0.06% (P = 0.007) (Negative control: 4.80 ± 0.06%). Thus, we conclude that 12BT40 increases omega-3 content of milk by protecting dietary omega-3 in flaxseed from ruminal biohydrogenation.

Key Words: flaxseed, lipid supplement, omega-3


The objective of this study was to examine the accuracy of urinary creatinine excretion as a predictor for urinary nitrogen (UN) output in dairy cows fed adequate and protein deficient diets. To determine dietary protein requirements and measure efficiency of N use in lactating dairy cows, N balance must be calculated. This requires measurement of N excreted in milk, feces and urine. Performing total urine collection via catheter to measure UN output is labor intensive and puts cows at risk for infection. As an alternative method, creatinine excretion is commonly used as a predictor of daily urine output. However, the accuracy of this method has been questioned, and previous research has not examined creatinine’s accuracy in cows fed adequate vs protein deficient diets. This study used 21 mid-lactation Holstein cows in 2 blocks. For 4 weeks, half the cows consumed protein-adequate diets and the other half consumed protein-deficient diets, then all cows were fitted with urinary catheters connected to total collection cans containing 50% sulfuric acid for 72h. Every 9 h, urine in the can was removed and measured, acid was re-added, and urine samples were taken from the cows and collection cans. Cows were milked 2× daily, and BW was taken 3× in the week before total collection. Urine samples for each cow from the collection can and cow were composited, and creatinine and N content were measured. Daily total creatinine output was estimated as 29 mg/kg BW. Data were analyzed by t-test and correlation. In both experiments, creatinine concentration in samples taken from cows accurately represented samples from cans. However, creatinine-estimated and actual daily urine output values were significantly different (P < 0.05), even though the correlations between them were moderate (0.51 in experiment 1 and 0.94 in experiment 2). When N output was calculated using creatinine-estimated vs actual urine output values, creatinine underestimated actual N output by 17% on average. Using creatinine to estimate urine volume can be used to see relative differences among cows but values are not always quantitative and should be used with caution.

Key Words: creatinine, urinary nitrogen
Global technologies and strategies have been implemented the past 60+ years and greatly enhanced mastitis control, resulting in improved quantities and quality of milk and dairy products. Continued demands for the highest quality, safest quality milk as a foundation for human nutrition is recognized and supported, and abilities to transport and export dairy products with greater shelf life has been a direct effect of this. While public health standards or SCC limits vary among countries globally, measurements assessing mastitis and milk quality such as SCC, milk nutrient composition values, antibiotic testing, and enhanced shelf life are similar among domesticated countries and milk supplies. Yet, many countries around the world with limited milk supplies, capital, and infrastructure can’t implement the simplest mastitis control strategies. The initial very successful 5-point mastitis control plan was developed in the 1960s by NIRD in the UK and then adopted globally. The aim was to reduce levels of subclinical and clinical mastitis primarily through the control of mastitis pathogens. Five points were (1) Record and treat clinical cases; (2) post milking teat disinfection; (3) dry cow therapy; (4) cull chronic cases; and (5) milking machine maintenance. With the control of contagious mastitis pathogens and the advent of environmental mastitis and other pathogens, focus on animal health and immunity and factors affecting this as well as pathogen exposure (nutrition, proper and clean animal environments) received attention and strategies and a 10-point plan. Over these years, our abilities to rapidly monitor milk components and disseminate information for monitoring and making changes rapidly has been astounding. Also, focused antibiotic use increased and so did enhanced continual regulatory testing for B-lactams and other compounds in the 1990s, with also some global concerns of antibiotic resistance. Our focused plans have resulted in an abundant supply of the highest quality, safest dairy products. Advent of genomic testing and large data handling, new therapeutic strategies and alternatives, and recognition and focus on personnel as the brains of genomic testing and large data handling, new therapeutic strategies and alternatives, and recognition and focus on personnel as the brains of milk quality exemplify our future opportunities.

Key Words: mastitis, control, milk quality, somatic cell counts

Genetics, genomics, and improving mastitis resistance. G. M. Pighetti*, University of Tennessee, Knoxville, TN.

Resistance to intramammary infection requires recognition of the invading organism, recruitment and activation of immune defenses, elimination of the organism, and return to homeostasis. The effectiveness of this response reflects a complex series of interactions among the cow’s immune system, her environment, and mastitis causing organisms. As such, the genetic contribution or heritability of mastitis is relatively low when compared with these other factors and genetic progress will be slow. To improve the rate of genetic change, gains must be made relative to reliability, selection intensity, and/or heritability of the trait itself or highly correlated traits. With changes in the industry and technology, we have newfound opportunities to increase the speed and accuracy of genetic gains for mastitis resistance. The keys to this opportunity reflect greater use of producer recorded data to improve reliability of genetic predictions, creation of novel phenotypes from currently recorded data such as SCC to improve reliability, selection intensity and/or heritability, as well as creation of novel measures of the immune system. These changes are coupled with another significant event in the industry, sequencing of the bovine genome, which has enabled the association of individual differences in DNA sequence with traits of interest – e.g., genomics. This has reduced the generation interval and thereby the rate of genetic change by identifying superior sires at younger ages, without the need for daughters to complete their first lactation. Furthermore, a DNA sequence is more “heritable” when compared with a phenotype which is influence by genetic and environmental factors. As we continue to progress, genomic evaluation of current and novel phenotypes will increase the speed and accuracy of selecting for mastitis resistance. Perhaps just as important, genomic markers can provide model systems to better understand the mechanisms that lead to mastitis resistance and enable a more targeted approach to new strategies against mastitis. This symposia will summarize recent advances in relation to historical practices.

Key Words: mastitis, genomic, immunity

Novel genomic and phenotypic strategies to improve mastitis resistance and milk quality. P. Martin1, H. Barkema2, S. G. Narayana1,2, and F. Miglior*1,3, 1CGIL, Dept of Animal Biosciences, University of Guelph, Guelph, ON, Canada, 2Dept of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada, 3Canadian Dairy Network, Guelph, ON, Canada.

Bacterial mastitis (inflammation of the mammary gland) is the most frequent and costly disease of dairy cattle. As disease-recording systems compiling data from large numbers of farms are not widely implemented, selection for mastitis resistance is often based on genetically correlated traits, including somatic cell count (SCC), udder depth and fore udder attachment. However, in Canada, a national health collection system for dairy cattle was started in 2007, with producers recording the incidence of 8 diseases, including mastitis. Participation is voluntary, but already includes >40% of all Canadian herds enrolled in milk recording. In several studies using these Canadian data, various traits with potential to predict mastitis resistance were investigated. The relationship between mastitis and SCC (and its variations during lactation) were also studied, with the most important being mean somatic cell score (SCS) in early lactation, standard deviation of SCS and excessive test-day SCC pattern. Genetic correlations between mastitis and other traits were also estimated. Selection for mastitis resistance would also improve resistance against other diseases, as well as enhance both fertility and longevity. However, milk yield was negatively correlated with clinical mastitis, emphasizing the importance of including health traits in genetic selection. Based on these studies, routine genomic evaluation for mastitis resistance was initiated in Canada (August 2014). The new evaluation incorporated mastitis, patterns of SCC, udder depth, fore udder attachment and body condition score. By including predictor traits in multiple-trait evaluations, EBV reliability increased 22 points. Furthermore, with genomics, reliability increased by 16 and 8 points for young and proven bulls, respectively. Further research, including the bacteriology of mastitis and development of a new female reference
population to improve reliability of genomic evaluation, are still under study and should result in further improvements.

**Key Words:** mastitis, genetics, genomics

177 Genome-wide association analyses identify loci associated with mastitis phenotypes generated from *Streptococcus uberis* experimental challenge data. L. Siebert*, M. E. Staton, S. P. Oliver, and G. M. Pighetti, University of Tennessee, Knoxville, TN.

Control and prevention of mastitis is a long standing goal of the dairy industry considering it affects 20 to 50% of any given herd and significantly decreases milk quality. Recent identification of genetic markers for mastitis have relied on somatic cell count (SCC) or clinical mastitis (CM) observation or averaged across a sire’s daughters. We propose utilizing novel phenotypes derived following an intramammary experimental challenge with *S. uberis*, which causes a high proportion of both clinical and subclinical mastitis cases. Aseptic milk samples were collected on each cow, n = 35 Holsteins, and used to determine both SCC and colony forming units (cfu) of *S. uberis* in milk. SCC was used to generate 3 novel phenotypes: area under the curve (AUC) of SCC for 0–7 d and 0–28 d post-challenge; and individual cows were placed in one of 3 categories (<21 d, 21–28 d, or > 28 d) based on when their SCC returned to below 200,000 cells/ml post-challenge. *S. uberis* cfu was used to create 4 additional phenotypes: *S. uberis* clearance *S. uberis* shedding cycles, and AUC of *S. uberis* cfu for 0–7 d and 0–28 d post-challenge. To identify loci of interest a 50K SNP chip analysis was performed using the BovineSNP50 v2 DNA Analysis BeadChip from Illumina and associations were tested using Plink. A total of 40 SNPs (P < 9.34 × 10−5) were identified across the 7 phenotypes. Of the SNPs identified, 12 are in regions with prior mastitis evidence lending validity to the use of our novel phenotypes to identify loci of interest. Furthermore, 14 of the SNPs identified are in genes with known functions linked to inflammation/immunity or regulation of gene expression, providing many potential candidate genes. These loci and candidate genes should be further investigated to identify potential roles in controlling *S. uberis* mastitis. Such investigations could lead to novel treatment or prevention compounds/protocols for *S. uberis* mastitis or genetic selection methods for cows with greater potential to resist *S. uberis* infection.

**Key Words:** mastitis, GWAS, phenotype

178 Mastitis therapy: Past successes, current challenges, and vision for the future. J. Middleton*, University of Missouri, Columbia, MO.

Mastitis is an inflammation of the mammary gland most often caused by a bacterial intramammary infection. Antimicrobials are often used for mastitis treatment and control, and dairy cattle receive more antimicrobial therapy for mastitis than for all other dairy cattle diseases combined. Mastitis can be categorized based on the severity of the inflammatory response as subclinical, having no overt clinical signs, or clinical, having overt changes in the milk, mammary gland, and/or cow. Clinical mastitis is divided into mild (overt changes in the milk), moderate (overt changes in the milk and udder inflammation such as redness, heat, pain, and swelling), or severe (overt changes in the milk, mammary gland, and systemic signs in the cow). Treatment decisions will not only be dictated by the severity of the disease, but also by the inciting agent and the predicted outcome of therapy based on empirical or scientific evidence. A common strategy is to try to clear the bacterial invaders with antibiotics in the hope of returning the mammary gland to normal function, but this strategy may not always be warranted or effective and thus understanding basic principles of therapy are critical. Challenges faced by the dairy industry include public concerns about cattle welfare and antimicrobial drug use and resistance. These concerns are driving policy aimed at reducing or eliminating certain types of antimicrobial use in food-producing animals because of real or perceived concerns about resistance in pathogens of human health importance. It is clear that antimicrobial use in food-producing animals is an evolving situation and one that likely will be more restrictive in the future. Prudent use of antimicrobial drugs, i.e., only applying antimicrobials where there are clearly demonstrated production and animal welfare benefits is a logical step in potentially reducing antimicrobial use on farms while preserving the ability to treat disease and optimize animal well-being. The future of mastitis treatment lies in optimizing management practices that promote animal health and reduce disease incidence while developing novel intervention strategies aimed at reducing antimicrobial usage.

**Key Words:** mastitis, therapy

179 Modulating adipose tissue lipolysis and remodeling to improve immune function in early lactation. G. A. Contreras*, C. Strieder Barboza, and J. De Koster, Department of Large Animal Clinical Sciences, East Lansing, MI.

Despite major advances in our understanding of transition and early lactation cow physiology and the use of advanced dietary, medical, and management tools, at least half of early lactation cows are reported to develop disease and 57% of cow deaths occur during the first week of lactation. Excessive lipolysis (measured as plasma FFA) is a major risk factor for the development of displaced abomasum, ketosis, fatty liver, and metritis, and may also lead to poor lactation performance. Lipolysis triggers adipose tissue (AT) remodeling that is characterized by enhanced humoral and cell mediated inflammatory responses, and changes in its cellular populations distribution and extracellular matrix composition. Uncontrolled AT inflammation perpetuates lipolysis as we have observed in cows with displaced abomasum. Efficient transition cow management ensures a moderate rate of lipolysis that is rapidly reduced as lactation progresses. Adequate formulation of pre- and postpartum diet reduces AT lipolysis intensity. Additionally, supplementation with niacin, monensin, and rumen-protected methyl donors (choline and methionine) during the transition period are reported to minimize FFA release into systemic circulation. Feeding saturated FA and other energy dense compounds during early lactation improves energy balance and increases insulin concentration that limit AT lipolytic responses. Independently of the type of intervention used to modulate lipolysis around parturition and early lactation, its benefits on immune function are well documented. In general, cows with moderated postpartum lipolysis exhibit improved immune response. For example, these animals have effective PMN phagocytic activity and adequate proliferation of mitogen stimulated PBMC compared with cows with high lipolysis. To further improve the modulation of AT lipolysis and remodeling, it is necessary to identify novel biomarkers of AT function as prepartum plasma FFA and BHB lack specificity and are retrospective in nature. Understanding the inherent characteristics of AT biology in transition and early lactation cows will reduce disease incidence and improve lactation performance.

**Key Words:** adipose tissue remodeling, lipolysis, early lactation

The bovine innate immune system has a strong repertoire of antimicrobial defenses to rapidly attack infectious pathogens that evade physical barriers of the udder. Exploration of the intracrine vitamin D pathway of bovine macrophages has generated a better understanding of the signals that initiate antimicrobial defenses that protect the udder. In the intracrine vitamin D pathway, pathogen recognition receptors upregulate CYP27B1 mRNA that encodes for the enzyme that converts 25-hydroxyvitamin D (25D) to the active vitamin D hormone, 1,25-dihydroxyvitamin D$_3$ (1,25D). The 1,25D, in turn, increases nitric oxide and b-defensin antimicrobial responses of bovine macrophages. Investigation of the intracrine vitamin D pathway using experimental models of mastitis of dairy cows has revealed that vitamin D signaling in innate immune cells contributes to defense of the mammary gland. Expression of the CYP27B1 and the vitamin D receptor genes rapidly increased in macrophages and neutrophils of the udder in experimental models of mastitis in dairy cows. Intramammary 1,25D treatment increased expression of inducible nitric oxide synthase and b-defensin genes in immune cells of the mammary gland. Furthermore, Intramammary 25D treatment slowed onset and decreased severity of experimental Streptococcus uberis mastitis. The evidence that vitamin D contributes to defense of the mammary gland provides a path for development for alternative solutions (i.e., nutritional, genetic, therapeutic) to increase mastitis resistance of dairy cows. Continued exploration of the intrinsic cellular pathways that specifically promote antimicrobial defenses of the udder, as demonstrated with the vitamin D pathway, is needed to support mastitis control efforts for dairy cows.

**Key Words:** vitamin D, innate immunity, mastitis

### 181 Challenges to milking efficiency: Quality versus quantity.
R. Erskine* and R. Moore-Foster, Michigan State University, East Lansing, MI.

The dairy industry is rapidly intensifying and becoming more diverse in terms of employment organization. Many dairy managers have limited human resource knowledge and experience; this often leads to frustration with protocol drift and a sense that employees are not motivated to engage in the success of the farm beyond prescribed instructions. Additionally, the role of immigrant labor can complicate communication barriers and management–employee relationships. Education, training, and translation tools have been developed by land grant universities, consultants and agricultural agencies. However, these programs were developed from a management-directed perspective with minimal input from employees. Furthermore, the effectiveness of employee training, or education programs, relative to farm protocols and productivity, has not been evaluated for short or long-term success. Dairy herds that ensure strict compliance of milking protocols have lower bulk tank somatic cell counts (BTSCC) than herds that have difficulties with protocol compliance. Thus, further advances in milk quality may be impeded by the gap between the human resource needs arising within the industry’s labor force and the capacity of producers and managers to address them. Superimposed on these labor issues, many dairy operations increase the primary output, fluid milk, is difficult to differentiate, increasing the need for alternative means of business differentiation. In “Competing on Analytics: The New Science of Winning,” Davenport and Harris (2007) pose that in industries with similar technologies and products, “high performance business processes” are one of the only ways that businesses can differentiate themselves. The basis for most of our mastitis decision tools thus far has been DHIA (Dairy Herd Information Association). DHIA records are an essential part of dairy herd management for many progressive dairy operations. Given the economic importance of both clinical and subclinical mastitis, early detection of mastitis is one of the most exciting precision dairy farming applications. Real-time data can be used for monitoring animals and creating exception reports to identify meaningful deviations. In many cases, dairy management and control activities can be automated. It’s important to remember that information obtained from precision dairy farming technologies is only useful if it is interpreted and utilized effectively in decision making. Integrated, computerized information systems are essential for interpreting the mass quantities of data obtained from Precision Dairy Farming technologies. This information may be incorporated into decision support systems designed to facilitate decision making for issues that require compilation of multiple sources of data. New technologies that measure SCC, LDH, conductivity, temperature, and behavior open up opportunities for additional data streams. Economic based decision tools may help farmers make more economically driven treatment and culling decisions.

**Key Words:** data, mastitis

### 182 Data, decisions, and mastitis.
J. M. Bewley*, University of Kentucky, Lexington, KY.

Adoption of sophisticated on-farm decision-making tools has been scant in the dairy industry to this point. Yet, the dairy industry remains a perfect application of decision science because (1) it is characterized by considerable price, weather, and biological variation and uncertainty, (2) technologies, such as those characteristic of precision dairy farming, designed to collect data for decision making abound, and (3) the primary output, fluid milk, is difficult to differentiate, increasing the need for alternative means of business differentiation. In “Competing on Analytics: The New Science of Winning,” Davenport and Harris (2007) pose that in industries with similar technologies and products, “high performance business processes” are one of the only ways that businesses can differentiate themselves. The basis for most of our mastitis decision tools thus far has been DHIA (Dairy Herd Information Association). DHIA records are an essential part of dairy herd management for many progressive dairy operations. Given the economic importance of both clinical and subclinical mastitis, early detection of mastitis is one of the most exciting precision dairy farming applications. Real-time data can be used for monitoring animals and creating exception reports to identify meaningful deviations. In many cases, dairy management and control activities can be automated. It’s important to remember that information obtained from precision dairy farming technologies is only useful if it is interpreted and utilized effectively in decision making. Integrated, computerized information systems are essential for interpreting the mass quantities of data obtained from Precision Dairy Farming technologies. This information may be incorporated into decision support systems designed to facilitate decision making for issues that require compilation of multiple sources of data. New technologies that measure SCC, LDH, conductivity, temperature, and behavior open up opportunities for additional data streams. Economic based decision tools may help farmers make more economically driven treatment and culling decisions.
Individual cow SCC) and a variety of independent variables that included employee factors such as, number of hours spent milking per day, total distance each employee moves per milking shift, employee turnover and cows milked per employee per hour. Negative correlations were found between the percent of cows with bimodal milking and the average teat stimulation and number of passes during the preparatory procedure. These results suggest that VaDia analysis of milking procedures can help identify opportunities to improve milking performance and that factors deemed to increase parlor throughput pressure on employees may result in decreased milking performance.

Key Words: mastitis, employees, education
Unmanned aerial vehicles (UAV, drones) have been used to observe beef cattle; flying below 33 m (100 ft) caused animal flight. Objectives were to learn to fly UAVs and evaluate UAV disease (Dz) monitoring and dairy cattle response. Current Dz detection and recording methods for cows and calves were studied. Experts in piloting and operating UAV assisted in model selection and hands on flight training; 2 UAVs were purchased, and flying, still and video photography, and data handling skills were gained. A questionnaire about Dz recording was developed for key farm personnel, and live color and thermal images and videos were captured by UAV on 4 dairy farms with large housing areas. Animal acclimation was achieved by first flying at 33 m, and then reducing flight altitude in 8 m increments; final tolerated height was approximately 4.3 m (14 ft). Animals in high-traffic areas adapted to UAV sooner than those in low-traffic areas, but all required <15 min. Animal ID was clear. Thermal imaging recorded cows’ skin temperature within 0.1°C, but black vs. white hide color, sun and shade affected the observed temperature by approximately 17°C. Cows with metritis or in estrus were detected by thermal imaging, but clinical mastitis was not readily detected by UAV. All farms used >1 method to record Dz. Cow records: notebook to computer 50% of farms, computer only 25%, not readily detected by UA V. All farms used >1 method to record Dz. Treatments: Cows: mastitis, metabolic, reproductive and GI Dz were recorded on all farms; cell phone 50%, text 25%. Calf records: white board 75%, cell phone 50%, text 25%. Calf records: white board 75%, cell phone 50%, text 25%. Calf records: white board 75%, chart 50%, hutch cards 50%, notebook 25%, kids tell 25%. Cows’ mastitis, metabolic, reproductive and GI Dz were recorded on all farms, keratitis 75%, twisted cecum 25%. Calves’ diarrhea and respiratory Dz were recorded on 75%, diarrhea severity and appearance 50%, respiratory severity 50%, Treatment (Tx) recording: Cows: mastitis, metabolic, reproductive Txs and surgery were recorded on all farms. Calves: diarrhea, respiratory, follow up Txs all were recorded on 75%, none 25%. Users of Dz records: herdsperson and calf feeders 100%, veterinarian 75%, owner 75%, head milker 50%. Three producers owned UAV, and all were interested in UAV for possible animal and facility monitoring including thermal imaging, an area needing further research.

Key Words: dairy cattle, drones

Calving is a crucial event in a productive cows’ life cycle and has significant influence on herd profitability and cow’s welfare. Calving detection is a key factor to ensure successful calving with minimal harm to the calf and the cow. It is used to decide if intervention is needed, when to move a cow to a maternity pen and to obtain a propercolostrum administration soon afterward. An automatic monitoring system to detect the onset of parturition could contribute to reduce calves morbidity and mortality and ensure better performance in the consequent lactation. The objective of this study was to test a real-time, automatic cow monitoring system for detecting calving in dairy cows based on rest and activity behaviors. The study was conducted on 4 Israeli dairy herds, between August 10 and October 22, 2015. Herds ranging from 356 to 1,012 Israeli Holstein milking cows. Cows were fitted with 2 tags (AfTag II, Afimilk, Israel) on front and rear legs, when moved to the close-up pen. Calving times were recorded by the herds’ teams. Calving alerts generated by the system (Af/Act II, Afimilk, Israel) were compared with the actual calving time. In total 231 and 187 successful calving detection alerts were recorded for cows fitted with tags on rear and front leg, respectively (not all the cows were fitted with tags on the front leg). Detection timing before calving were similar for front and rear legs. The distribution was about 35.5%, 28%, 26.5%, 8% and 2% for the last 1 h, 1–2 h, 2–4 h, 4–8 h and more than 8 h before calving, respectively. In all 4 herds, 50% and more of the alerts were provided in the 2 h preceding calving for both legs (range 50%–79%) and more than 80% of the alerts were in the last 4 h before calving (range 81.9–94.8%). The average time from detection to calving was about 2 h for both front and rear legs (range 01:18–02:38 h). These results suggest that a real-time automatic monitoring system based on cows’ rest and activity behavior can be a useful tool for detecting calving events in dairy cows. The use of such a system can help improve calving management and human interventions.

Key Words: calving detection, rest time, real-time system

The aim of the present longitudinal field study was to compare the efficiency of 2 analytical approaches for the sanitation of *Staphylococcus aureus* genotype B (GTB)-positive dairy herds. *S. aureus* is one of the most widespread mastitis pathogens worldwide. Typically, it causes subclinical, chronic mastitis leading to reduced quality and production of milk, and to substantial economic loss in the dairy industry. In Switzerland, different genotypes of *S. aureus* have been identified, whereby the genotype B was demonstrated to be the only contagious subtype, causing herd problems. Furthermore, this pathogen can cause food poisoning because of enterotoxin production. As the efficacy of antibiotic therapy and vaccination against *S. aureus* is not satisfactory, the most promising strategy for controlling this udder pathogen is the implementation of specific sanitation programs. In the present study, a new qPCR assay (very sensitive and specific for *S. aureus* GTB) was evaluated in the field for the sanitation of GTB-positive herds and compared with classical bacteriology. Both analytical methods were demonstrated to be effective, although the qPCR approach showed some key advantages, which enable the sanitation of entire herds in short time. The use of clean, instead of aseptically collected, milk samples facilitates sample collection in terms of time and cost, enabling the sampling of even big herds during a normal milking time. Because of the high sensitivity of qPCR, the rate of false-negative results is minimal, so that each GTB-positive cow can be correctly identified at any time point during lactation. The conclusive identification of GTB-positive cows can be accomplished within 2 d after sampling: This allows farmers to immediately build milking groups and to maintain the correct milking order. Milk sample analysis becomes easier, faster, more objective, and suitable for routine application. Additionally, all steps of the analytical procedure are suitable for automation, from sample preparation to the final qPCR reaction. This allows for the first time the implementation of sanitation programs at a broader, regional level, instead of being limited to the herd level.

Key Words: mastitis, *Staphylococcus aureus*, sanitation

Effects of dexamethasone and opsonized *Mycoplasma bovis* on bovine neutrophil function in vitro. H. A. Alabdullah*,1, L. K. Fox1, J. M. Gay1, G. M. Barrington1, and R. H. Mealey2,
The objective was to determine if in vitro glucocorticoids treatment of bovine neutrophils would impair their function to phagocytize and kill opsonized M. bovis. We hypothesized that in vitro treatment of bovine neutrophils by glucocorticoids impairs phagocytosis of opsonized M. bovis compared with non-treated neutrophils and such impairment would be a function of M. bovis strain differences. Neutrophils isolated from 20 mid-lactation cows were treated with $5 \times 10^{-4}$ M dexamethasone (TX) or non-treated (CX). After treatment neutrophil function included: percentage reduction in $\log_{10}$ of M. bovis cfu/mL, percentage of phagocytizing neutrophils, phagocytized M. bovis per neutrophil, and killed M. bovis per neutrophil were quantified by incubating one of TX (NDM1–4) or CX (NM1–4) neutrophils group with one of 4 opsonized M. bovis strains. Least squares means of all neutrophil groups were contrasted using linear mixed-effects models. Overall means $\pm$ SEM for the dependent variables of percentage reduction in $\log_{10}$ of M. bovis cfu/mL, percentage phagocytizing neutrophils, phagocytized M. bovis per neutrophil, and killed M. bovis per neutrophil were: $17 \pm 1.19, 69 \pm 1.48, 8.3 \pm 1.23$, and $1.57 \pm 0.67$ for control neutrophils and $14 \pm 1.3, 34 \pm 1.4, 2.6 \pm 0.82$, and $0.59 \pm 0.53$ for neutrophils treated with dexamethasone respectively. Effects due to strain, treatment, and their interaction on neutrophil function measured by the number of phagocytized M. bovis per neutrophil and number of killed M. bovis per neutrophil were different ($P < 0.05$). However, there was no strain by treatment interaction effect on percentage reduction in $\log_{10}$ of M. bovis cfu/mL or strain and treatment by interaction effects associated with the dependent variable of percentage phagocytizing neutrophils. Dexamethasone consistently decreased all neutrophil function tested ($P < 0.0001$). These findings might explain in part the association of stressful events with subsequent outbreaks of Mycoplasma bovis associated bovine diseases.

**Key Words:** dexamethasone, Mycoplasma bovis, neutrophil function

189 Pegbovigrastim affected gene expression in neutrophils of transition cows indicating increased neutrophil function. A. Heiser1, S. LeBlanc*2, and S. McDougall1, 1AgResearch, Palmerston North, New Zealand, 2Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, 3Cognosco, AnexaFVC, Morrinsville, New Zealand.

Treatment of transition cows with granulocyte colony stimulating factor (G-CSF) has been shown to increase neutrophil count and function. It was hypothesized that prepartum under-nutrition may reduce the effect of a commercial recombinant bovine G-CSF product (pegbovigrastim; IMR). Hence this study was undertaken to test the effect of under-nutrition and IMR treatment on gene expression in neutrophils. Pasture-fed cows (n = 99) in New Zealand were blocked by calving date and BCS and randomly assigned in a $2 \times 2$ factorial design to be fed prepartum to exceed energy requirements or restricted to 85% of energy requirements. Half of the animals in each group were injected with IMR or saline at approximately 7 d before expected calving and again on the day of calving. Blood samples were collected 7 d pre-calving (D-7) and samples from blood, uterus and milk were on D4 and D7 after calving. Gene expression analysis was performed for 21 genes using Nanostring. Effects of time and IMR treatment were observed but feeding did not affect gene expression. On average, cows showed higher expression of almost all selected genes at D4 compared with D-7 including genes for migration and inflammation markers (L-selectin, ICAM-1 and TLR 2 and 4; $P < 0.05$) indicating an ongoing neutrophil response to the hormonal and metabolic stresses of the parturition and postpartum infections. IMR treatment enhanced the effect by further increasing expression of ICAM1 and TLR2 ($P < 0.05$) suggesting increased neutrophil efficiency. In uterine fluid and to a lesser degree in milk IMR lowered expression of migration markers and increased expression of genes for other neutrophil functions, including myeloperoxidase, FAS, and caspases 2 and 9 ($P < 0.05$) potentially increasing neutrophil effectiveness. IMR treatment resulted in significant increases in the expression of genes involved in inflammation, phagocytosis, respiratory burst, degranulation, and apoptosis/survival of neutrophils in blood, uterine fluid and milk, and also migration of blood neutrophils.

**Key Words:** transition cow, neutrophil, pegbovigrastim

188 Changes in galectin gene expression in bovine blood during the periparturient period. E. Asiamah*1, S. Adjei-Fremah1, K. Ekwemalor1, M. Worku1, L. Sordillo2, and J. Gandy2, 1North Carolina A&T State University, Greensboro, NC, 2Michigan State University, East Lansing, MI.

Galectins (Gal) are a family of proteins that bind to β-galactoside sugars found on parts of other proteins, either on the cells’ surface or in the extracellular matrix. There are 15 galectin protein subtypes that all share the characteristic of carbohydrate recognition. Galectins are also involved in adhesion, migration and inflammation markers (L-selectin, ICAM-1 and TLR 2 and 4; $P < 0.05$) indicating an ongoing neutrophil response to the hormonal and metabolic stresses of the parturition and postpartum infections. IMR treatment enhanced the effect by further increasing expression of ICAM1 and TLR2 ($P < 0.05$) suggesting increased neutrophil efficiency. In uterine fluid and to a lesser degree in milk IMR lowered expression of migration markers and increased expression of genes for other neutrophil functions, including myeloperoxidase, FAS, and caspases 2 and 9 ($P < 0.05$) potentially increasing neutrophil effectiveness. IMR treatment resulted in significant increases in the expression of genes involved in inflammation, phagocytosis, respiratory burst, degranulation, and apoptosis/survival of neutrophils in blood, uterine fluid and milk, and also migration of blood neutrophils.

**Key Words:** transition cow, neutrophil, pegbovigrastim
was undertaken to test the effect of undernutrition for one month before calving on the response to IMR. Cows (n = 99) on pasture in a research herd in New Zealand were blocked by expected calving date and BCS and randomly assigned in a 2 by 2 factorial design to be fed to exceed energy requirements prepartum (FULL), or restricted to approximately 85% of prepartum energy requirements (RES). At approximately 7 d before expected calving and on the day of calving, half the cows in each feed group were injected with IMR while the remaining half were injected with saline. Blood samples were collected pre-and post-calving for complete blood count, biochemistry and in vitro assessment of PMN function including phagocytosis, myeloperoxidase (MPO) release and oxidative burst. Energy restriction prepartum resulted in lower body weight (96.1 ± 0.4% vs 101.0 ± % of initial body weight for RES vs FULL cows at calving; P < 0.001), and a higher proportion of cows with elevated concentrations (i.e., > 0.4 mmol/L) of fatty acids (35/41 (85.4%) vs 23/41 (56.1%) elevated for RES vs FULL cows at 7 d before calving; P < 0.001). Treatment with IMR increased PMN count (9.8 ± 0.2 vs 3.9 ± 0.2 × 10^3/mL; P < 0.001). There was a time x IMR interaction (P < 0.001) for proportional release of MPO by PMN, with higher release at 4 d post-calving in IMR cows (0.80 (95%CI = 0.72–0.88) vs 0.59 (95%CI = 0.53–0.64)). There was no effect of prepartum energy restriction, nor energy restriction x IMR interactions for any of the white cell counts or functional tests. It is concluded that IMR treatment resulted in significant increases in PMN count, and enhances PMN function as indicated by increased MPO release. The response to IMR was not affected by restricted pre-partum energy intake.

Key Words: pegbolavigastim, energy balance, neutrophil function

191 Epidemiology of bovine respiratory disease in pre-weaned dairy calves in California. S. A. Dubrovsky*1, A. L. Van Eenennaam1, B. M. Karle2, T. W. Lehenaus3,4, and S. S. Aly3,4, 1Department of Animal Science University of California Davis, Davis, CA, 2University of California Cooperative Extension, Orland, CA, 3Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA, 4Department of Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis, Tulare, CA.

Bovine respiratory disease (BRD) is one of the leading causes of pre-weaning death in dairy heifers. The objective of this prospective cohort study was to characterize the epidemiology of BRD in preweaned dairy calves and to identify management practices that reduce the incidence of BRD. Calves were chosen for the study based on management practices, calf records, location, and size. A total of 6 calves, ranging in size from 700 to 3,200 milking cows, in 6 counties across California’s Central Valley, were enrolled for at least one year. A total of 11,945 calves were born on the study dairies and followed from birth to weaning. Incidence of BRD was estimated using treatment records. A comprehensive calf management survey and prevalence estimate was performed by trained study personnel once every season. A shared frailty model was used to explore the associations between management practices and BRD. A total of 11,470 calves with complete records were included in the final models. The overall BRD prevalence across the study herds was 22.84% (95% confidence interval, CI = 0.22–0.24). The mean incidence density rate (IDR) for all calves was 0.17 cases (95% CI = 0.16–1.74) per calf-month at risk. The shared frailty model found that feeding only waste or saleable milk (compared with use of milk replacer), feeding over 3.8 L of milk per day to calves under 21 d of age, frequent changing of maternity pen bedding, and administration of both modified live or killed BRD vaccines to dams before calving significantly reduced the risk of BRD. Risk factors for developing BRD included if the calf was a twin and if calf managers held the opinion that dust was perceived to occur “regularly” compared with no dust in the calf raising area. All 4 seasons were analyzed and it was found that both summer (HR = 1.15 (95% CI = 1.01–1.32)) and spring (HR = 1.26 (95% CI = 1.11–1.44)) were associated with a higher risk of BRD as compared with winter. Our study found that specific housing and feeding practices could be modified to help decrease the risk of BRD and a strong effect for season on a calf’s risk of developing BRD.

Key Words: bovine respiratory disease (BRD), dairy calves, pneumonia

192 The effect of lung consolidation, as determined by ultrasonography, on first lactation milk production in Holstein dairy calves. T. R. Dunn*s1, T. L. Ollivett2, D. L. Renaud3, and D. F. Kelton1, 1Department of Population Medicine, University of Guelph, Guelph, ON, Canada, 2Department of Medical Sciences, University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI.

Bovine respiratory disease (BRD) is a complex disease process and early diagnosis can be difficult because of inconsistent or absent clinical signs. Many reports emphasize the negative implications of clinical BRD. Diagnosing subclinical respiratory disease through thoracic ultrasonography has the potential to improve animal welfare and productivity. The objective of this cohort study was to determine if lung consolidation in young dairy calves was associated with a decline in first lactation milk production. A total of 215 female calves from 3 dairy herds in Southwestern Ontario were enrolled and assessed weekly during their first 8 weeks of life for evidence of lung consolidation (CON) through the use of thoracic ultrasonography (US) (Ibex Pro, Loveland, CO). Consolidation was measured within the first 10 intercostal spaces on both sides of the thorax, using gridlines on the screen of the US. Calves were considered CON positive if 3cm or more of consolidated lung was present. A multivariable linear regression model was used to identify significant risk factors associated with first lactation 305 milk production. The study population, the following calfoth conditions were detected: twins (4%; n = 8), diarrhea in the first 21 d of life (31%; n = 66), rib fractures (7%; n = 14), lung abscesses (3%; n = 6), and at least one diagnosis of CON (57%; n = 123). Overall, 7% (n = 15) of calves died, and 18% (n = 38) of animals were sold before the end of first lactation. For every one-month increase in age at first calving, milk production in first lactation increased by nearly 140 kg (P = 0.01). The presence of CON, at least once in the first 8 weeks of life, was associated with a 525kg decrease in first lactation 305 milk production (P = 0.027). These results indicate that lung consolidation during the first 56 d of life may have a possible long-term impact on dairy calves, manifested as reduced milk production during first lactation.

Key Words: lung consolidation, ultrasound

193 Associations between respiratory disease type and average daily gain in preweaned group-housed dairy calves. M. C. Cramer*s1 and T. L. Ollivett2, 1University of Wisconsin-Madison, Department of Dairy Science, Madison, WI, 2University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI.

The study objective was to determine associations between average daily gain (ADG) in dairy calves and 6 forms of respiratory disease (RESP), defined by combinations of clinical and thoracic ultrasound scores. Preweaned dairy calves (n = 280) on a commercial herd in Ohio, USA were enrolled at entry to an automated milk feeder barn and housed in groups of 13 ± 3 (mean ± stdev). Calves were 21 ± 6 d old at enrollment
and were followed for 6 weeks. Twice weekly health exams included a clinical respiratory score (CRS), thoracic ultrasound score (USS), fecal score, and body weight. For the CRS, the nose, eyes, ears, cough, and rectal temperature were assigned a score (0–3) and was considered positive when at least 2 areas scored ≥2. The USS ranged from 0 (normal) to 5 (abnormal) based on the degree of lung consolidation. The CRS and USS were combined to create 6 RESP types: clinical lobular pneumonia (CLL; USS = 2, CRS+; n = 10), clinical lobar pneumonia (CL; USS ≥ 3, CRS+; n = 31), subclinical lobular pneumonia (SLL; USS = 2, CRS-; n = 82), subclinical lobar pneumonia (SCL; USS ≥ 3, CRS-; n = 88), upper respiratory tract disease (URT; USS < 2, CRS+; 40), and normal (USS < 2, CRS-; n = 29). A multivariable linear regression model was fit to determine if ADG was associated with RESP after controlling for sex, breed, and scour. A significant interaction existed between RESP and scours (P = 0.01). For calves without scours, ADG was significantly lower for CL (0.46kg) compared with SCL (0.69kg, P = 0.03), SLL (0.72kg, P = 0.008), and URT (0.79kg, P = 0.002); there was no difference in ADG among CL, CLL, or normal calves (P > 0.41). For scurrying calves, there was a tendency for lower ADG in URT calves (0.44kg) compared with SCL calves (0.70kg, P = 0.06); ADG did not differ among CL, CLL, SLL, URT, or normal calves (P > 0.95). This research is the first step in understanding the impacts of types of RESP, identified with CRS and USS, on calf performance. Findings suggest that calves with clinical lobar pneumonia are most severely affected. Further research is needed to understand if these calves should be managed differently than calves with other types of respiratory disease.

Key Words: bovine respiratory disease (BRD), calf, ultrasound

194 Time lost to disease in dairy cattle: Associations between two consecutive lactations. P. Bacigalupo-Sanguesa*, C. McConnel2, F. Garry1, J. Lombard3, and P. Pinedo4, 1Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO. 2Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA. 3USDA-APHIS-VS-Center for Epidemiology and Animal Health, Fort Collins, CO. 4Department of Animal Sciences, College of Agricultural Sciences, Colorado State University, Fort Collins, CO.

The objective of this study was to evaluate the association between days lost due to specific diseases and total days lost due to disease in 2 consecutive lactations using a new measure called the disease-adjusted lactation (DALact). The DALact is a health measure that incorporates morbidity and removal (death or culling) into a single time-based summary measure and it represents a new approach for assessing the impact of diseases in a lactation. Health and removal data were obtained from a Colorado dairy with approximately 1,400 lactating cows. Multiparous cows (n = 805) that calved, were sold, or died from July 1, 2015, through June 30, 2016, were selected. Individual cow health data were collected from Dairy Comp 305 for the recently completed lactation and from the previous lactation. Health events included calving injury, displaced abomasum, diarrhea, hypocalcemia, ketosis, lameness, mastitis, metritis, musculoskeletal injuries, pneumonia, and retained placenta. All cow-level data were imported into SAS for validation, calculation of DALact, and analysis. The DALact was calculated adding the Days Lost due to Premature Death or Culling (DLRD) and the Days Lost due to Illness (DLI). DLRD was calculated as the difference between the average completed lactation days in milk for the herd and the days in milk at culling or death for individual cows. The DLI was the product of the number of cases of each disease multiplied by established disability weights and estimated disease durations (days) for a specific disease. The association between DALacts in 2 consecutive lactations was evaluated using PROC GLM. P-values < 0.05 were considered significant. Positive significant associations (P < 0.001) were found between DALacts in 2 consecutive lactations for lameness and mastitis. Similarly, the total DALacts in 2 consecutive lactations were also significantly associated (P < 0.001). Identification of diseases and reasons for removal that significantly affect time lost during 2 consecutive lactations will help producers focus management and preventive measures on diseases having the greatest impact on future productivity and wellbeing.

Key Words: dairy cattle, disease, DALact

195 Metagenomic analysis of fecal microbiomes in cattle infected with Mycobacterium avium ssp. paratuberculosis. N. Indugua*, D. Pitta, B. Bhukya, B. Vecchiarelli, M.-E. Fecteau, and R. Sweeney, University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, PA.

Johne’s disease (JD) is a chronic gastrointestinal infection of cattle caused by Mycobacterium avium ssp. paratuberculosis (MAP). We hypothesized that cattle naturally infected with MAP acquire gastrointestinal dysbiosis which may play a role in the pathogenesis of JD. To this end, we compared the fecal microbiomes of 20 naturally infected fecal samples (positive group), 10 JD-negative herd mates (exposed group) and 10 JD-negative cows from a MAP-free herd (negative group). Metagenomic DNA libraries were constructed and sequenced on Ion Torrent platform and assembled using NextGENe (V2) program. Phylogenetic assignments and functional annotations of assembled contigs were performed with RefSeq and COGs database respectively using MG-RAST Server. Bray-Curtis dissimilarity distance based analysis showed significant differences (P < 0.05; PERMANOVA) between positive, exposed and negative groups. Taxonomic annotations revealed the abundance of bacteria at up to 85%. Although the same phylotypes were commonly present among all 3 groups, their relative abundance varied (P < 0.05; Wilcoxon), particularly in the positive group. Notably, Actinobacteria was highly abundant (30% of the total bacteria) in the positive group, whereas it constituted less than 1% in the other groups. Further, only a small proportion of sequences (100 sequences; 0.002%) were detected as MAP sequences in the positive group, but were not detected in the other 2 groups. Functional annotations showed the abundance of metabolism pathways at up to 25% of gene content. Among metabolic pathways, gene sequences associated with energy production, amino acid metabolism, lipid metabolism, mineral metabolism and secondary metabolites biosynthesis were significantly higher (P < 0.05; Wilcoxon) in the MAP positive group compared with the other 2 groups. While elevated lipid pathways in the MAP positive group denotes that MAP relies heavily on lipid-based substrates such as cholesterol for its growth. An increase in other metabolic pathways probably denotes adaptation mechanisms of MAP in the host.

Key Words: fecal-microbiome, Johne’s disease, MAP
In this presentation, I draw upon my own work and experiences as Dairy Public Affairs lead for Monsanto and Elanco as well as peer-reviewed research published by others in working in this area. I will also incorporate survey work done by Deloitte, Nielsen, Center for Food Integrity and others to demonstrate the public’s values as they relate to dairy management practices. Management practices on the dairy farm have traditionally been done in a manner consistent with the owner's desires and the “standard of practice” of the day. Consumers were primarily interested in the price and taste of food, and accepted that food was safe. In the past many consumers had a connection to the farm through a relative or neighbor. They trusted the person who was the caretaker on the dairy or farm and accepted that they managed the animals appropriately. In many cases they had been on that farm. That relationship led to a level of trust which is not present between most consumers and producers today. Today, many consumers do not have a direct connection to the farm. These consumers are subject to asymmetrical information with regard to food production. That is to say, they cannot see it, feel it, and touch it. This creates a need for them to get their information from other sources. Couple this with the fact that many consumers are interested in value drivers beyond price taste and convenience. They are interested in multiple areas of the food production process. They hold all participants in the food production chain responsible for everything from animal well-being to environmental impact to food safety. Marketers in food companies, grocery stores and restaurants have responded to that consumer interest in several ways. These include the advertising direction taken, process labeling of foods, and setting specific demands on suppliers. This session will explore how we arrived at where we are today with regard to management practices on dairy farms. It will look at the impact public perceptions has on the practices we use today, and how that perception may impact what management practices dairy producers will be able to utilize in the future. It will also propose ideas and methods to potentially impact those public perceptions in ways that benefit the producer and the consumer.

Key Words: public, marketing, practices

Dairy farming is a highly complex and demanding activity. Dairy farmers’ responsibilities include crop, animal and business management, as well as their interactions. Because of that degree of complexity, skilled managers often rely on guidance from specialists such as agronomists, animal scientists and veterinarians, to name a few, each contributing specific bits of information. The producer then weighs the various opinions, often contradictory, to determine what course of action to take. For instance, land-grant universities and the National Academy of Science constantly evaluate and periodically publish updated recommendations in nutrient requirements of dairy cattle and fertilizer recommended rates for common crops and soil types across the US. In the past, however, cheap feeds and fertilizers relative to milk prices led to relaxed attitudes toward feed allowances (quantity and quality) to cows and in fertilizer applications to crops. If feasible, consultants recommended and producers followed up by allowing a wide safety margin to ensure that yields would not be limited, often ignoring nutrient recycling with the farm. Disconnection between fertilizer and nutritional recommendations further exacerbate nutrient imbalances, resulting in farming systems increasingly dependent on costly nutrient imports to the farm and potentially contribute to excess nutrient losses to the environment. Since then, feed costs soared and milk prices have become more volatile, making it ever more important to plan carefully and keep expenses and excesses very close to the minimum necessary. Society now demands that animal welfare and environmental sustainability also be added to that list of dairy farming responsibilities. Sustainability is a continuously evolving concept inextricably associated with social, economic and environmental aspects, the interactions of which have challenged scientists, producers and society. In this presentation we will review the scientific literature on recommended practices for the sustainability of dairy production systems in the US. I will incorporate results of our research on dairy nutrition and waste management in Louisiana small grazing dairies. We will discuss potential limitations of those practices and suggest future research to address gaps in knowledge.

Key Words: dairy, sustainability, environment

Dairy production and agriculture overall faces many challenges in the 21st century- the need for increasing food production to meet a growing population, consumer demands for social and ethical production, and a growing need to consider environmental sustainability simultaneously. Farmers face numerous decisions on their farms across varying issues and time scales and make decisions based on numerous internal and external influences. Understanding how and why farmers make decisions to pursue strategies that can increase sustainability across environmental, social and economic realms is important to understand for the dairy industry, policymakers and researchers. Such information can provide insight into strategies and programs that may foster an increased adoption of sustainable practices, which may confer benefits to both the farmer, their farm system and society. In this presentation, I draw upon my own work and others to understand what influences farmer’s likelihood and actual adoption of practices to achieve sustainable farming systems. I will incorporate research from my own work in California, Vermont, New Zealand and the US more broadly based on farmer surveys and interviews, and also bring in additional research to highlight from a social science perspective the factors that can influence farmer’s implementation of a range of sustainability practices. The presentation will discuss these findings in the context of implications for the dairy industry and policymakers who may want to provide resources and other strategies to assist farmers in implementing new strategies for on-farm sustainability.

Key Words: sustainability, farmer adoption

Antibiotic resistance is a critical issue in public health. Reducing the volume of antibiotics used in animal agriculture has long been advocated to limit the spread of antibiotic resistant pathogens, or the dissemination of horizontally transferable genetic determinants of resistance.
Yet, antibiotics are necessary to treat and prevent bacterial infections in livestock. On dairy farms, producers make most routine decisions on when to initiate antibiotic therapy; therefore, the advocated reductions in antibiotic use from current levels will require changes in producer treatment behaviors. Many surveys of livestock producers, however, show a consistent doubt about public health impact of antibiotic use in agriculture. For instance, the majority (58%) of conventional dairy producers disagreed that antibiotic use in agriculture led to resistant bacterial infections in people. Likewise, 88% of beef producers believed that antibiotic use in the beef industry contributes little or nothing to antibiotic resistance in general. Undeniably, the relative contribution of veterinary antibiotic use to the incidence of resistant infections in people is unknown, though not zero. By contrast, consumer surveys and purchasing behaviors indicate substantial concern about the use of antibiotics in livestock. For instance, a survey of the general public showed that most (60%) of people agreed with the statement that “The use of antibiotics for livestock leads to resistant bacteria in meat that can make people sick.” Other literature showed that consumers support the idea of antibiotics for the treatment of disease, but are unsupportive application of antibiotics to healthy animals. Clearly, consumers are not likely to be well-informed on the complexity of antibiotic resistance, and may not distinguish the overlapping but distinct issues of antibiotic residues and of antibiotic resistance. Nonetheless, the evolving consumer attitudes and changing purchasing behaviors are likely to continue to influence antibiotic treatment practices. Sustainable dairy production will require a proactive and holistic approach toward antibiotic stewardship to meet consumer expectations.

**Key Words:** antibiotic residue, sustainability
Breeding and Genetics I: Fertility and Efficiency

200 Genetic dissection of bull fertility in dairy cattle. Yi Han¹, Paula Nicolini²,³, and Francisco Peñagaricano*¹. ¹University of Florida, Gainesville, FL, ²University of Nebraska-Lincoln, Lincoln, NE.

Improving reproductive performance of dairy cattle has become one of the major challenges of the dairy industry worldwide. Most studies have investigated cow fertility while bull fertility has received much less attention. However, there is growing evidence that the service sire represents an important source of variation for conception rate in dairy cattle. As such, the main objective of this study was to perform a comprehensive analysis to reveal the genomic architecture underlying male fertility in dairy cattle. Sire Conception Rate was used as a measure of bull fertility including records in both Holstein and Jersey bulls. The analysis included the application of alternative genome-wide association mapping approaches and the subsequent use of gene set enrichment tools. The association analyses identified several genomic regions strongly associated with bull fertility. Most of these regions harbor genes, such as CCT6A, CKB, IGF1R, KAT8 and TDRD9 with functions related to sperm biology, including sperm development, motility and sperm-egg interaction. Some regions showed marked dominance effects, which provide more evidence for the importance of non-additive effects in fitness traits such as male fertility. Moreover, gene set analyses revealed many significant Gene Ontology and Medical Subject Headings terms, including fertilization, sperm motility, calcium channel regulation, and SNARE proteins. Most of these terms are directly implicated in sperm biology and male fertility. Our study contributes to the identification of genetic variants and biological pathways responsible for the genetic variation in bull fertility in Holstein and Jersey breeds. Additionally, our findings may provide opportunities for improving dairy bull fertility via marker-assisted selection.

Key Words: sire conception rate, association analysis, gene set enrichment

201 Predicting bull fertility using genomic data and biological information. R. Abdollahi-Arpanahi¹,², G. Morota³, and F. Peñagaricano*¹. ¹University of Florida, Gainesville, FL, ²University of Tehran, Tehran, Iran, ³University of Nebraska-Lincoln, Lincoln, NE.

The use of genomic data has revolutionized the prediction of complex traits in animal breeding in the last decade. Genomic prediction is generally considered as a black box because it ignores any available information about functional features of the genome. However, it is believed that genomic prediction can be more accurate and more persistent by integrating biological information. As such, the main objective of this study was to evaluate alternative models for predicting a complex trait such as bull fertility using both genomic and biological information. Sire conception rate (SCR) was used as a measure of bull fertility. The data set included 8k Holstein bulls with SCR records and 55k single nucleotide polymorphisms (SNPs) spanning the whole genome. Different subsets of SNPs were evaluated, including SNPs within or near genetic regions (n = 20k), SNPs linked to genes in the Gene Ontology (GO) term reproduction (n = 0.9k), SNPs linked to genes that belong to Medical Subject Headings (MeSH) terms related to sperm biology (n = 0.3k), and SNPs that were marginally associated with SCR (n = 18k). Both linear and Gaussian kernels were constructed for each set of SNPs and fitted in the models either separately (single kernel) or simultaneously (multi-kernel). Predictive ability was evaluated by mean-squared error (MSE) and predictive correlation (COR) in 5-fold cross-validation. Interestingly, the entire set of SNPs achieved good SCR predictions in the testing set (MSE = 4.13, COR = 0.35). Neither genomic regions nor GO or MeSH gene sets achieved predictive abilities higher than their counterparts using random sets of SNPs. Notably, kernel models fitting significant SNPs showed better predictive ability (MSE = 4.04 and COR = 0.36) than the whole-genome approach in both single and multi-kernel analyses. Models fitting Gaussian kernels outperformed their counterparts fitting linear kernels irrespective of the set of SNPs. Overall, our findings suggest that genomic prediction of bull fertility is feasible in dairy cattle. Pre-filtering SNPs based on testing marginal associations seems a promising alternative to avoid fitting the whole set of SNPs. The potential inclusion of gene set results into prediction models deserves further research.

Key Words: prediction of complex traits, gene set, functional genomics

202 Evaluation of conception rates of sex-sorted semen in commercial dairy farms over the last five years. C. Heuer*, D. Kendall, C. Sun, J. Deeb, J. Moreno, and R. Vishwanath, ST Genet- ics, Navasota, TX.

The objective of this study was to investigate the development of sexed semen conception rates in the field and compare those to the conception rates of conventional semen over the last 5 years (2012–2016). Conception rates were estimated for conventional and sexed semen using field data from 63 commercial dairy farms. In this period, sex-sorted semen underwent many significant changes with the introduction of SexedULTRA processing as well as improvements through the use of high throughput digital sorting technology. The total numbers of inseminations in the data set were 2,214,246 for conventional and 343,154 for sexed semen from 2,508 Holstein sires. The percentage of sexed semen used in those herds increased from 8% in 2012 to 23% in 2015. A linear mixed model was fitted to the data that included an interaction term between year-month of insemination and semen type, the insemination number (1–3) and the age of the service sire at the time of insemination. Random effects included the service sire and a herd-year-season of insemination effect. The model was fitted separately for heifers and cows, whereas the cow model included the lactation number (1–3) as additional fixed effect. Least squares means (LSM) on the interaction between year-month and semen type were used to describe the changes in conception rates over time while averaging over the remaining fixed effects. The cow conception rates showed strong seasonality which is consistent over years with any semen type. That seasonality was not observed in heifers. The LSM conception rate in cows in January 2012 was 0.38 while sexed semen reached 0.25. In heifers this difference in conception rates was even more pronounced with 0.58 using conventional and 0.38 with sexed semen. Through the years the technological and biological advances in sexed semen processing that were introduced seems to have improved the conception rates starting in 2013. In June 2014 conventional and sexed semen conception rates were almost at the same level for the first time (0.34 and 0.33). In 2015 the conception rates of sexed semen were stable and at 0.88 and 0.86 of conventional semen conception rates in cows and heifers, respectively.

Key Words: sexed semen, conception rates, fertility
Mathematical modeling can combine a wide range of information sources and facilitate the research of scenarios that would not be feasible to evaluate empirically. We have developed a stochastic model using genetic and physiological data from over 70 published reports on aspects of fertility in dairy cows. The model simulates cow pedigree, random mating allocation, correlated breeding values and interacting phenotypic variables. It was used to generate a large (200,000 cows replicated 100 times) data set of herd records for up to 5 parities within a seasonal dairy production system. From these data, a genetic evaluation of sires based on genetic merit for lifetime reproductive success (LRS) and the impact of high-LRS (Hi-LRS) or low-LRS (Lo-LRS) sires were investigated. LRS was defined as the number of times, during her lifetime, a cow calved within the first 42 d of the calving season. The proportion of daughters which calved (calving rate) in the 2nd parity was the strongest predictor of sire genetic merit for LRS ($R^2 = 0.81$). When 2nd parity calving date was included, the power of the prediction increased substantially ($R^2 = 0.97$). Reasonable predictions could also be made from 1st parity records. A predictive model containing 1st parity records for overall calving rate, and calving rate within the first 21 d, provided a good ($R^2 = 0.76$) LRS estimation when growth rate from weaning until first estrus was also included. Comparison of simulated daughters from widespread industry use (1000 daughters/bull) of sires with high ($n = 100$, $\mu = +0.70$) and low ($n = 100$, $\mu = -0.73$) breeding values for LRS, indicated that 12 of the 14 underlying genetic traits were divergent between the sire lines. Phenotypically, the daughters from the Hi-LRS sires displayed first estrus 34.1 d younger than their Lo-LRS contemporaries. Hi-LRS cows calved ~15 d younger at each parity and, despite producing less milk per season (~155L) than Lo-LRS cows, produced more milk over their lifetime (+33%) owing to additional lactations before culling. In summary, this simulation model suggests that lifetime reproductive success contributes substantially to cow productivity, and can be accurately predicted at a young age.

Key Words: breeding, reproduction, modelling

204 Estimating epistatic and dominance genetic variances for fertility and reproduction traits in Canadian Holstein cattle. K. Alves*1, M. Sargolzaei2, C. Baes1, A. Robinson1, and F. Schenkel1, 2Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada, 3The Semex Alliance, Guelph, ON, Canada

Non-additive genetic effects are usually ignored in animal breeding programs due to data structure (e.g., incomplete pedigree), computational limitations, and over parameterization of the model. However, non-additive genetic effects may play an important role in the expression of complex traits in livestock species, such as reproduction and fertility traits. We assessed the use of pedigree and SNP-marker-based models to estimate additive and non-additive genetic variances for reproduction and fertility traits in Canadian Holstein heifers ($n = 5,825$) and cows ($n = 6,090$). Four traits were analyzed including age at first service for heifers (AFS), calving to first service interval for cows (CTFS), and 56-d non-return rate for heifers and cows (NRR). Four linear models were used (1) additive genetic model (MA); (2) a model including both additive and epistatic (additive by additive) genetic effects (MAE); (3) a model including both additive and dominance effects (MAD); and (4) a full model including additive, epistatic, and dominance genetic effects (MAED). The models which included non-additive genetic effects for AFS and CTFS indicated that epistasis, dominance, or a combination thereof, are as important as additive effects, and sometimes contribute a larger proportion to the total phenotypic variance than the contribution of the additive effects. The partitioning of variance components resulted in a re-ranking of animals in the top 10% based exclusively on the additive genetic effects between models. The change in rank indicates that adjusting for non-additive genetic effects could change selection decisions made in dairy cattle breeding programs. These results suggest that non-additive genetic effects play an important role in some reproduction and fertility traits in Canadian Holsteins and their inclusion in genetic evaluations may improve accuracy of selection based on additive genetic effects.

Key Words: Holstein cattle, low-heritability trait, non-additive genetic effect

205 Discovery of a haplotype affecting fertility in Ayrshire dairy cattle and identification of a putative causal variant. D. J. Null*, J. L. Hutchison1, D. M. Bickhart2, P. M. VanRaden1, and J. B. Cole1, 1Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD, 2U.S. Dairy Forage Research Center, ARS, USDA, Madison, WI.

The goal of this research was to identify the causal variant associated with a haplotype affecting fertility in Ayrshire cattle. The US dairy population is routinely monitored to identify cases where homozygotes for a minor allele are expected, but never observed, which can indicate the presence of a genetic defect causing embryonic death. Such a haplotype was identified on chromosome 17 in January 2013. A second haplotype reached the threshold for statistical significance (7 expected homozygotes and none observed) in October 2013. Sire conception rate was 6.1% lower for matings of carrier sires to cows with carrier maternal grandsires, but the effect was not significant. As of December 2016, 23.5 homozygotes were expected but none had been observed. Most carriers trace back to OAK-RIDGE FLASHY KELLOGG (AYUSA000000125168), born in 1961, but many Canadian carriers only trace back as far as WOODLAND VIEW PARDNER ET (AYCAN000000811799), born in 1994. These bulls share an ungenotyped ancestor in OAK-RIDGE LIGHTNING (AYUSA000000120135), born in 1958. This haplotype has been designated Ayrshire haplotype 2 (A2H), and its frequency has increased from 6% in animals born in 1990 to 21.7% for animals born since 2008. Whole-genome sequence data ranging from 9 × to 13 × read-depth for 8 Ayrshire bulls, 3 carriers and 5 non-carriers, was used to search for the causal variant. The analysis used a short-read sequence data analysis pipeline including BWA (v. 0.7.10) and Samtools (v. 1.3). Likely false positive variants and variants with small predicted functional effects were removed after annotation with SNPeFF (v. 4.3). A splice acceptor variant at 51,267,548 bp in the RNA Polymerase 2 Associated Protein (RPAP2) gene was the most likely causal variant in the haplotype. RPAP2 is an essential component of the RNA polymerase 2 holoenzyme necessary for transcription of snRNA species. Experiments with mouse knockouts also found a deficiency of homozygotes, suggesting that RPAP2 is necessary for embryonic development. These findings provide strong evidence for the existence of a new, lethal recessive in Ayrshire cattle. AH2 carrier status should be reported to the industry routinely and its effect on fertility confirmed.

Key Words: Ayrshire, fertility, genetic disorder

206 Predictions for workability and reproductive traits using two-step and single-step genomic BLUP in Canadian Holsteins.
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For low heritability traits, large reference populations are required to achieve high reliability of genomic EBV (GEBV). By including genotyped and non-genotyped animals simultaneously in the evaluation, the single-step GBLUP (ssGBLUP) has the potential to yield more accurate and less biased evaluations. The aim of this study was to compare the reliability and bias of genomic predictions for various workability and reproductive traits in Holstein cattle using 2-step GBLUP (tsGBLUP) and ssGBLUP. A total of 33,568 bulls and 6,849 cows were genotyped. Genomic predictions were assessed using genotypes only for bulls or for bulls and cows under different approaches: (1) GEBV estimated by ssGBLUP with a default blending of 5% of the pedigree relationship matrix among genotyped animals (A22) and 95% of the genomic relationship matrix (G); (2) Direct genomic value (DGV) estimated by tsGBLUP where 5% or 20% of A22 was blended into G; (3) GEBV as an index combining EBV and DGV, where the latter was obtained from tsGBLUP with 5% or 20% blending. Regular BLUP without genomic information were also carried out and EBV served as benchmark for comparisons. Reliabilities were obtained with forward prediction, following the Interbull validation method. Validation bulls had at least 50 daughters in 2014. Including genomic information improved reliability, on average, by 14.5 pts for ssGBLUP and 12 pts for tsGBLUP compared with BLUP. Overall, ssGBLUP predictions had 3.3 pts greater reliabilities and were 0.15 pts less biased compared with tsGBLUP. When a 20% blending was used in stGBLUP, predictions were less biased, but no differences in reliability were observed compared with a 5% blending. Adding genotypes for cows had a small, positive impact of 1.5 pts in reliability for ssGBLUP and 1.6 pts for tsGBLUP; bias was reduced by 0.002 and 0.023, respectively. When genomic information is available for cows, predictions for both workability and reproductive traits can be slightly improved. Single-step GBLUP leads to more accurate and less biased predictions compared with 2-step GBLUP.

Key Words: genomic EBV, reliability, single-step GBLUP


Feed conversion efficiency is important for the profitability of dairy farms, but measuring dry matter intake to estimate it directly is expensive and time consuming. Consequently, fast and inexpensive measurements of genetically correlated traits are desirable as predictors. In this study, we describe the utility of thermal imaging to predict feed conversion efficiency. We conducted a 30–40 d feeding trial on 6 to 9 mo old Friesian bulls (n = 75) and their half-sisters (n = 246) and estimated feed conversion efficiency as residual feed intake (RFI: the residual from a regression of daily dry matter intake on average daily gain and mid-trial metabolic weight). We also took thermal images to measure heat loss from the eye, cheek, and muzzle. Using univariate animal models, we estimated the heritability of RFI for each sex (0.13 for heifers and 0.18 for bulls), and using a bivariate animal model that treated RFI in bulls and heifers as different traits, we estimated the between-sex genetic correlation (0.93). To investigate the utility of thermal traits as predictors, we fit single-trait animal models for all 8 heat loss measurements (maximum and mean temperature of the eye, eye corner, cheek, and muzzle) to estimate heat loss EBVs and then used multiple regression of heat loss EBVs on RFI EBVs to produce prediction equations. Even over-fit regression models using all 8 predictors resulted in low coefficients of determination ($r^2$) of 0.35, 0.40, and 0.19 for both sexes, heifers and bulls respectively. To simulate selective RFI phenotyping of bulls only, we fit a 3-trait animal model treating RFI and the first 2 principle components (PCs) of the 8 heat loss traits as separate traits using all data on bulls, but only the heat loss PCs on heifers to estimate RFI BVs in both sexes. These RFI EBVs estimated using only 75 direct measurements on bulls were highly correlated with those from univariate models using 321 measurements on both sexes with $r^2$ values of 0.85, 0.84, and 0.94 for both sexes, heifers and bulls respectively but with lower reliabilities. Both strategies would require extensive independent validation to justify routine measurement of RFI and its incorporation into the national breeding objective.

Key Words: feed efficiency, dairy, genetics

208 Determining the economic value for efficiency traits. C. Richardson*, C. Baes*, P. Amer*, C. Quinton, P. Hely*, P. Martin*, V. Osborne, J. Pryce**, and F. Miglior1,2, 1University of Guelph, Guelph, ON, Canada, 2Canadian Dairy Network, Guelph, ON, Canada, 3AbacusBio Limited, Dunedin, Otago, New Zealand, 4Development, Jobs, Transport and Resources, Agribio, Bundoora, VIC, Australia, 5La Trobe University, Agribio, Bundoora, VIC, Australia.

Altering management practices and production designs to create a more sustainable global system has been highlighted in government policies and corporation objectives. In the dairy market, the inefficient utilization of feed and high production of emissions associated with raising and maintaining cattle are scrutinized for contributing to environmental degradation, and therefore, have become targeted areas for improvement. Feed is a major expense for the Canadian dairy industry, representing over 50% of all production costs. A new prospect for decreasing the environmental footprint of the dairy industry is to increase feed efficiency and decrease methane emissions through the selection of genetically superior animals, as these traits are favorably correlated. Current selection programs have been successful in achieving breeding objectives related to production, health and longevity. It is also important to develop a selection program that includes an aim to increase total system efficiency, thereby, maintaining a viable industry both economically and environmentally. Thus, the economic value of efficiency traits to be used in selection programs must be determined. The methodology introduced below was used to determine the total savings associated with selecting for a decrease in the total dry matter intake of a lactating first parity dairy cow; including the economic value of the reduction in feed-related methane production. The lifespan of a typical Canadian dairy animal was divided into stages based on significant changes in cost of ration or energy requirements by the animal. Regression coefficients defined as a ratio of the mean total feed intake across production animal life stages relative to a first parity lactating cow, were used to determine the economic effect of decreasing feed intake. The resulting coefficients were applied to determine the cost savings associated with the lower output of emissions. Using this approach, the approximate lifetime savings generated by a more feed efficient production animal can be estimated.

Key Words: feed efficiency, methane, production, economics

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Genomic predictions of transmitting ability (GPTAs) for residual feed intake (RFI) were computed using data from 4,621 42-d and 202 28-d feed intake trials of 3,947 US Holsteins born 1999–2013 in 9 research herds. The 28-d records had 8.5% larger error variance than 42-records and received less weight (0.92 vs. 1.0) in the evaluation. The RFI averages were already adjusted to remove phenotypic correlations with milk energy output, metabolic body weight, and body weight change and for several environmental effects including other nutrition experiments during the feed intake trials. Traditional breeding values (BV$s) for RFI of 74.3 million Holsteins were obtained by an animal model that also included effects for age-parity group, trial date, herd management group, permanent environment, herd-sire interaction, and regressions on inbreeding and on genomic evaluations for milk energy and body weight composite (BWC). The milk energy and BWC terms were specified with the intent to remove positive genetic correlations that remained after the phenotypic correlations were removed. Estimated heritability was 0.17 and repeatability across lactations was 0.42. Genomic BV$s for RFI included 60,671 genetic markers for 1.4 million Holsteins and produced calculated genomic reliabilities for young animals averaging 19% compared with traditional reliabilities of 5%. A parallel test using somatic cell score (SCS) records for these same 3,947 cows indicated lower observed than expected genomic reliability (11% vs. 19%). The economic value of RFI is very large and could receive >20% of total economic value. This extra feed eaten per day to feed saved per lactation. Additional feed intake records could make feed saved a very important trait in future selection indexes for dairy cattle.

Key Words: feed intake, genomic evaluation, dairy efficiency

211 A comparison of feed intake, production, body condition score, body weight, and frame size of ProCROSS crossbred versus Holstein cows during the first 150 days of first lactation. B. N. Shonka-Martin*1, B. J. Heins2, and L. B. Hansen1, 1University of Minnesota, St. Paul, MN, 2West-Central Research and Outreach Center, Morris, MN.

Three-breed (Montbéliarde, Viking Red, Holstein) rotational crossbred (ProCROSS) cows (n = 44) were compared with Holstein cows (n = 41) for feed intake, production, and body traits during the first 150 d of first lactation. Cows calved in the University of Minnesota campus herd from September 2014 to April 2016. A total mixed ration (TMR) was fed twice daily, and refusals were weighed once daily. All cows were individually fed the same TMR on an ad libitum basis. Feed intake was recorded and dry matter intake (DMI) was calculated. Body weights were recorded weekly, body condition score (BCS) was evaluated once weekly, and height at both the withers and the hips was measured once a month. Best prediction (BP) was used to estimate production from data collected from routine milk recording. Mean body weight and BCS were calculated for monthly periods. Statistical analysis of total 150-d DMI included the fixed effects of year, age at calving, breed, and the interaction of year and age at calving and the random effect of cow nested within breed. A similar model was used for production traits except age at calving was removed because this variable is adjusted for by BP. Month nested within breed was an additional fixed effect for the body traits. Crossbred cows (2,770 kg) consumed significantly (P < 0.01) less DMI during the first 150 d of lactation than Holstein cows (2,922 kg). Fat plus protein production was not different (P = 0.54) for crossbred (338 kg) and Holstein cows (328 kg), but the crossbreds were numerically higher. Crossbred cows (3.39) had significantly higher (P < 0.01) BCS than Holstein cows (3.16) during the first 150 d of lactation. However, body weight was not different (P = 0.33) between the crossbreds (556 kg) and Holsteins (545 kg). Crossbred cows (136 cm) were shorter (P < 0.01) for height at the withers than Holstein cows (138 cm), but similar (P = 0.51) for height at the hips (143 cm vs. 144 cm, respectively).

Key Words: crossbreeding, feed intake
212 Impact of pasture versus indoor feeding systems on quality characteristics, nutritional composition, sensory and volatile properties of full-fat Cheddar cheese. T. O’Callaghan1,2, D. Manion1, D. Hennessy3, S. McAuliffe3, M. O’Sullivan4, N. Leeuwendaal1, T. Beresford1, P. Dillon2, K. Kilcauley1, J. Sheehan1, R. P. Ross2,3, and C. Stanton*1,2, 1Teagasc Food Research Center, Fermoy, Co. Cork, Ireland, 2APC Microbiome Institute, University College Cork, Cork, Ireland, 3Teagasc Animal and Grassland Research Center, Fermoy, Co. Cork, Ireland, 4University College Cork, Cork, Ireland.

The purpose of this study was to investigate the effects of pasture versus indoor total mixed ration (TMR) feeding systems on the chemical composition, quality characteristics and sensory properties of full fat Cheddar cheeses. Fifty-four multiparous and primiparous Friesian cows were divided into 3 groups (n = 18) for an entire lactation. Group 1 was housed indoors and fed a TMR diet of grass silage, maize silage and concentrates, Group 2 was maintained outdoors on perennial ryegrass only pasture (GRS), while Group 3 was also maintained outdoors on perennial ryegrass/white clover pasture (CLV). Full fat Cheddar cheeses were manufactured in triplicate at pilot scale, from each feeding system in September and were analyzed over a 270 d ripening period at 8°C for chemical composition, textural characteristics, sensory and volatile properties. Pasture derived feeding systems were shown to produce Cheddar cheeses more yellow in color than that of TMR, which was positively correlated with increased cheese β-carotene content. Feeding system had a significant effect on the fatty acid composition of the cheeses. The nutritional composition of Cheddar cheese was improved through pasture based feeding systems with significantly lower thrombogenicity index scores and a greater than 2-fold increase in the concentration of vaccenic acid and the bioactive conjugated linoleic acid c9t11, while TMR derived cheeses had significantly higher palmitic acid content. Such alterations in the fatty acid profile of the cheeses resulted in pasture derived cheeses having reduced hardness scores at room temperature. Feeding system and ripening time had a significant effect on the volatile profile of the Cheddar cheeses. Pasture derived cheeses have significantly higher concentrations of the hydrocarbon toluene. Ripening period resulted in acids, alcohols, aldehydes and ester based compounds. Fatty acid profiling of cheeses coupled with multivariate analysis showed clear separation of Cheddar cheeses derived from pasture-based diets (perennial ryegrass or perennial ryegrass/white clover) from that of a TMR system.

Key Words: pasture, total mixed ration, Cheddar cheese

214 Withdrawn

215 Chemical and sensory characteristics of Chanco cheese from dairy cows supplemented with olive oil and partially hydrogenated vegetable oil. E. Vargas-Bello-Pérez*,1, C. Garido2, C. Geldsetzer-Mendoza1, M. S. Morales2, P. Toro-Mujica1, R. A. Ibáñez1, and P. C. Garnaesworthy3, 1Departamento de Ciencias Animales, Pontificia Universidad Católica de Chile, Santiago, Chile, 2Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile, 3School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, United Kingdom.

The aim of this study was to assess the effects of unrefined olive oil (OO; unsaturated fatty acid source) and hydrogenated vegetable oil (HVO; saturated fatty acid source) in dairy cow diets on chemical and sensory characteristics of cheese. Fifteen multiparous Holstein cows were used during a 9-wk study. Cows averaging 189 ± 28 d in milk (average ± SD) at the beginning of the study were assigned to different treatments that included a control diet with no added lipid (n = 5), and fat-supplemented diets containing OO (n = 5; unrefined oil; 30 g/kg of dry matter; DM) and HVO (n = 5; manufactured from palm oil; 30 g/kg DM). Diets were based on corn silage and alfalfa silage. Either extract (g/kg DM) was 50 for control and 70 for both OO and HVO diets. Individual milk samples were taken on d 21, 42 and 63 for proximate analysis. During the same sampling days, milk collected from individual cows from the same treatment was pooled and made into cheese (n = 4) and ripened for 7 d and analyzed for fatty acid (FA) profile. Sensory evaluation of cheeses was carried out in relation to appearance, odor, flavor, and texture. Milk production (35.0 ± 1.8 kg/d) milk fat (3.1 ± 0.16 g/100g) and milk protein (3.3 ± 0.11 g/100g) were not affected by dietary treatments. Moisture (51.3 ± 2.2 g/100g), fat (23.1 ± 1.2 g/100g), total protein (20.7 ± 1.7 g/100g) and ash (2.3 ± 0.1 g/100g) from cheeses were not affected by dairy cows would not result in adverse effects in milk composition or in quality of baby Swiss cheese produced from that milk. Thirty-five multiparous and lactating Holstein dairy cows were assigned randomly to 1 of 2 dietary treatments in a 2 × 2 crossover design. Each period lasted 35 d. Treatment 1 was a standard corn/corn silage/hay diet and treatment 2 was based on the same diet with the exception of 20% of dry matter (DM) being RF-DDGS. Diets were isonitrogenous, isenergetic, and contained similar limiting dietary amino acids. There was no treatment effect on milk yield (35.66 and 35.39 kg/day, control and RF-DDGS, respectively), milk fat percentage (3.65 and 3.61%), lactose percentage (4.62 and 4.64%), and milk total solids (12.19 and 12.28%). However, milk protein percentage increased (3.01 and 3.11%). For cheese production, milk was collected and pooled 6 times for each dietary treatment. Regarding appearance, cheeses were atypical, but the only significant differences were in eye size, with the control being closer to ideal than RF-DDGS cheese. There were significant interactions between production day and treatment, but no clear trend emerged, indicating that the make procedure has a greater impact on cheese quality than RF-DDGS. These results indicate that RF-DDGS can effectively be fed to cows at a 20% inclusion rate (DM) without negatively influencing suitability of milk for production of quality baby Swiss cheese.

Key Words: dried distillers grains with solubles (DDGS), late-blowing, sensory
dietary treatments. Compared with control and HVO, OO decreased \( (P < 0.05) \) contents \((g/100g \text{ total FAME})\) of C4:0 and C10:0, and increased \((P < 0.05) \) C18:1trans11, C18:1cis9, C18:2cis 9, trans11 and C18:3n3 in cheeses. Compared with control and HVO, OO reduced \((P < 0.05) \) number of holes, overall odor and acidity whereas HVO increased \((P < 0.05) \) cow milk odor, bitterness and acidity. OO and HVO increased \((P < 0.05) \) salty flavor. In conclusion, supplementation of dairy cow diets with HVO or OO did not affect milk production, milk composition and cheese composition. From a human standpoint, OO improved the FA profile of cheeses. However, attributes related to appearance, odor, flavor and texture were affected by OO and HVO.

**Key Words:** cheese, milk, sensory characteristics

### 216 Evaluation of electrical bioimpedance spectroscopy in estimate the milk composition, SCC, and milk ethanol stability—Preliminary results

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Electrical bioimpedance spectroscopy (EBS) is a fast, easy and low-cost methodology used for measurement of electrical properties of biological materials and quality of food. The present study aimed to evaluate an automatic equipment based on EBS for measurement of milk quality. Individual composite milk samples from 2 daily milkings were collected from 25 Holstein cows averaging \((\text{mean} \pm \text{SD})\) \(25 \pm 5 \text{ kg of milk/d and 150 \pm 6 \text{ DIM}}\). Milk samples were stored for 24 h at 5°C. Milk ethanol stability (MES) was performed at following concentrations \((\text{vol/vol})\): 64, 68, 70, 72, 74, 76, 78, 80, 82 and 84% ethanol. Results were expressed by the lowest concentration of ethanol in which coagulation occurred. The EBS analysis made by Milkspec FS317 (Bionexus, Brazil) required only 20 mL of raw milk and the bioimpedance spectra were obtained in less than a minute. The Bionexus InterCurve EBS Model software for curve fitting calculated the parameters list fitted to the impedance model and temperature compensation. At the end of this first step, milk samples were heated at 37°C in attempt to study the efficiency of milk heat on accuracy of EBS predicted milk quality. Fat, lactose and protein were determined by infrared absorption. The mean spectra parameter related with EBS curve behavior fitted by Bionexus InterCurve were obtained and associated with MES, milk composition and SCC by correlation and regression model. MES was better predicted using cooled samples by the mean of bioimpedance expressed in ohms \((R^2 = 0.49; P < 0.0001; \text{RMSE} = 4.31)\). Milk composition was better predicted at 37°C, as it was possible estimated the milk concentrations of lactose \((R^2 = 0.63; P < 0.0001; \text{RMSE} = 0.2790)\), fat \((R^2 = 0.23; P = 0.0292; \text{RMSE} = 0.837)\) and log 10 of SCC \((R^2 = 0.6; P < 0.0001; \text{RMSE} = 0.339)\) using parameters of EBS estimated from Bionexus InterCurve EBS Model. Milk protein were not associated with EBS. This preliminary results suggest that an automatic equipment based on EBS may be used in dairy farms and dairy plants to estimate milk quality parameters, with easier operating procedures and lower cost than traditional methods.

**Key Words:** electrical bioimpedance spectroscopy, mastitis detection, milk quality

### 217 Determination of native lactoferrin in milk using HiTrap Heparin HP column coupled with HPLC

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Lactoferrin is increasingly supplemented in foods for its multiple functions. It is necessary to establish a reliable analytical method for nutritional assessment and quality control. A method for determination of native lactoferrin in milk using HiTrap Heparin HP column coupled with HPLC was developed and validated. Native lactoferrin was separated from denatured lactoferrin by centrifuging the samples that were adjusted to pH 4.6. Response surface design was used to find the optimal conditions for lactoferrin using HiTrap Heparin HP column as below: 10.35 mmol L\(^{-1}\) disodium hydrogen phosphate for equilibration solutions, pH 6.02 for equilibration solutions, 1.78 mol L\(^{-1}\) NaCl in elution solutions, and pH 7.26 for elution solutions. Subsequently lactoferrin was quantified using HPLC-PDA. A linear range from 2 to 100 mg L\(^{-1}\) of lactoferrin standards was obtained, with a value of \(R^2\) equal to 0.9989. The limit of detection (LOD) and quantification (LOQ) were 2.00 and 6.67 mg L\(^{-1}\) for lactoferrin standard, and 0.57 and 1.90 mg L\(^{-1}\) for milk sample considering enrichment factor involved in the pre-treatment procedures. In addition, milk spiked with lactoferrin at 3 concentration levels (2, 5 and 10 mg/L) showed that overall mean recovery were 88.3%, 90.2 and 95.1%, respectively. Relative standard deviation of intra-day and inter-day precision was 1.3–4.8%, and 2.1–5.7%, respectively, demonstrating good performances of the proposed method. The developed method was subsequently applied to determine lactoferrin in raw milk and processed milk. Results showed that this established method can be used to determine lactoferrin in different processed milk. Levels of lactoferrin in raw milk and processed milk were ranging from around 0.8 to 44.9 mg/L, indicating its level was strongly relying on the actual heat load that milk samples were exposed to. As expected, increasing the temperature from 72.5°C to 120°C resulted in lower native lactoferrin in milk. In addition, quantification of lactoferrin in raw milk and processed milk using this method displayed the usefulness and effectiveness of the proposed method.

**Key Words:** lactoferrin, heparin, HPLC

### 218 Development and quality enhancement of cottage-type cheese made from Nili Ravi buffalo postpartum milk (colostrum).

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Cottage-type cheese was prepared from Nili Ravi buffalo first 5 d colostrum and the results were compared with cottage cheese made from normal buffalo milk (Control). The milk pasteurization temperature was optimized after many trials for all treatments (63°C for 30 min), for optimization different time and temperature combinations were applied for different days colostrums, physico-chemical quality was analyzed and then various steps for Cottage cheese manufacturing were finalized. Pragmatic gel formation took 3 h in T1 whereas in T2, T3, T4, T5 and control after 45 min firm coagulum was produced. After cutting in cubes, cooking at 55°C and whey drainage was performed; it was found that percentages of all chemical contents decreases with the increase in age (days) of colostrum. The data collected were analyzed through one way ANOVA under completely randomized Design and means were compared through Duncan’s Multiple Range test with the help of SAS 9.1 (Statistical Software). Results showed that maximum cheese yield was obtained in T1, then T2, T3, T4, and T5 respectively. Cottage cheese

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Flavor profile of UHT conjugated linoleic acid-enriched milk based on headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry. M. Leal-Davila¹, J. Curtis¹, M. Saldaña¹, and S. Martinez-Monteagudo*¹, ²Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, ²Dairy and Food Science Department, South Dakota State University, Brookings, SD.

There is industrial interest to reformulate dairy products by increasing the concentration of certain trans fatty acids, consumption has been associated with improved human health. Examples of such fatty acids are conjugated linoleic acid (CLA, C18:2), trans-vaccenic acid (TVA, C18:1 n11), eicosapentaenoic acid (EPA, C20:5 n3), and docosahexaenoic acid (DHA, C22:6 n3). Unfortunately, the concentration of such fatty acids in milk is rather low, which limits the use of milk fat as a primary source of bioactive lipids in the human diet. Ruminant scientists have developed cattle nutritional guidelines that increase the concentration of bioactive lipids through rumen biohydrogenation, offering commercial opportunities to formulate healthier dairy products. However, changes in the milk fat composition that are desirable from nutritional purpose may have significant impact on the flavor profile during thermal processing. This study evaluated the impact of UHT (125–145°C/2–20 s) on the flavor profile of CLA-enriched milk. Headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS) was used to characterize the volatile compounds, followed by multivariate analysis. After UHT treatments, 18 volatiles were identified, including linear aldehydes (propanal, hexanal, heptanal, octanal, nonanal and decanal), branched aldehydes (3-methylpropanal, 2-methylbutanal, 3-methylbutanal, furfural and benzaldehyde), ketones (diacetyl, 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone, 2-decanone and 2-undecanone) and dimethyl sulfide. The concentration of total aldehydes increased up to 4-fold with respect of methyl ketones when increasing temperature from 125 to 145°C. Heptanal was the most abundant volatile, resulting from UHT processing and a potential suitable marker for heat treatment of enriched-CLA milk.

Key Words: UHT, flavor profile, CLA

This study explored the effectiveness of a training workshop for animal-based measures (ABM) of welfare in dairy cows, in which 14 people were trained to evaluate 6 ABM: hock injuries (HOCK), lameness (LAME) in freestalls and tie-stalls, body condition score (BCS), and udder, flank, and leg cleanliness (CLEAN). All scoring systems were modified to a dichotomous outcome: acceptable (AC) or unacceptable (UN). AC hocks: no swelling and/or hair loss. UN hocks: swelling and/or scab. AC BCS: < 2, on a 5 point scale. UN BCS: ≥ 2. AC cleanliness: up to minor splashing. UN cleanliness: distinct plaques to a solid manure plaque. LAME was evaluated using locomotion score (LS) or in-stall lameness score (SLS), depending on cow housing. UN lameness: ≥ 3 LS, on a 1 to 5 scale, where 3 = mild lameness or ≥ 2 SLS, where 2 out of 4 behavioral indicators of lameness were detected. Classroom instruction took place on day 1 of training. Day 2: live group assessment of LAME (n = 25 cows), live group assessments of HOCK/CLEAN/BCS (n = 30 cows), followed by live individual assessment of HOCK/CLEAN/BCS (n = 20 cows). Day 3 included live individual assessment of HOCK/CLEAN/BCS (n = 33 cows), and individual video assessment of LAME (n = 27 cows). An additional training video for LAME was sent to trainees 3 weeks after the workshop, and another follow-up assessment of LAME took place via video (n = 37 cows). Repeatability and accuracy of the trainees was assessed using Fleiss’s Kappa (FK) and byrt’s Kappa (BK) to examine group inter-rater agreement and expert-trainee agreement, respectively. Both Kappa systems use a scale of poor (<0), slight (0.01–0.20), fair (0.21–0.40), moderate (0.41–0.61) substantial (0.61–0.80), or almost perfect (0.81–1.00). At the conclusion of the workshop, FK = 0.66 and 0.41 for HOCK and LAME. BK mean = 0.81 (from 0.63 to 1.00) and 0.62 (from 0.56 to 0.85) for HOCK and LAME. Each trainee achieved a BK of “almost perfect agreement” for BCS and CLEAN. After the follow-up video, trainees achieved a FK = 0.73 and a BK mean = 0.72 (from 0.63 to 0.78). Multiple assessors can achieve substantial agreement for ABM with adequate training.

Key Words: lameness, hock injury, inter-rater reliability

221 Comparison of online, hands-on, and a combined approach for teaching cauterity disbudding technique, including administration of a cornual nerve block, to dairy producers. C. Winder*1, S. LeBlanc1, D. Haley1, K. Lissemore1, M. Godkin1, and T. Duffield1, 1Dept. of Population Medicine, University of Guelph, Guelph, ON, Canada, 2Ontario Ministry of Agriculture, Food, and Rural Affairs, Elora, ON, Canada.

The use of pain control for disbudding and dehorning is important from both an animal- and industry-centered perspective. Best practices include the use of local anesthetic, commonly given as a cornual nerve block (CNB), and a non-steroidal anti-inflammatory drug (NSAID). Approximately 40% of Canadian dairy producers do not use local anesthesia, perhaps in part due to lack of knowledge regarding CNB technique. Although this skill is typically learned in person from a veterinarian, alternative methods may be useful for hard to reach producers. The objective of this trial was to determine if there were differences in the efficacy of online training (n = 23), hands-on training (n = 20), and a combined approach (n = 23) for teaching producers to successfully administer a CNB and disbud a calf. The primary outcome was block efficacy, defined as a lack of established pain behaviors during iron application. Secondary outcomes were background knowledge (assessed by a written quiz), CNB and disbudding technique (evaluated by rubric scoring), time taken, and self-confidence before and after evaluation. Associations between training group and outcome were assessed with univariable logistic regression and Cox-proportional hazard models. Block efficacy was not different between groups, with 91% successful in both combined and online groups, and 75% in the hands-on trained group. Online and combined groups tended to have higher written scores, but online learners also tended to have poorer technical scores compared with hands-on (P < 0.10). Time to block completion was not different between groups, but disbudding completion was fastest for the hands-on group compared with both online (P = 0.03) and the combined group (P = 0.08). The combined group had the highest pre-evaluation confidence score (P < 0.05), and after evaluation remained higher than online (P = 0.04) and tended to be higher than hands-on (P = 0.10). While we saw statistical differences in time taken and self-confidence between groups, absolute differences were small and block efficacy was similar. This suggests online training may be a useful tool for motivated producers who lack access to hands-on training.

Key Words: dehorn, education, pain control

222 Bovine respiratory disease prevalence estimation in pre-weaned dairy calves using a mobile application. B. M. Karle1, S. S. Aly2,3, D. R. Williams4, J. W. Stockhouse1, A. L. Van Eenennaam2, and T. W. Lehenbauer2,3, 1University of California Cooperative Extension, Orland, CA, 2Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, 3UC Davis Veterinary Medicine Teaching and Research Center, Tulare, CA, 4University of California Cooperative Extension, Eureka, CA.

Bovine respiratory disease (BRD) accounts for 22% of pre-weaned dairy calf deaths. Dairy calf raisers frequently do not accurately diagnose or track the incidence of BRD in pre-weaned calves. The objective of this project was to estimate the sample size required to survey a herd of pre-weaned dairy calves for BRD and develop a user-friendly interface to aid calf caretakers, producers, veterinarians, and consultants in accurate diagnosis of this disease and assist in improved control measures. A mobile application (“app”) was developed to aid in BRD detection and prevalence estimation using the California BRD scoring system. Study personnel designed the application’s survey methodology based on a simple random sample for prevalence estimation. Once outlined by study personnel, application specifications and design layout were implemented into the app by software developers (Vestra Resources Inc., Redding, CA). The app automates the prevalence estimation based on a user specified bound (width of the confidence interval), assumed prevalence and total number of pre-weaned calves on site. After the designated sample number of calves is evaluated, the app generates a report indicating the estimate and 95% confidence limits for prevalence of BRD in the calf herd. In addition, descriptive analyses, a histogram of clinical signs present in the calves evaluated, and identification numbers of cases and non-cases are reported and can be self- emailed or shared with a consultant. Users can review the history of BRD status results with the ability to review the data collected for each calf at each sampling session, and for consultants, by herd. The mobile application

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is currently available free of charge in both English and Spanish on the iOS App Store and Google Play. The use of a mobile application to train calf caretakers on BRD detection, and to automate BRD prevalence estimation in pre-weaned dairy calves will allow producers, veterinarians and consultants to more effectively track the respiratory disease status of their dairy calf herds.

Key Words: bovine respiratory disease, calves, app

223 Bringing udder health to life: Using data visualization to improve student and dairy producer learning. S. Roche1,2, D. Kelton3, A. Godkin4, K. Hand4, and D. Shock5. 1ACER Consulting, Guelph, ON, Canada, 2University of Guelph, Guelph, ON, Canada, 3Ontario Ministry of Food, Agriculture and Rural Affairs, Guelph, ON, Canada, 4Precision Strategic Solutions, Guelph, ON, Canada, 5Main Street/Upper Grand Veterinary Services, Guelph, ON, Canada.

The primary objective of this extension education project was to develop an innovative teaching tool to mobilize research on udder health and milk quality in the Canadian dairy industry. More specifically, data visualization and real-space animation techniques were used to develop a unique and engaging video to (a) support conventional teaching materials at the University of Guelph, and (b) educate Canadian dairy producers about milk quality in Canadian dairy herds and the impact of evidence-based management on udder health. The video superimposes animations in real-space to create an interactive on-screen environment, and explores the use of somatic cells for evaluating milk quality and udder health, discusses optimal somatic cell counts (SCC), and reviews effective management practices for maintaining low SCC. Furthermore, the video uncovers provincial patterns and trends in Ontario bulk tank somatic cell count (BTSCC) at an industry level from 1985 (~12,000 dairy farms; average BTSCC: 294,000 cells/milliliter) to present day (~3,700 dairy farms; average BTSCC: 217,000 cells/milliliter), and highlights the impact of penalty programs to improve milk quality.

The video was filmed in December 2016, and made available online in February 2017 at bit.ly/BTSCCC17. A webpage housing the video and supplementary content, and a social media communication strategy utilizing Twitter, YouTube and other platforms will be implemented in March 2017 to disseminate the video online. A classroom learning series is also being developed to explore the efficacy of the video as a teaching tool in undergraduate, graduate and veterinary medicine courses. An industry series is also being developed to explore the efficacy of the video as a teaching tool in undergraduate, graduate and veterinary medicine courses.

Key Words: NRCS, feed management, nutrient management


NRCS has a Conservation Practice Standard 592 for Feed Management available to dairy producers. The goal of this standard is to provide rations to minimize feeding nitrogen (N) and phosphorus (P) above animal requirements. This can lower the N and P excreted in manure. One component of this program is a requirement for development of a feed management plan by a certified feed management planner. However, there is uncertainty regarding the amount of time required to develop a plan. A pilot program was funded by NRCS in New York to determine the time required to write a plan. Eighteen New York dairy farms were enrolled in this study. Herds were selected to represent a range in size and management practices. Number of cows per herd ranged from 35 to 900. Daily milk production varied between 35 and 95 pounds per cow. Eleven herds fed a total mixed ration while the other herds were component fed. Forages fed varied from 43 to 95% of total ration dry matter. All plans were written by 2 individuals that were certified planners. Farm visits were made to obtain animal, ration and feeding management information. The planners kept records of travel time, on-farm time and office time required to develop the plans. The average time required to develop a plan was 53 h with a range of 22 to 79 h. There was no relationship between herd size and management system in terms of the time required to develop a plan. The key factor that influenced the time required to write a plan was the quality and ease of access of the farm records. Eleven herds had milk urea nitrogen values >12 mg/dL compared with the guideline range of 8 to 12 mg/dL. Fifteen herds had ration CP >16.5% and ration P >100% of requirements. The results of this project can be used as a base to determine the time needed to develop a plan and establish a payment rate for planners.

Key Words: NRCS, feed management, nutrient management

225 Dairy employee training: A new extension educational approach. M. Rovai1*, H. Carroll2, R. Foos3, T. Erickson1, and A. Garcia1. 1Dairy and Food Science Department, South Dakota State University, Brookings, SD, 2Animal Science Department, South Dakota State University, Brookings, SD, 3Department of Occupational Safety and Ergonomics, Colorado State University, Fort Collins, CO.

Growth in today’s dairies has led to increasing dependence on Latino immigrant workers. Shortage of qualified personnel prompts owners to assign training to tenured employees which can perpetuate bad habits. New educational trainings to improve dairy practices are required. The aim was to create similar training courses used by other industries, with a strategic approach to environmental sustainability, animal health and wellbeing, milk quality, and workers’ health. “Dairy Tool Box Talks” program was conducted over 10-week period in Spanish at 3 SD dairies. Employees (n = 75) involved in milking operations had weekly talks included a 1-h cattle handling demonstration and 9 30-min classroom trainings covering basics understanding of animal care and employee safety. Throughout this period bulk tank milk was tested for milk quality. To evaluate the employees understanding a final group assessment was conducted at wk 10 using Turning Technologies. Average daily milk yield was 33 kg/cow and bulk milk SCC ranged from 159,000 to 270,000 cells/mL with a significant increase (P < 0.001) expected by the season (summer). Coliforms did not differ by farm (4.48, 3.54 and 3.74 log cfu/mL for farms A, B and C, respectively); however, there were lower counts during the last 4 weeks (P < 0.05; 3.21, 2.78 and 0.77 log cfu/mL for farms A, B and C, respectively), suggesting improved hygiene practices. The trainings resulted in significant outcomes from a better understanding of farming practices to a higher milk quality harvested throughout the study. Nearly 85% agreed sessions helped with job confidence and 76% considered the program length adequate as well as the topics. Furthermore, 95% desired more involvement during sessions from farm management. Positive changes in employee behav-
ior, better working relations and hygiene awareness were noted during the employer’s interview. These changes should increase employee’s productivity, reduce costly and even fatal farm accidents and improve employee retention. The sessions were highly effective since they were offered in the workers’ native language and were tailored to weekly single topic trainings.

**Key Words:** dairy worker, training, Latino employees

226 The fact and fiction about dairy personnel training and performance. G. M. Schuenemann*, J. D. Workman, J. M. Piñeiro, B. T. Menichetti, A. A. Barragan, and S. Bas, *Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH.*

It is common to observe great variation in dairy personnel turnover and performance within and between dairy herds. The objective was to assess the types of training requested by stakeholders for their dairy personnel and the actual problems reported by personnel. A total of 1,100 individual written requests for dairy personnel training were determined the perceived needs for training by stakeholders (farm owners, managers, veterinarians, or consultants). All training sessions consisted of ~1 h lecture followed by ~1–2 h of demonstration and supervised hands-on practice designed to improve knowledge and skills. At the conclusion of each training session, dairy personnel were asked to list the problems that they believe should be addressed to improve their work performance. Written feedback from 2,900 individual workers representing 450 dairy herds (conventional and certified organic) were assessed to determine the actual needs by personnel responsible to execute the daily tasks. The top 5 requests for personnel training, according to stakeholders, were (1) milking routine and mastitis control, (2) nutrition management (TMR and feed bunk), (3) health screening for cows and calves, (4) replacement heifers (calving, colostrum), and (5) development of protocols. The top 5 areas to improve work performance, according to personnel, were (1) lack of communication with coworkers or managers, (2) lack of written protocols and resources for the tasks, (3) lack of facility maintenance (e.g., broken gate or hose), (4) properly organize and schedule tasks, and (5) schedule regular meetings to communicate and discuss tasks. While dairy personnel agreed (48%) or strongly agreed (52%) that the content of the training sessions were relevant to their work, and they gained significant knowledge and skills (*P* < 0.05); their self-reported areas of improvement were not necessarily associated with lack of knowledge and skills in the areas requested by stakeholders. Fully trained workers know what to do and how to do it; however, the self-reported areas likely affected their attitude which in turn reduces their overall work performance. Training for personnel is an essential management tool; however, the trainer must take into account the underlying problems negatively affecting performance.

**Key Words:** personnel, training, dairy cattle

227 Validation of dryer bag as a new method to estimate moisture content in feedstuffs. W. da Silva Machado* and M. I. Marcondes, *Federal University of Viçosa, Viçosa, Minas Gerais, Brazil.*

Procedures to determine moisture content accurately are expensive and not practical to be used in the farm routine. Thus, the Dryer Bag (DB) method was developed as an instrument that uses common commercial hair dryer to determine moisture content of feeds. To test its accuracy, 13 feedstuffs moisture contents were compared when estimated by DB, 55°C Forced-Air-Oven (55FAO) for 72 h, and 105°C oven (105O) for 24 h. Samples of alfalfa (AL), perennial peanut (PP), *Brachiaria* grass (BG), sugarcane (SC), elephant grass (EG), coast-cross grass (CCG), corn whole plant (CWP), corn silage (CS), *mombaça* grass (MG), total mixed ration (TMR), corn meal (CM), soybean meal (SM), and commercial concentrate with 21% crude protein (21CP) were used. Data were analyzed as a completely randomized design, with 3 replications, and significance was declared at *P* < 0.05. Moisture estimated by DB was similar to 105O for all feedstuffs (*P* > 0.05). However, both DB and 105O differ from 55FAO for 21CP, CM, SM and TMR (*P* < 0.05). As previously described in the literature, 55FAO is not able to remove entirely the moisture from concentrate feeds and TMR, thus DB and 105O are indicated to determine moisture in feedstuffs in the farm routine.

**Table 1 (abstract 227).** Dry matter (%) of feedstuffs determined by dryer bag (DB), 105°C oven (105O; 24 h) and 55°C forced-air-oven (55FAO; 72 h) methods

<table>
<thead>
<tr>
<th>Feedstuff</th>
<th>DB</th>
<th>105O</th>
<th>55FAO</th>
<th>SE</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>18.2</td>
<td>18.0</td>
<td>18.0</td>
<td>0.26</td>
<td>0.308</td>
</tr>
<tr>
<td>Perennial peanut</td>
<td>22.3</td>
<td>22.5</td>
<td>22.7</td>
<td>0.23</td>
<td>0.087</td>
</tr>
<tr>
<td>Brachiaria grass</td>
<td>27.6</td>
<td>27.7</td>
<td>27.8</td>
<td>0.23</td>
<td>0.342</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>23.5</td>
<td>23.9</td>
<td>24.0</td>
<td>0.28</td>
<td>0.095</td>
</tr>
<tr>
<td>Elephant grass</td>
<td>14.8</td>
<td>14.9</td>
<td>14.9</td>
<td>0.23</td>
<td>0.815</td>
</tr>
<tr>
<td>Coast-cross grass</td>
<td>22.9</td>
<td>23.0</td>
<td>23.1</td>
<td>0.26</td>
<td>0.539</td>
</tr>
<tr>
<td>Corn whole plant</td>
<td>24.2</td>
<td>24.5</td>
<td>24.4</td>
<td>0.26</td>
<td>0.236</td>
</tr>
<tr>
<td>Corn silage</td>
<td>25.2</td>
<td>25.0</td>
<td>25.4</td>
<td>0.23</td>
<td>0.080</td>
</tr>
<tr>
<td>Mombaça grass</td>
<td>20.9</td>
<td>21.1</td>
<td>21.1</td>
<td>0.23</td>
<td>0.351</td>
</tr>
<tr>
<td>Total mixed ration</td>
<td>37.4A</td>
<td>37.5A</td>
<td>39.9B</td>
<td><em>P</em> &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Corn meal</td>
<td>88.4A</td>
<td>88.2A</td>
<td>90.5B</td>
<td><em>P</em> &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Soy meal</td>
<td>87.9A</td>
<td>87.9A</td>
<td>90.2B</td>
<td><em>P</em> &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>21CP</td>
<td>85.2A</td>
<td>85.1A</td>
<td>88.1B</td>
<td><em>P</em> &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

*ABC* Different superscript letters in the same row indicate difference at *P* < 0.05. 121CP commercial concentrate with 21% crude protein.

**Key Words:** forced air oven, hair dryer, farm routine

228 Maximizing income over feed cost by grouping cows with mixed-integer programming. Y. Wu*, V. Cabrera, and R. Shaver, *University of Wisconsin-Madison, Madison, WI.*

Study objective was to maximize income over feed cost (IOFC) by mixed-integer programming (MIP), an optimization engine with objective function: max(IOFC) = Σ(g1i × MYi × P) − Σ(g1i × DMI × Path(g1i × NECi) × C1) − Σ(g1i × DMI × Pbl(g1i × CPCi) × C2) + Σ(g2i × MYi × P) − Σ(g2i × DMI × Pbl(g2i × NECi) × C1) − Σ(g2i × DMI × Pbl(g2i × CPCi) × C2), s.t. g1, g2 ∈ {0,1}, g1 + g2 = 1, where j is group, i is cow. Milk yield of cow i (MYi) is constant. NECi, CPCi and DMIi are cow i’s NE requirement (Mcal/kg DM), CP requirement (%) and daily dry matter intake (kg/cow), respectively calculated according to NRC. Path() is the value ranking as a*0.01*(vector length) and “a” is between 0 and 100. P, C1 and C2 are milk (0.35 $/kg), NE (0.07 $/Mcal) and CP (0.4 $/kg) prices, respectively. BW was calculated with Korver function as described by van Arendonk. We compared MIP with cluster (by NE) strategy in even and uneven grouping using same IOFC function, groups sizes and percentiles of NE and CP. The daily data of 471 cows from the University of Wisconsin-Madison Dairy Science herd (BW = 604.6 ± 28.9kg, DIM = 152 ± 103days, Milk yield = 43.1 ± 10.4kg/day) were used. MIP had greater average IOFC than cluster strategy in both even grouping (10.175 vs. 9.996 $/cow per d) and uneven grouping (10.383 vs. 10.175 $/cow per d).
vs. 10.193 $/cow per d). Compared with cluster strategy, MIP had 202 (42.89%) and 150 (31.85%) cows grouped differently in even and uneven grouping. FCM, feed efficiency (FCM/DMI) and IOFC were compared (Table 1). MIP approach seems to have better performance than cluster nutritional grouping, but animal experiments are needed.

**Key Words:** nutritional grouping strategy

### Table 1 (abstract 228). Summary of MIP and cluster grouping strategy

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>Group</th>
<th>MIP (low)</th>
<th>Cluster (low)</th>
<th>t</th>
<th>P-value</th>
<th>MIP (high)</th>
<th>Cluster (high)</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Even grouping</strong></td>
<td>FCM (kg/d/cow)</td>
<td>44.5 ± 9.1</td>
<td>40.1 ± 7.8</td>
<td>-5.6</td>
<td>&lt;0.001</td>
<td>43.5 ± 10.7</td>
<td>47.9 ± 10.3</td>
<td>4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>FCM/DMI</td>
<td>1.7 ± 0.3</td>
<td>1.5 ± 0.2</td>
<td>-8.9</td>
<td>&lt;0.001</td>
<td>1.7 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>7.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>IOFC ($/cow per d)</td>
<td>10.3 ± 2.8</td>
<td>9.0 ± 2.3</td>
<td>-5.3</td>
<td>&lt;0.001</td>
<td>10.1 ± 3.2</td>
<td>11.0 ± 2.7</td>
<td>3.3</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Uneven grouping</strong></td>
<td>FCM (kg/d/cow)</td>
<td>44.0 ± 9.4</td>
<td>43.8 ± 9.5</td>
<td>-0.2</td>
<td>0.9</td>
<td>44.1 ± 12.1</td>
<td>44.5 ± 11.7</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>FCM/DMI</td>
<td>1.7 ± 0.31</td>
<td>1.6 ± 0.2</td>
<td>-4.4</td>
<td>&lt;0.001</td>
<td>1.8 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>10.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>IOFC ($/cow per d)</td>
<td>10.3 ± 3.0</td>
<td>10.2 ± 2.9</td>
<td>-0.4</td>
<td>0.7</td>
<td>10.7 ± 3.4</td>
<td>10.0 ± 3.2</td>
<td>-1.3</td>
<td>0.2</td>
</tr>
</tbody>
</table>
229 The role of nutrition in dairy cow health and welfare in grazing systems. J. Roche*, 1, G. Zobel*, 2, J. Huzyz*: 1, and J. Loor4, 1DairyNZ, Hamilton, New Zealand, 2AgResearch, Hamilton, New Zealand, 3Cal Poly, San Luis Obispo, CA, 4University of Illinois, Urbana, IL.

Animal welfare measures must consider a cow’s health and production (i.e., functional state), how she feels about her situation (i.e., affective state), and whether her management allows for “natural” behaviors. Although there are accepted, quantitative measures of functional state, affective state and naturalness are typically only assessed using indirect, behavior-based measures. The most unique factors likely to affect welfare differentiating cows in grazing systems from their housed counterparts fed TMR are climatic variation, a lack of shade and shelter, and the relatively low DMI of grazing cows. Both access to and quality of feed is also less consistent and predictable in grazing cows. From a functional state perspective, fluctuating DMI is associated with risk of disease, particularly in early lactation; when affective state is considered, hunger becomes a concern as well. Although grazing cows eat less, they also produce less milk; the lack of a considerable difference in negative energy balance between unsupplemented and supplemented grazing cows in early lactation likely indicates that output is reduced to match nutrient intake. The risk of disease is minimized by ensuring that animals are adequately managed in late lactation and the dry period, are not too fat at calving, and that pre-calving energy intake is controlled. Hunger is more difficult to assess and, is often, emotively anthropomorphized. Measurements of the neuro-endocrine factors that respond to nutrient intake and expenditure have been established; however, these should be paired with behavioral measures, such as vocalizations, changes in activity and motivation to access feed. As measures are further developed for assessing general animal affective state (e.g., cognitive bias, anticipatory behavior) these should be included when assessing welfare. Although its role in the functional state of cows has been extensively investigated, further research is required to better understand the role of nutrition in cows’ quality of life in grazing systems.

Key Words: pasture, affective state, functional welfare

230 The impact of forages and their quality on the efficiency of dairy production. R. D. Shaver*, Department of Dairy Science, University of Wisconsin, Madison, WI.

Average 2016 production efficiency in USA exceeded 10,000 kg milk per cow (USDA-NASS); about 5% of WI dairy herds on DHI test exceeded 13,500 kg milk per cow. Some projections suggest that average production efficiency in USA dairy herds will reach this level within 20 yr. In a survey of feeding programs from selected WI high-producing (≥13,500 kg milk per cow) herds, 63% of milk production, on average, was attributed to dietary forages. Estimated percentages of dietary nutrients provided by forages, on average, were as follows: fiber (≥75%), protein (45%), energy (50%) and starch (40%). The foregoing points denote the importance of improved forage quality. Key forage quality indicators are reduced NDF content and greater NDF digestibility (ivNDFD) for decreased fill limitation of DMI to allow for production gains from forages or the feeding of higher forage diets. In a multi-commercial lab, multi-year survey of corn silage analyses, NDF% (DM basis) and ivNDFD (30 h; % of NDF) were 41 to 36% (mean – 1 SD) and 54 to 60% (mean + 1 SD), respectively. Starch, influenced by grain yield, contributes greatly to the energy value of corn silage. In the lab survey, starch was 32 to 39% (DM basis; mean + 1 SD). The digestibility of starch in corn silage is influenced by harvest maturity, kernel processing, time in storage before feeding, and kernel endosperm properties. The ivNDFD in corn silage is influenced primarily by hybrid type and growing environment. High cutting can decrease NDF% and increase ivNDFD and starch%. Optimal chop length questions have increased in concert with the feeding of higher corn silage diets. Key forage quality indicators for legume forages also include greater CP% for reducing supplemental protein. In the lab survey, legume forage NDF, ivNDFD and CP were 42 to 37% (mean – 1 SD), 46 to 57% of NDF and 21 to 24% CP (mean + 1 SD), respectively. Reduced-lignin alfalfa varieties are now available commercially for either increased ivNDFD or extended harvest windows for increased yield. New questions involve harvest and feeding strategies when combining reduced-lignin alfalfa silage and brown midrib corn silage.

Key Words: forage, corn silage, alfalfa

231 The filling effect of forages and its effect on feed intake of lactating cows. M. Allen*, Michigan State University, East Lansing, MI.

Forages provide physically effective fiber, which is required for proper rumen function. However, forage neutral detergent fiber (NDF) is filling and can limit dry matter intake (DMI) by distention from undigested feed residues within the gastrointestinal tract. The rumen is generally regarded as the site within the gastrointestinal tract at which distention limits DMI. A signal from distention is integrated in brain feeding centers and increasingly limits DMI as milk yield increases. Therefore, less filling rations should be fed to cows with high milk yield. Forage NDF has a longer ruminal retention time than NDF from other sources because of longer initial particle size. While grinding and pelleting forages generally increases DMI, decreasing particle size of chopped forages generally has less effect unless chopped very finely. Digestibility of NDF varies among forage type (e.g., grasses vs. legumes) and generally decreases as forages mature and become more lignified. Greater in vitro or in situ NDF digestibility of forages has been related positively to DMI and milk yield of lactating cows. Within a forage type, NDF that is more fermentable clears from the rumen faster and is less filling, allowing greater DMI when limited by distention. However, this applies only within forage type; NDF from perennial grasses is generally much more digestible than NDF from legumes but is also more filling and more likely to limit DMI. This is because grasses are more resistant to particle size reduction by chewing during eating and ruminating. Faster particle size reduction will increase the mass of particles below the threshold size to pass from the rumen as well as decrease the ability of the rumen to selectively retain those particles by decreasing the size of the rumen mat. Therefore, in vitro NDF digestibility should be used to compared within forage type only. The overall filling effect of diets is determined primarily by forage NDF content, forage particle size, fragility of forage NDF, and NDF digestibility within a forage family.

Key Words: rumen distention, forage NDF, forage fragility
The influence of forage feeding on the ruminal microbiome of dairy cattle and its implications for dairy production. P. J. Weimer*1,2, 1USDA-ARS, Madison, WI, 2University of Wisconsin-Madison, Madison, WI.

Forage utilization is the key distinguishing feature of ruminant agriculture, and the ruminal microbiome is the agent by which these forages are converted to VFA and microbial cell protein that nourish the ruminant host. Culture-independent studies, based on phylogenetic classification of sequences of small-subunit ribosomal RNA molecules, have shown an impressive diversity in species composition of the ruminal microbiome. Because forages are compositionally and structurally more diverse and more complex than are grains and other concentrates, they have the potential to support a greater diversity of microbes within the rumen, and this diversity is extended further when forages are combined with concentrates in a TMR. Although microbiome composition varies across individual animals fed the same diet, certain forage-specific patterns in community composition have emerged. High-forage diets generally increase the relative abundance of phylum Firmicutes relative to phylum Bacteroidetes, consistent with higher ruminal ratios of acetate/propionate expected from known differences in the physiologies of cultured members of these phyla. Worldwide ruminal microbiome census studies have shown that forage-fed ruminants also host elevated populations of uncultured Bacteroidiales, Clostridiales and Ruminococcaceae. Subtle differences in forage form may also affect microbiome composition. For example, orchardgrass fed as pasture selectively increases the abundance of genus Butyrivibrio compared with feeding the same forage as hay, and the parallel observed increase in butyrate production may enhance development of ruminal papillae essential for efficient VFA absorption. Bacterial species differ in their affinities for particular plant tissue types, further adding to the complexity of microbial interactions during forage degradation. Several recent studies with cows on mixed rations have shown associations between specific bacterial taxa and important production metrics such as feed efficiency and milk composition, but such studies have not yet been carried out on all-forage diets.

Key Words: forage, microbiome, rumen
Physiology and Endocrinology II

233 Hepatic mTORC2 synchronizes glucose and fatty acid metabolism to sustain cellular energy status. S. I. Arriola Apelo1,5, X. Guo2,6, A. Lin1,5, E. J. Meyer1,5, N. E. Cummings3,5, C. P. Pumper1,5, D. J. Pagliarini1,6, and D. W. Lamming1,5.

The mechanistic target of rapamycin (mTOR) protein kinase exists in 2 different complexes. Genetic inhibition of mTORC2 in the liver disrupts whole body glucose homeostasis. The objective of this study was to determine the mechanism of mTORC2 regulation of hepatic glucose metabolism. We performed quantitative mass spectrometry analysis of cultured primary hepatocytes from 9-wk-old C57BL/6 wild type mice or mice which Rictor, an essential protein of mTORC2, had been deleted exclusively in the liver. Using 5% false discovery rate, we detected 1703 proteins significantly affected by Rictor deletion. As previously described, we found that gluconeogenic enzymes were upregulated, while proteins involved in fatty acid synthesis were downregulated in Rictor knock out (RKO) hepatocytes. Additionally, we found that rate limiting glycolytic enzymes were downregulated, while enzymes involved in TCA cycle, mitochondrial fatty acid transport and oxidation, ketone bodies and cholesterol synthesis were upregulated in RKO hepatocytes. Wild type and RKO hepatocytes were cultivated and 24 h later oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) as a mean of glycolytic function, were measured in vitro. While OCR was not affected by Rictor deletion, ECAR and ECAR/OCR were significantly reduced, suggesting that RKO hepatocytes reprogram energy sources to sustain respiration rate. We did not detect changes in OCR in the presence of a mitochondrial fatty acid transport inhibitor. Therefore, we measured plasma levels of BHBA and cholesterol, 2 other potential metabolic fates of acetyl CoA, in addition to TCA cycle, derived from fatty acid oxidation. While plasma free cholesterol and cholesterol esters were not affected by hepatic Rictor deletion, fasting plasma BHBA was significantly increased. Our results suggest that to sustain energy levels under dysregulated gluconeogenesis, RKO hepatocytes increase fatty acid oxidation and ketone body production.

Key Words: mTORC2, hepatocyte, energy metabolism

234 Association between bone and energy metabolism in caleidial treated dairy cows. R. M. Rodney1,2, N. P. Martinez1, P. Celi2,3, J. E. P. Santos1, D. R. Fraser2, and I. J. Lean1,2,5.

There are beneficial effects of vitamin D and DCAD interventions in the pre-calving period on production, health and reproduction of cattle. How interventions during this time can have extended effects in the following lactation is not yet clear. Time series analysis was used to examine responses between metabolites in peri-parturient cows to evaluate roles of bone metabolism in the adaption to lactation. Holstein cows (n = 32) were blocked by parity and milk yield and randomly allocated to diets containing either 25-OH-D3 or vitamin D3 (3 mg/11 kg of DMI) and positive (+130 mEq/kg) or negative (−130 mEq/kg) DCAD from 255 d gestation to parturition. Blood was sampled every 3 d pre- and postpartum (14 samples/cow). Concentrations of 25-OH-D3, vitamin D3, osteocalcin (OC), crosslaps, IGF-1, glucose and insulin were determined. Feeding 25-OH-D3, compared with vitamin D3, increased blood concentrations of 25-OH-D3 pre- (264.2 ± 61.3 ng/mL) and postpartum (170.8 vs 51.3 ± 6.2 ng/mL) and decreased concentrations of vitamin D3 (1.2 vs 14.5 ± 0.6 ng/mL pre- and 1.9 vs 3.2 ± 0.6 ng/mL postpartum for 25-OH-D3 and vitamin D3 respectively). Pre-partum, cows fed the negative DCAD diets had decreased blood concentrations of vitamin D3 and glucose. Nulliparous cows had increased concentrations of OC, crosslaps, IGF-1, glucose and insulin when compared with multiparous cows. Data for each cow were used to produce approximately stationary series and time series cross correlations between variables at 3d lags calculated for use in a meta-analytical evaluation of associations between metabolites over time. Associations identified included those between 25-OH-D3 and IGF-1 (ES 0.2 to 0.5 at 3 d before and ES = −0.1 to −0.4 at −9 d before) and between vitamin D3 and IGF-1 (positive on the same d and 3 before, and negative at 6 d before and 6 d after). Associations between IGF-1, uOC, oOC, crosslaps, and glucose were also present. A feedback loop between bone and energy metabolism was identified as indicated by alternative positive and negative effect size estimates at different lags. The positive association between OC and IGF-1 may indicate an effect that could influence future production.

Key Words: IGF-1, osteocalcin, vitamin D

235 Blue light from light-emitting diodes (LEDs) directed at a single eye elicits a dose-dependent suppression of melatonin in dairy cows. B. A. Murphy1, M. M. Herlihy2, M. B. Nolan1, and S. T. Butler*2, 1University College Dublin, Belfield, Dublin, Ireland, 2Teagasc, Moorepark AGRIC, Cork, Ireland.

The objective of this study was to determine the minimum light intensity directed at a single eye required to suppress melatonin (MT) levels to concentrations observed under indoor lighting systems in dairy cows. Following a 14-d environmental conditioning period comprising 8 h of light, 16 h of dark (LD8:16), where mean ±SD white light intensity levels by day (08:00 – 16:00) at eye level were 237 ± 68 lx, 5 nonlactating Holstein-Friesian cows were exposed to each of the following light intensities for 8 additional hours (16:00 – 00:00) using a 5 × 5 Latin Square design; <1 lx; 70 lx; 125 lx; 175 lx and 225 lx. Light was administered via head worn masks fitted with LEDs emitting short wavelength blue light (465 nm) to the right eye. Each treatment night was followed by a break night, where the animals again received LD8:16. Two days after completion of the different light intensity treatments, all cows were exposed to the indoor lighting system until 00:00 (LIGHTS ON). Blood samples were collected from indwelling jugular catheters at 16:00, 17:00, 18:00, 20:00, 23:00, 00:00 and 01:00 on treatment nights and at 18:00 and 22:00 on break nights. Plasma samples were assayed for MT by radioimmunoassay. MT data were log-transformed, and analyzed using mixed models with treatment, period, hour and treatment × hour as fixed effects and cow as a random effect. A dose-dependent effect of treatment on mean circulating MT concentrations (and 95% CI) between 16:00 and 00:00 was observed [9.1 (6.8, 12.2), 4.9 (3.7, 6.6), 4.4 (3.2, 5.8), 3.3 (2.5, 4.4), 1.8 (1.4, 2.5) and 1.9 (1.4, 2.5) ng/mL for 0, 70, 125, 175, 225 lx].
225 lx and LIGHTS ON treatments, respectively). Only the 225 Lux mask treatment did not differ from LIGHTS ON, and hence 225 lx is the minimum light intensity required. Future studies will examine the effect of photoperiod manipulation in grazing animals.

Key Words: melatonin, photoperiod

236 The effects of blood composition and age on PBMC mitochondrial enzyme activity in prewean dairy calves. A. M. Niesen* and H. A. Rossow, University of California Davis, Davis, CA.

Mitochondria are central to metabolism, nutrition and health but many factors can influence their efficiency. The objective of this study was to determine if mitochondrial enzyme activity rates of peripheral blood mononuclear cells (PBMCs) were affected by changes in blood composition, aging or breed. Data from 23 Holstein and 23 Jersey heifer calves was collected from age 4 to 66 d. Blood samples were collected at 1, 2 and 9 wk of age and analyzed using a Hemavet 950FS Hematology Analyzer (Drew Scientific, Miami Lakes, FL) to determine levels of neutrophils (NE, K/ul, %), lymphocytes (LY, K/ul, %), monocytes (MO, K/ul, %), eosinophils (EO, K/ul, %), and red cell distribution width (RDW, %). Additional blood was used to determine plasma total protein (TP, g/dl) and obtain crude mitochondrial extracts from the PBMC fraction using a mitochondria isolation kit from Abcam (Cambrige, MA). Enzyme activities for citrate synthase (CS), Complex I (CI), Complex IV (CIV) and Complex V (CV) were all determined using kits from Abcam (Cambrige, MA). Activity rates were compared by time point, breed, and blood parameters and were analyzed using the MIXED procedure of SAS (v:9.4) with repeated measures, breed as a fixed effect and time point as a random effect. Breed was not significant for all comparisons. CI activity was not different between time points but was affected by RDW (P < 0.02). CIV (P < 0.04) and CV (P < 0.01) showed an increase in activity with time point while CS (P < 0.01) decreased. An increase in EO and MO immune cell production increased CIV activity (P < 0.01) and CV activity (P < 0.01) respectively. As white blood cell (WBC) differential fluctuated CV activity increased as NE% (P < 0.01) and MO% (P < 0.01) decreased and LY% (P < 0.01) and EO% (P < 0.01) increased. These findings suggest that changes in mitochondrial enzyme activity is impacted by fluctuations in NE, LY, MO, EO cell populations and changes as calves age.

Key Words: PBMC, mitochondria, dairy

237 Effect of delayed colostrum feeding on plasma concentrations of glucagon-like peptide 1 and 2 in calves. Y. Inabu*, A. Fischer, T. Sugino*, M. Oba, L. L. Guan, and M. Steele, 1The Research Center for Animal Science, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan, 2Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada.

Glucagon-like peptide 1 (GLP-1) is involved in glucose homeostasis by stimulating insulin secretion while GLP-2 increases mucosal growth of the small intestine. The objective of this study was to evaluate effect of delayed colostrum feeding on plasma concentrations of GLP-1 and 2. Holstein bull calves (n = 27) were fed colostrum at 7.5% of BW within 1 h of birth (0H), 6 h after birth (6H) or 12 h after birth (12H; n = 9 for each treatment), and fed a milk replacer at 2.5% of BW at 12 h after the colostrum feeding and every 6 h thereafter. Blood samples were obtained before the colostrum feeding and every 3 h after the colostrum feeding for a 36-h period, and plasma concentrations of GLP-1, GLP-2 and glucose were measured. Data were analyzed by ANOVA to determine effects of treatment, time as repeated measures, and their interaction. Time by treatment interaction was observed for plasma GLP-1 concentrations (P = 0.04); GLP-1 concentrations increased after colostrum feeding (P < 0.01) regardless of treatment, but they tended to peak faster (P = 0.10) for 0H (12.7 ± 2.6 h; LSM ± SEM) than 6H (20.7 ± 2.6 h) or 12H (18.7 ± 2.6 h) calves, and its concentrations at 12 h after colostrum feeding were higher for 0H (3.37 ± 0.53 ng/mL) than 12H (2.11 ± 0.53 ng/mL). In addition, area under the curve (AUC) for GLP-1 concentrations was higher (P < 0.01) for 0H (6.65 ± 0.82 ng/mL × h) than 6H (3.12 ± 0.82 ng/mL × h) or 12H (2.23 ± 0.82 ng/mL × h). Similarly, plasma concentrations of GLP-2 and glucose increased after colostrum feeding (P < 0.01), and reached their peak concentrations at 15.4 ± 3.1 and 26.6 ± 1.9 h after the feeding, respectively. However, treatment did not affect plasma concentrations of GLP-2 and glucose, time to reach their peak concentrations, and their AUC for a 36-h period after colostrum feeding. In conclusion, delayed colostrum feeding may decrease plasma GLP-1 concentrations for a 36-h period after colostrum feeding without affecting plasma concentrations of GLP-2 and glucose.

Key Words: glucagon-like peptide 1 (GLP-1), glucagon-like peptide 2 (GLP-2), colostrum

238 Systemic administration of bovine recombinant interleukin-8 induces long-term insulin resistance in Holstein bull calves. M. Ziniola* and R. Bicalho, Cornell University, Ithaca, NY.

This study was conducted to assess the effects of systemic administration of bovine recombinant interleukin-8 (rIL-8) on whole-body insulin resistance in Holstein bull calves. Additionally, blood metabolites and complete blood cells count were evaluated. Holstein bull calves (30 ± 6 d of life) were individually housed and randomly allocated into 1 of 2 treatment groups; rIL-8 (rIL-8, n = 10) and controls (CTR, n = 8). Calves assigned to the rIL-8 group received a total of 7 rIL-8 injections consecutively from 1 to 4 (one s.c. injection followed by 6 i.v. injections at a dose of approximately 4 μg of rIL-8 per kg of body weight). Calves in the CTR group also received 7 injections but containing only sterile saline solution. To determine whether calves injected with rIL-8 were developing insulin resistance, 2 intravenous glucose tolerance tests (IVGTT) were conducted at 12 h, and 7 d after treatment cessation. Blood samples were collected at 30 (before injection, 0830h), 30, 60, 120, 240 and 360 min after daily treatments during treatment days (d 1 to 4). Additionally, blood samples were collected daily (0830h) from d 5 to 10 (end of follow-up period). Serum was harvested and the following parameters were measured; β-hydroxybutyrate (BHB), nonesterified fatty acid, glucose, insulin, plasma urea nitrogen, haptoglobin, and complete blood cells count. Calves treated with rIL-8 had higher concentrations of insulin and higher positive area under the curve (AUC) in response to the glucose challenge when compared with controls. Moreover, BHB was significantly higher for rIL-8 calves than for CTR calves (rIL-8 = 3.54 ± 0.10 mg/dL; CTR = 2.99 ± 0.12 mg/dL). Treatment also affected the count of lymphocytes (rIL-8 = 4.52 ± 0.12; CTR = 3.84 ± 0.14 × 10^3/μL), monocytes (rIL-8 = 0.87 ± 0.03; CTR = 0.67 ± 0.04 × 10^3/μL), and granulocytes (rIL-8 = 3.54 ± 0.22; CTR = 2.66 ± 0.24 × 10^3/μL). We conclude that systemic administration of rIL-8 induces strong and long lasting insulin resistance in milk fed Holstein bull calves. Moreover, we observed that systemic administration of rIL-8 alters blood metabolites and white blood cell population.

Key Words: insulin, metabolism, interleukin-8
239 Luteolysis and concentrations of estradiol during preovulatory period in 7-day or 5-day Co-Synch with one or two doses of prostaglandin in dairy heifers. C. Navamukkara*, V. Khunthusaeng, N. Kogram, and S. Uriyapongson, Agricultural Biotechnology Research Center for Sustainable Economy (ABRCSE), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand.

The objective of this study was to determine if there were different in luteolysis and circulating estradiol (E2) concentrations in 7-d or 5-d Co-Synch with one or two doses of prostaglandin (PGF2α). Dairy heifers (n = 60) were randomly assigned to receive 7-d or 5-d Co-Synch protocols. Animals received 100 µg GnRH and a CIDR insert on d 0 for 7 and 5 d, respectively. Heifers in 7-d Co-Synch received a single dose (25 mg) of PGF2α at CIDR removal, 100 µg GnRH at 54 h after CIDR removal, and were inseminated concurrently, 54 h after CIDR removal. Heifers in 5-d Co-Synch received 25 mg PGF2α at CIDR removal, the second dose 8 h later, 100 µg GnRH at 72 h after CIDR removal, and were inseminated concurrently, 72 h after CIDR removal. Blood collected on d 0, 7 or 5, and 9 or at the AI time was assayed for progesterone (P4) and E2. Defined luteolysis occurred when P4 was ≥1 ng/mL on d 7 or 5 and 72 or 54 h (d 9) was <1 ng/mL. Concentrations of P4 did not differ between groups. Luteolysis was not different between the groups [86.7% (25/30) vs. 90.0% (27/30)]. Diameter of follicle at the second GnRH did not differ (P > 0.05) between treatments (13.2 and 12.7 mm, respectively). Although treatments did not affect the conception rate (P > 0.05), concentrations of E2 were greater (P < 0.01) on d 9 or before fixed time AI in 5-d Co-Synch group than 7-d Co-Synch (6.5 vs. 4.7 pg/mL). These results provide evidence that 5-d Co-Synch with 2 doses of PGF2α affects greater circulating E2 concentrations during preovulatory period and may increase conception success following a fixed time AI.

Key Words: luteolysis, estradiol, fixed-time AI

240 Differences of blood biochemical parameters among jugular, subcutaneous abdomen and coccygeal veins and between coccygeal artery and veins in dairy cows compare. Z. H. Wei*, B. X. Zhang, X. H. Wu, and J. X. Liu, Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, China.

Two trials were conducted to compare the differences of blood biochemical parameters from different sites in dairy cows. In trial 1, 10 animals were randomly selected from 35 Holstein cows (DIM = 202 ± 38.7; milk yield = 24.5 ± 3.8 kg/d; parity = 2.9 ± 1.2). Blood samples were collected from jugular vein, subcutaneous abdomen vein (SADV) and coccygeal vein into heparinized tubes at approximately 2 h after feeding, respectively, to investigate the differences of biochemical parameters among 3 sites of vein. In trial 2, 18 early lactation cows (DIM = 46 ± 12.6; milk yield = 42.6 ± 15.3 kg/d; parity = 3.3 ± 1.9) were used to collect the blood samples from coccygeal artery and vein into heparinized tubes at approximately 4 h after feeding, respectively. No hemolysis was found in both trials. ANOVA for biochemical parameters were performed using PROC GLM of SAS, and regression analysis was conducted using CORR of SAS. No differences were found in concentrations of creatinine, total bilirubin, total protein, albumin, and globulin, and activities of alanine transaminase, aspartate aminotransferase, and alkaline phosphatase among the 3 sites of veins (P > 0.05). Nonesterified fatty acids (NEFA) was higher (P = 0.02) in SADV than in jugular and coccygeal vein with no difference between jugular and coccygeal veins. β-hydroxybutyric acid (BHBA) was higher in jugular than in coccygeal vein, with higher value in coccygeal vein than in SAV (P < 0.01). Glucose concentration was higher in jugular vein than in SAV (P = 0.02). No differences were found in most of biochemical parameters between coccygeal artery and vein (P > 0.05), with higher concentration of glucose, NEFA and BHBA in coccygeal artery than in coccygeal vein (P < 0.01). Concentrations of glucose, NEFA and BHBA in coccygeal artery was closely correlated with those in coccygeal vein (R² = 0.842, 0.989 and 0.901, respectively, P < 0.01). The results from this study indicate that some of biochemical parameters may be different from blood site to site. Attention should be paid to these differences when the blood samples are analyzed.

Key Words: sampling sites, blood biochemical parameters, dairy cows

241 Effect of Dalmavital on pregnancy rate in CIDR-synchronized Nili-Ravi buffaloes. A. Sattar*, B. Munawwar, N. Ahmad, A. Rehman, S. Murtaza, M. R. Yousaf, U. Arshad, M. Ijaz,* A. Riaz1, 1Department of Theriogenology, University of Veterinary and Animal Sciences, Outfall Road, Lahore, Pakistan, 2Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Outfall Road, Lahore, Pakistan.

Nili-Ravi buffalo is the most valuable dairy animal in Pakistan. It is well known as black gold of Pakistan. Production efficiency of the breed is compromised due to its reproductive failure most of the time. This will lead to prolong calving interval which enhances input cost of farmer. To overwhelm the low reproductive competence, the objective of this study was to manage the reproduction by using Dalmavital (antioxidant) in CIDR synchronized protocol to increase pregnancy rate. For this purpose, 86 animals with healthy reproductive tract and good body conditions of mixed parity were recruited into 2 groups; (G1): CIDR; G2: CIDR+Dalmavital as control and treatment respectively. CIDR were placed at d 0 and removed at d 7 while PGF2α was injected at d 6. GnRH was injected 36 h after CIDR removal. Dalmavital was injected at CIDR insertion in G2. AI was performed at standing estrus, morning and evening at intervals of 12 and 24 h after GnRH injection. Pregnancy was monitored via ultrasonography at d 30. Data collected was analyzed through Chi-Square and Binary logistic regression model. Results revealed that Estrous intensity was significantly (P < 0.05) higher in CIDR-D than CIDR group. Pregnancy rates in CIDR and CIDR-D had no noteworthy difference (P > 0.05). There was no statistically difference when comparison was made parity wise in CIDR and CIDR-D groups. However, overall there were 57.33% pregnancy rates in CIDR-D as compared CIDR which were 51.5%. Pregnancy rates were improved in fourth and fifth parities (older) as compared with first, second and third parities. It is concluded that Dalmavital has positive effect on estrous intensity but has narrow role in pregnancy rate. However in aged animals pregnancy rates were improved. That may be due to antioxidant nature of Dalmavital which neutralize reactive oxygen species (ROS).

Key Words: Dalmavital, CIDR, buffalo


Peptide transporters, also named proton-coupled oligopeptide transporters (POTs), play a great role in mediating cellular uptake of di/tripeptides...
and peptidomimetic drugs via the inwardly directed proton motive force. However, the expression profile and transport kinetics of the transporters in bovine mammary gland remain unknown. Polymerase chain reaction (PCR) and Western blotting were used to investigate expression of POTs (PepT1, PepT2, PhT1, PhT2) in BMECs. Immunofluorescence was used to determine the location of the peptide transporters in bovine mammary gland tissue. Uptake and transport kinetic studies were performed with stable metabolically β-alanyl-l-lysyl-Nε-7-amino-4-methyl-coumarin-3-acetic acid (β-Ala-Lys-AMCA) in BMECs. The effects of time, pH, concentration and inhibitors on β-Ala-Lys-AMCA uptake were studied. All experiments were performed in 3 replicates. Data were analyzed using GLM procedure of SAS software. The results showed PepT2 and PhT1 are expressed in bovine mammary gland by using PCR and Western blotting. The immunofluorescence results showed that PhT1 and PepT2 are both located on the apical membrane and basolateral membrane in BMECs. The uptake of β-Ala-Lys-AMCA was linear during the 60 min incubation period and reached plateau at 60 min. The optimal pH for the uptake of β-Ala-Lys-AMCA in BMECs was 6.5. The transport kinetic study suggested that the uptake of β-Ala-Lys-AMCA is saturable over the tested concentration. The binding affinity (Km) and the maximal velocity (Vmax) exhibited in BMECs is 82 µmol and 124 pmol/min/mg protein. The competitive inhibition result showed Gly-Sar, Met-Gly and Met-Met significantly inhibited β-Ala-Lys-AMCA uptake. However, histidine had no effect on β-Ala-Lys-AMCA uptake indicating that PhT1 may not be involved in β-Ala-Lys-AMCA uptake. Furthermore, knockdown of PepT2 significantly inhibited β-Ala-Lys-AMCA uptake. The above results showed that PepT2 may be involved in peptide transport, but PhT1 may not function in peptide transport in bovine mammary gland.

**Key Words:** transport kinetics, PepT2, PhT1

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Fetuin-A (FETA) is a FFA carrier and an acute-phase protein (APP) that enhances lipid-induced inflammation in AT of monogastrics. Little is known about its role and potential use as a biomarker in transition cows. We hypothesized that serum and AT FETA content increases as the periparturient period progresses and is enhanced by high lipolysis rate. Blood and subcutaneous AT were collected from 10 multiparous cows through the transition period at far off (FO: −51 ± 3d) and close-up dry (CU: −14 ± 2d), and early lactation (EL: 7 ± 0.5d). FETA was analyzed by ELISA in serum, and by RT-qPCR and protein blotting in AT. Contrary to our hypothesis, serum FETA concentration and AT gene and protein expression were greatest at FO compared with EL (P ≤ 0.05) when FFA concentration was the least (P < 0.01). Serum FETA concentration was 1.11 ± 0.08, 1.08 ± 0.08, and 0.98 ± 0.08 mg/mL at FO, CU and EL (P = 0.05), respectively, and was positively associated with serum albumin (r = 0.27; P = 0.03) and calcium (r = 0.32; P = 0.05). Circulating FETA was negatively associated with FFA (r = −0.25; P = 0.05) and BCS loss (r = −0.73; P < 0.001) over the transition period, and adverse health events at EL (r = −0.42; P = 0.05). AT FETA expression dynamics through FO, CU and EL was analogous to adipogenic and lipogenic genes PPARY, FASN, FABP4, and SCD1, and negatively correlated with AT inflammatory markers SPP1 (r = −0.46; P = 0.01) and CD68 (r = −38; P = 0.04). To test adipocyte inflammatory response to FETA in vitro, primary bovine adipocytes were treated with 0, 50, 100 or 200 µg/mL of FETA (8h), or LPS (25 ng/mL; 4h). Adipocytes treated with FETA had lower CCL2 expression than LPS (P = 0.03), and reduced adipocyte IL6 transcription (P = 0.01) when treated with 100 µg/mL of FETA compared with 0. These results indicate that FETA is a negative APP inversely linked to AT lipolysis and health events in transition cows. Contrary to monogastrics, FETA plays a beneficial role in AT inflammation in cows by modulating the expression of pro-inflammatory cytokines by adipocytes.

**Key Words:** adipose marker, inflammation, lipolysis
The objective was to assess the association between colostrum and plasma insulin concentration around parturition and milk yield in Holstein dairy cows. Our hypothesis was that low plasma insulin concentration (PI) around calving and during early lactation, as well as high insulin concentration in colostrum would be associated with milk production. Two experiments were conducted. In experiment 1, blood samples (BS) from cows (n = 48) were collected at −7, 0, 3 and 7 d relative to the day of parturition. Additionally, colostrum samples were collected immediately after calving. In experiment 2, BS from primiparous and multiparous cows (n = 237) were collected at 0, 3 and 7 d relative to the day of parturition. Insulin was measured using a bovine ELISA kit. Weekly milk yields were recorded for the first 7 weeks after calving. In experiment 1, cows were grouped according to the PI at d 0 based on the median (0.42 ng/mL), into LOW (PI < 0.42 ng/mL, n = 23) and HIGH (PI > 0.41 ng/mL, n = 25) cows. Insulin concentration at −7, 3 and 7 d did not differ between groups. However, LOW cows had significantly lower PI at d 0 (LOW, 0.28 ± 0.07; HIGH, 0.71 ± 0.06) compared with HIGH cows. No differences were found in colostrum insulin concentrations between groups. Relative to HIGH cows, LOW cows produced 5 kg/d more milk (LOW, 42 ± 1.15; HIGH, 37 ± 1.34). In experiment 2, cows were grouped according to the PI levels at −7 d in quartiles (1stQ, PI < 0.29 ng/mL, n = 57; 2ndQ, 0.29 ng/mL ≤ PI ≤ 0.34 ng/mL, n = 60; 3rdQ, 0.34 ng/mL < PI ≤ 0.43 ng/mL, n = 58; 4thQ, PI > 0.43 ng/mL, n = 62). Primiparous cows in the 4thQ had significantly higher PI at d 0, and produced significantly less milk (1stQ, 32.1 ± 2.4; 2ndQ, 33.6 ± 1.6; 3rdQ, 35.1 ± 4.1; 4thQ, 29.5 ± 1.1) compared with cows in the other quartiles. Multiparous cows in the 4thQ had significantly higher PI at d 0 compared with cows in the other quartiles. Additionally, cows in the 1stQ produced 5 kg/d more milk (1stQ, 47.6 ± 1.2; 2ndQ, 42.8 ± 1.3; 3rdQ, 42.5 ± 1.4; 4thQ, 41.3 ± 1.7) compared with cows in the other quartiles. PI at 3 and 7 d among the quartiles did not differ between groups. We conclude that low plasma insulin concentration at the day of parturition seems to be critical for milk yield in the long term.

Key Words: insulin, milk, parturition

The effect of nipple bottle vs. esophageal tube feeding of colostrum on absorption of IgG and plasma glucagon-like peptide-2 concentrations. M. Desjardins-Morrisette*1, J. K. van Niekerk1, D. Haines2, T. Sugino3, M. Oba1, and M. A. Steele1, 1Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, 2The Saskatoon Colostrum Co. Ltd., Saskatoon, SK, Canada, 3The Research Center for Animal Science, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan.

The objective of this study was to determine if feeding colostrum to newborn calves through an esophageal tube, compared with a nipple bottle, affects passive transfer of IgG and plasma glucagon-like peptide-2 (GLP-2) concentration. Twenty newborn Holstein bull calves (birth BW = 44.8 ± 4.13 kg; mean ± SD) were fed 3L of colostrum replacer (200 g IgG) through either an esophageal tube or nipple bottle at 2 h after birth. The following meals of pooled whole milk were fed at a volume of 3L at 12, 24, 36 and 48 h after birth. A 2" jugular catheter was placed in each calf 1 h after birth to allow frequent blood samples to be taken for analysis of IgG and GLP-2 concentrations. All response variables were evaluated for effect of colostrum feeding method using the ANOVA procedure of JMP 13. Maximum concentration (Cmax) of serum IgG was 24.2 and 24.7 ± 0.58 mg/mL, time to maximum concentration (Tmax) was 786 and 966 ± 161 min, and apparent efficiency of absorption of IgG (AEA) was 52.7 and 53.2 ± 1.63% for the bottle and tube treatments, respectively. There was no difference between treatments for serum IgG maximum concentration (P = 0.56), Tmax (P = 0.44), or AEA (P = 0.84). There was also no significant treatment effect on GLP-2 Cmax (P = 0.74), Tmax (P = 0.20), or area under the curve (P = 0.33). There was a significant time effect (P < 0.001), however, indicated by an increase in GLP-2 concentrations following colostrum feeding. These results indicated that feeding colostrum at a volume of 3L through either an esophageal tube or nipple bottle accomplishes successful passive transfer of IgG and increases GLP-2 plasma concentrations after colostrum feeding.

Key Words: colostrum, esophageal tube, nipple bottle

Use of phytogetic feed additives in pre-weaned dairy calves. B. Miller* and P. Gott, Biomin USA, San Antonio, TX.

Increased pre-weaning gain rates have been shown to have positive effects on subsequent milk production. Feeding programs that can increase lean growth rate can provide economic benefits to both the calf operation and subsequent dairy production. Phytogetic feed additives (PFA) have been identified as natural growth promoters which can potentially replace antibiotic growth promoters. A feeding trial was conducted to test the performance of calves that received PFAs in both the milk replacer and the calf starter diets. Holstein calves were randomly assigned to one of 2 treatment groups, Control (Con) and Phytogetic (PFA). Two open-sided barns each containing 4 rows of 12 hutches were used. A total of 96 calves, 48 per treatment were enrolled in the study. Calves were received at the facility and began the trial between 2 and 3 d of age. Calves were fed for approximately 8 weeks. A commercial milk replacer was used. PFA calves received the same milk replacer as the Con diet with the addition of a commercial phytogenic product (Biomin USA.) at the rate of 0.1 L per L reconstituted milk replacer. PFA calves received the same dry calf starter mash, but were supplemented with 300g/MT of a commercial phytogetic product (Biomin USA). Growth rate was determined based upon entry weight and weaning weight. Initial body weights differed. Because this was a commercial facility, calves were fed to similar final size, resulting in differing time on feed. Con calves initially weighed 40.9 kg while PFA calves initially weighed 38.7 kg (P = 0.041). As a result, initial calf weight and days on feed were used as a covariates in analyzing the data under PROC MIXED (SAS). Final body weights did not differ, Con = 76.6 kg and PFA = 78.2 kg (P = 0.385). Average daily gain did not differ 0.66 kg (Con) vs. 0.69 kg (PFA), (P = 0.385). Total weight gain was greater for the PFA group (39.2 kg vs. 36.0 kg (Con), (P = 0.009), but feeding period also differed, 54 d (Con) and 58 d (PFA), (P = 0.0003).

Key Words: phytogetic, calf, weaning

Heifer calf health and management on Canadian dairy farms. C. Winder*, T. Duffield, C. Bauman, and D. Kelton, Dept. of Population Medicine, University of Guelph, Guelph, ON, Canada.
Phase I of the 2015 Canadian National Dairy Study was a cross-sectional survey which included questions on heifer calf health and rearing practices. The objectives of this section were to describe these practices and explore factors associated with different strategies. The questionnaire was open to all dairy producers in Canada and had an 11% response rate (n = 1,157). Fifty-one percent of respondents reported never allowing heifer calves to nurse their dam; 16% always removed calves within 30 min of birth. Sixty-eight percent reported always feeding heifer calves 4 L of colostrum within 12 h of birth; 17% always fed colostrum between 9 p.m. and 5 a.m. Five percent pasteurized all colostrum fed on farm. Seventy-seven percent of respondents reported calving and stillbirth information for 2014; mean reported stillbirth rate was 4.9% (SD = 3.3). The majority of respondents housed calves in pens (73%), while 23% used hutches and 3% tethered calves. Pre-weaned calves were housed in groups on 37% of farms, with a mean of 8 calves per group (SD = 13). The maximum amount of milk offered to calves per day during the pre-weaned period was a mean of 8 L (SD = 3). Fifty percent of respondents reported mortality data for 2014; mean pre-weaned mortality was 6.4% (SD = 8.3), and post-weaned mortality was 2.5% (SD = 4.4). Over 95% of producers reported disbudding practices; 86% used cautery, 11% used surgical amputation, and 9% used caustic paste. Twenty-eight percent reported disbudding at less than 3 weeks of age, 61% at 3 to 8 weeks, 22% at 8 to 16 weeks, and 5% reported dehorning at more than 16 weeks of age. Sixty-eight percent of cauterers reported use of local anesthetic, 32% used sedation, and 26% used a non-steroidal anti-inflammatory drug. Multi-variable regression models showed use of local anesthetic was associated with social media activity (OR = 2.3, P < 0.001), while sedation and NSAID use were associated with geographic region and age at dehorning (P < 0.01). Exploring heifer rearing management practices, including adoption of best practices, may help focus future education and extension efforts. Poor reporting of mortality data may reflect a lack of this information being kept on farm.

**Key Words:** national dairy study, mortality, colostrum

### 247 Cow-level responses to two commercial dry cow mastitis preparations.

E. Cox*, 1 T. Bilby, 1 S. Kieser, 2 B. Petersen, 1 J. Laporta, 3 and R. Chebel 4, 5, 1 Merck Animal Health, De Soto, KS, 2 Dairy Vet Services, Sunnyside, WA, 3 Sunrise Veterinary Services, Dalhart, TX, 4 Department of Animal Sciences, University of Florida, Gainesville, FL, 5 Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL.

Objectives of this experiment were to compare the efficacy of 2 intramammary (IM) antibiotic formulations in reducing the incidence of IM infections (IMI) during the dry period at the cow-level. Secondary objectives of this experiment were to evaluate the cure of IMI during the dry period at the cow-level and the performance of cows treated with different antibiotic formulations. Cows from 2 herds were used in this experiment. At dry-off, cows were randomly assigned to the ORB (n = 1,310; benzathine cloxacillin; Orbenin-DC, Merck Animal Health, Madison, NJ) and the SPC (n = 1,354; cefotiofur hydrochloride; Spectramast LC, Zoetis, Parsippany-Troy Hills, NJ) treatments. Composite milk samples were collected at dry-off and at parturition and submitted for microbiology laboratory analysis. Data were analyzed using SAS. The likelihood of IMI at dry-off and at parturition, the likelihood of a new IMI during the dry period, the likelihood of IMI cure during the dry period, the likelihood of a clinical case of mastitis in the first 100 DIM, and the likelihood of pregnancy after the first 2 postpartum inseminations were analyzed by logistic regression using PROC GLIMMIX. Milk yield, milk composition, and somatic cell count (SCC) were analyzed by ANOVA for repeated measures using PROC MIXED. Dairy was used as the random effect. SPC treatment was used as reference. There was no (P = 0.24) difference between treatments regarding the prevalence of IMI at dry-off (OR = 1.11, 95% CI = 0.94, 1.31). There was no effect of treatment on the likelihood of new IMI during the dry period (OR = 0.91, 95% CI = 0.76, 1.09; P = 0.32), of cure of IMI during the dry period (OR = 0.99, 95% CI = 0.77, 1.28; P = 0.93), of IMI at calving (OR = 0.96, 95% CI = 0.80, 1.15; P = 0.67), and clinical mastitis up to 100 DIM (OR = 0.85, 95% CI = 0.61, 1.17; P = 0.32). Fat corrected milk yield (ORB = 44.1 ± 0.2 vs. SPC = 44.2 ± 0.2 kg/d; P = 0.21) and linear SCC (ORB = 20.2 ± 0.03 vs. SPC = 20.7 ± 0.03; P = 0.85) in the first 100 DIM were not different between treatments. Similarly, treatment did not (P = 0.81) affect likelihood of pregnancy after the first 2 postpartum inseminations (OR = 0.98, 95% CI = 0.83, 1.16). No differences were observed between ORB and SPC in regards to the aforementioned outcomes.

**Key Words:** dry cow preparations, intramammary infection

### 248 Evaluating the effect of two hoof-trimming techniques on lesion incidence.

G. Stoddard*, 1 N. Cook, 2 S. Wagner, 3 and G. Cramer 1, 1University of Minnesota Twin-Cities, St. Paul, MN, 2University of Wisconsin Madison, Madison, WI, 3North Dakota State University, Fargo, ND.

Lameness is detrimental to the well-being and productivity of dairy cattle. One recommended procedure to prevent lameness is hoof trimming (HT). The research surrounding the efficacy of HT however is currently limited. The objective of this project was to compare the functional HT method (LIT) to more modeling of the weight bearing claw (BIG) on lesion presence at mid-lactation. This randomized trial was conducted on a convenience sample of 3 farms using free-stall housing, recycled sand bedding and had a regular HT schedule. Professional hoof trimmers were trained on the techniques and to evaluate the presence of a lesion at 100–150 DIM. LIT model animals were modeled within 32mm of the abaxial wall and BIG model animals were modeled within 18mm of the abaxial wall. Cows with no hoof lesions were allocated to LIT or BIG model on the week level at their dry off trim. The outcome was a lesion occurring before or one being present at the evaluation. Logistic regression was used to evaluate the effect of trim (BIG/LIT) on the odds of lesion development at the P < 0.05. A total of 382 cows from 1 study farm were used in this preliminary analysis (LIT = 201 BIG = 190). Cows were examined on average at 114 DIM with an average lactation of 3. The incidence of lesions was 15% and 13% in the LIT and BIG groups respectively. The final logistic regression model included treatment, breed and lactation. BIG decreased the odds of having a lesion by 24% (OR = 0.76, CI = 0.42–1.38, P = 0.39). Crossbreds had a 1.94 (CI = 1.08–3.63, P = 0.03) higher odds of a lesion compared with Holsteins. Second lactation and greater was associated with a 1.32 (CI = 1.05–1.70, P = 0.02) higher odds of a lesion. These preliminary results indicate that the BIG technique decreased the odds of lesions at mid-lactation. Though this result was not significant, a large reduction in the odds was observed, which is biologically significant for the animal. The effect this procedure has on locomotion score, culling risk, and milk yield still needs to be determined, to establish which procedure is more efficacious.

**Key Words:** hoof trimming, technique, locomotion

### 249 Effects of intramuscular injection of vitamin B12 and dietary biotin addition on feed intake and milk performance of dairy cows.

B. X. Zhang, D. M. Wang*, and J. X. Liu, Institute of...
Biotin and vitamin B_{12} (VB_{12}) play an important role in regulating activity of hepatic gluconeogenesis related enzymes for the periparturient cows and hence had positive effects on health status and productivity of lactating dairy cows. However, their interactive effects remain unclear. Thus, this study was conducted to investigate the interactive effects of introducing biotin and VB_{12} on health status and productivity in dairy cows. Forty-eight multiparous pre-partum dairy cows were allocated into 12 blocks based on parity (2.75 ± 0.89, mean ± SD) and daily milk yield (31.2 ± 3.28, mean ± SD kg/d), and were then randomly assigned to one of the 4 treatments: basal diet without, and with 30 mg/d dietary biotin, intramuscular injection of VB_{12} at 10 mg/week once, or biotin addition (30 mg/d) plus VB_{12} injection (10 mg/week). Treatments started from 3-week pre-partum (expected) to 8-week post-calving. Dry matter intake and milk productivity (yield and composition) were recorded weekly after calving. Blood from coccygeal vein were sampled biweekly after calving to determine serum nonesterified fatty acids, β-hydroxybutyric acid, and superoxide dismutase. Data were analyzed using the MIXED procedure of SAS. Body weight were measured at the beginning and end of the study. Injection of VB_{12}, but not dietary biotin, significantly increased feed intake and yield of milk, milk protein and lactose (P < 0.01), compared with the cows fed the basal diet. However, no interactive effects (P > 0.05) were observed on dry matter intake and milk yield between biotin and VB_{12}. Serum nonesterified fatty acids, β-hydroxybutyric acid, and superoxide dismutase and body weight change did not differ across all the treatments (P > 0.05). In conclusion, biotin and VB_{12} had limited interactive effect on dairy productivity. Introducing VB_{12} in pre-partum dairy cows can increase milk yield rapidly without body weight loss, and is potentially beneficial to whole lactation cycle.

**Key Words:** milk productivity, biotin, vitamin B_{12}

### Nutritional recovery strategies from severe nutrient restriction after milk and blood parameters of dairy cows

V. Fischer*1, D. Werncke1, F. A. Schmidt2, and A. Thaler Neto2, 1Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, 2Universidade do Estado de Santa Catarina, Lages, SC, Brazil.

This work aimed to investigate the effect of different nutritional strategies used in the recovery from severe nutrient restriction on milk and blood parameters. Twelve Holstein cows (milk yield, MY, 18.9 ± 5.5 L/day; parity 2.7 ± 1.6; days in milk 146 ± 50; body condition score, BCS, 2.7 ± 0.3 and body weight, BW 575 ± 70 kg) received a diet that supply 100% of energy and protein requirements on d 1 to 13 (NRC, 2001), were restricted in 50% of their energy and protein requirements on d 14 to 17 and, they were assigned to one of the following treatments: full supply of only energy (100E+50P), full supply of only protein (50E+100P) or full supply of both energy and protein (100E+100P) requirements on d 18 to 24. Milk and blood measurements were taken on the last day of recovery phase. Diets were formulated with different proportions of corn silage, Tifton hay, soybean meal, toasted soybean. Data were analyzed as a Latin square design (12 cows, 4 Latin squares, 3 periods, 3 treatments). Cows supplied with 100E+100P showed higher values for MY and stability than the other cows. Cows supplied with 100E+100P showed higher values for MY, BCS, BW but lower values for FPR, MUN, albumin than cows supplied with 50E+100P. Cows supplied with 100E+50P presented higher values for MY, BCS, BW but lower values for FPR, MUN and albumin than cows supplied with 50E+100P. There were no differences (P > 0.10) for haptoglobin and BHBA concentrations. Only full supply of both nutrients overcomes the adverse effects of nutrient restriction on MY, stability and MUN.

**Table 1 (abstract 250).**

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<tr>
<th>Item</th>
<th>Treatment</th>
<th>100E+100P</th>
<th>100E+50P</th>
<th>50E+100P</th>
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<td>14.4b</td>
<td>12.1c</td>
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<td>2.7a</td>
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<tr>
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<td>525a</td>
<td>471b</td>
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<td>1.2a</td>
<td>1.4b</td>
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<td>MUN (mg/d)</td>
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<td>2.1c</td>
<td>17.7a</td>
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<tr>
<td>Milk stability (°GL)</td>
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<td>69.8b</td>
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<td>Haptoglobin (g/L)</td>
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<td>Albumin (g/L)</td>
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<tr>
<td>BHBA (mmol/dL)</td>
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<td>0.4a</td>
<td>0.5a</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*a,b,c*Means in the same row followed by different letters are distinct (P < 0.05).

**Key Words:** blood traits, nutritional restriction, milk composition

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Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, China.
Colonizing microbiome influences early intestinal development in newborn dairy calves. N. Malmuthuge1,2, G. Liang1,3, and L. L. Guan1,1 Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, 2Vaccine and Infectious Disease Organization-International Vaccine Centre, University of Saskatchewan, Saskatoon, SK, Canada, 3Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

The understanding on pre-ruminant gut microbiome is very limited. Therefore, this study characterized the small intestinal (jejunum and ileum) microbiome of pre-weaned calves, aiming to understand the dynamics of postnatal microbial establishment within the first 6 weeks of life and how potentially it interacts with host mucosal immune system. Next generation sequencing of digesta- and tissue-associated communities revealed remarkable variations in the microbial composition and the relative abundance of detected bacterial groups among individuals. Despite the highly individualized microbiome, we identified 2 taxonomic-based clusters of calves that were comprised of either high levels of Lactobacillus or Bacteroides. Moreover, Bacteroides, Prevotella, Roseburia, Ruminococcus, and Veillonella were low abundant or absent in the ileum of Lactobacillus-dominated calves. The analysis of metagenome profiles revealed that calves can be grouped to 2 function-based clusters with either high protein metabolism (cluster1) or sulfur metabolism (cluster2). When the small intestinal transcriptome was profiled, it indicates that the first week after birth is a very dynamic developmental period for the intestinal mucosal immune system. Similar changes were observed in the expression of miRNAs and microbiome during the first week of life suggesting that the changes observed at transcriptome level may be regulated by both miRNAs and microbial colonization. Besides, the ileal transcriptome of the calves belonged to 2 taxonomic-based clusters revealed varied immune responses. The present study revealed that an establishment of small intestinal-specific microbiota from birth, and there are microbial markers (microbial functions and taxonomy) that can be used to broadly categorize calves, regardless of the highly individualized early microbiome. Findings from this study indicate that the colonizing microbiome is an essential factor regulating the rapid development of the mucosal immune system during the first week of life.

Key Words: gut microbiome, gut development, pre-weaned dairy calf

Host-rumen microbe interactions may be leveraged to improve productivity of dairy cows. D. M. Bickhart* and P. J. Weiner, USDA-ARS DFRC, Madison, WI.

The cattle rumen serves as a digestive bioreactor for the dairy cow, yet our knowledge of the microbial contents, ecology and host-selection within the rumen is only cursory. This is despite the knowledge that the volatile fatty acids (VFA) and microbial crude protein (MCP) produced by rumen microbiota serve as the precursors to milk fat and protein, respectively. By creating models that predict rumen microbial population presence and predict the flux of metabolites in the rumen, it may be possible to develop intervention therapies designed to relieve milk fat depression, negative energy balance and/or disease. These therapies will need to be designed with the properties of the cattle rumen in mind, as recent research indicates that the community is dynamic during lactation phases but adopts a profile that is unique to each individual cow. Development of probiotics to elicit permanent changes in the cattle rumen may therefore be difficult as probiotic species tend to be undetectable in the rumen contents after a 24 - 48 h period. Rumen content exchanges between a host and donor cow similarly show a reversion of rumen pH and VFA profiles within a day’s time. This suggests a strong selection of microbial content by the host cow and/or selection by the residual microbial species that remain after rumen content evacuation. This presents an intriguing possibility with respect to the inheritance of milk fat and protein traits in the genetic selection of dairy cattle, as much of the heritability of these traits may be due to the host’s selection and maintenance of microbes in the rumen. It is challenging to test this hypothesis, as we are unable to efficiently genotype dairy cattle rumen microbial communities for use in large population-scale models. Instead, we present the preliminary results of a meta-analysis of publicly available, whole genome shotgun (WGS) sequencing of the rumen microbial community. Our intent is to generate an indexable profile of singly-unique microbial reads that would be suitable for rapid genotyping in future WGS sequencing experiments. By using this reference in larger sampling surveys, it may be possible to identify key quantitative trait loci within the cattle genome that select for rumen microbial contents.

Key Words: metagenomics, rumen, sequencing

Can the rumen microbiome be manipulated to enhance feed efficiency in dairy cows? I. Mizrahi*, The Department of Life Sciences & the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

The mammalian gut microbiota is essential in shaping many of its host’s functional attributes. Relationships between gut bacterial communities and their mammalian hosts have been shown in recent years to play an important role in the well-being and proper function of their hosts. A classic example of these relationships is found in the bovine digestive tract in a compartment termed the rumen. The rumen microbiota is necessary for the proper physiological development of the rumen and for the animal’s ability to digest and convert plant mass into basic food products, making it highly significant to humans. I will discuss some of our recent findings regarding this ecosystem’s development, and interaction with the host.

Key Words: mammalian gut, microbiome, bovine digestive tract

Understanding dietary-microbe interactions to enhance the productivity of dairy cows. D. Pitta*, University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, Kennett Square, PA.

Ruminants are dependent on the microbiota (bacteria, protozoa, archaea and fungi) that inhabit their reticulo-rumens for digestion of feedstuffs. Nearly 70% of energy and up to 85% of the protein requirements for dairy cows are met from microbial fermentation in the rumen, emphasizing the need to characterize the role of microbes in feed breakdown and nutrient utilization. Over the past 2 decades, next generation sequencing technologies have allowed for rapid expansion of knowledge concerning microbial populations and alterations in response to forages, concentrates, supplements and probiotics in the rumen. Advances in
gene sequencing and emerging bioinformatic tools have allowed for increased throughput of data to comprehend the functional relevance of microbial genomes. Particularly, metagenomics can identify specific genes involved in metabolic pathways, and metatranscriptomics can describe the transcriptional activity of microbial genes. These powerful approaches help untangle the complex interactions between microbes and dietary nutrients to more fully understand the physiology of feed digestion in the rumen. The need to increase energy and protein content of feeds to meet high nutrient demands of lactation in the dairy sector are well appreciated. Application of these genomic-based approaches offers the promise of unraveling specialized microbial niches and their gene repertoires that have the potential to enhance fiber digestion, microbial protein synthesis and healthy biohydrogenation. New information on microbial genomics and interactions with dietary components will more clearly define pathways in the rumen to positively influence milk yield and components.

Key Words: dairy cow, dietary-microbe interaction, productivity

255 Leveraging next-generation sequencing technology to identify the functional role of rumen microbiome in dairy cows. T. Snelling*, I. Tapio¹, F. Strozzi², D. Fischer¹, A. Bayat¹, P. Garnsworthy³, P. Huhtanen², K. Shingfield⁶, and J. Wallace⁵, ¹Natural Resources Institute Finland (Luke), Helsinki, Finland, ²Swedish University of Agricultural Sciences, Umeå, Sweden, ³University of Nottingham, Loughborough, UK, ⁴Università Cattolica del Sacro Cuore, Piacenza, Italy, ⁵University of Aberdeen, Aberdeen, UK, ⁶Aberystwyth University, Aberystwyth, UK, ⁷Enterome Bioscience, Paris, France.

RuminOmics is a European Commission funded project that aims to connect the animal genome, gastrointestinal microbiomes and nutrition to improve digestion efficiency and the environmental impacts of ruminant livestock production. A major undertaking of the project was to take rumen digesta samples from 1000 dairy cows from herds kept in UK, Italy, Finland and Sweden. Comprehensive phenotype data were recorded including milk yield, dietary intake, digestibility and methane emissions. The animals were ranked according to methane yield data expressed as g CH4 per kg dry matter intake (DMI) corrected for live weight and a selection was made of 5 the highest and lowest methane emitters from each country. Microbial DNA was extracted from digesta of each of the selected animals for metagenome sequencing using Illumina HiSeq 2000 to a depth of 90 million quality paired-end reads per sample. A reference set of nearly 4 million putative genes was generated and mapped to the UniProt KB with a relatively small proportion, just over 500000 genes, with high sequence similarity (E-value <1e-10 and identity > = 75%). MDS plots of relative gene abundance by taxonomy and functional annotation clustered according to country. However separation data by country revealed the clustering of the metagenomes according to methane emissions. KEGG orthologs (KO) involved in acetoclastic methanogenesis K13788 and K01895 were found to be significantly different in the Italian and Swedish cows respectively and all the genes involved in hydrogenotrophic methanogenesis were consistently higher in relative abundance in high methane emitters. VFA production (mmol/L) showed no significant differences and this was reflected in the relative abundance of microbial genes (cel, xyn, and amy) involved in carbohydrate degradation. Similarly, no significant differences were found in milk yield and composition (kg/day). As part of the effort to reduce the environmental impact of livestock, the results from this study showed the selection for low methane emitting animals need not necessarily come at the expense of productivity.

Key Words: metagenome, methane, rumen
256 Low and high methane emitting cows hold their ranking over different feeding strategies. A. R. Bayat*1, T. Luukkanen1, P. Kairenius1, H. Leskinen1, T. Hurme2, S. Ahvenjärvi1, and J. Vilkki1, 1Green Technology, Natural Resources Institute Finland (Luke), Jokioinen, Finland, 2Natural Resources and Bioproduction, Natural Resources Institute Finland (Luke), Jokioinen, Finland.

To study whether the cows’ ranking based on methane (CH4) emission is defined by the host animal irrespective of dietary strategy, 100 Nordic Red cows in mid-lactation were ranked according to CH4/DMI emission by using respiratory chambers. Two groups of 5 low- and 5 high-emitters were selected, fitted with rumen cannulas and subjected to different diets in 3 35-d periods. High grass (HG, 70:30), low grass (LG, 30:70) and red clover (RC, 50:50) based-diets differing in forage to concentrate ratio were fed. Proc GLIMMIX of SAS was used to analyze the data with a model that included random effect of cow, fixed effects of period, diet, group, and interaction of diet and group. Intakes of DM and gross energy tended (P ≤ 0.12) to be lower for the low- compared with high-emitter cows (23.7 vs 25.5 kg/d and 421 vs 455 MJ/d). Intakes of DM and gross energy were higher (P < 0.01) for LG compared with HG and RC (26.5 vs 23.4 and 23.7 kg/d; 476 vs 416 and 423 MJ/d). Intake of NDF was higher for HG compared with LG and RC (8.65 vs 7.95 and 7.78 kg/d; P < 0.01). Both groups had similar milk yield. HG had lower milk yield than LG and RC (34.2 vs 39.3 and 38.4 kg/d; P < 0.01). Digestibility of OM (71.4 vs 73.0%; P < 0.01) and NDF (55.6 vs 59.2%; P < 0.05) was lower for low- compared with high-emitters. Low-emitters tended (P = 0.08) to have lower CH4/DMI than high-emitters (20.9 vs 22.5 g/kg). LG had lower CH4/DMI than HG and RC (19.7 vs 23.4 and 22.0 g/kg; P < 0.01) and the interaction of diet and group was not significant. High-emitters consuming HG and LG had higher CH4 emission (g/d) than low-emitters consuming LG (581 and 580 vs 466 g/d; P = 0.06 for group × diet interaction). Rumen DM fill, measured by rumen evacuation, did not differ between diets or groups but rumen contents were collected on d 14 and 17 for volatile fatty acid (VFA) analysis and protozoal populations. Enteric methane emissions were measured on d 18 to 21 using open circuit chambers. Diet digestibility was measured on d 24 to 27. Methane production was reduced from 26.2 (CON) to 19.6, 17.9, and 12.7 g/kg of DM, for OIL, NOP, and NOP+OIL, respectively (P < 0.01). Total VFA concentrations (mM) were greatest for CON (101.3), similar between OIL (94.8) and NOP (94.8), and lowest for NOP+OIL (88.3) (P < 0.01). A decrease in acetate (A) and increase in propionate (P) proportions, and therefore a decrease in the A/P ratio was also observed with the OIL, NOP, and NOP+OIL treatments compared with CON (P < 0.01). The OIL and NOP+OIL treatments had a reduction in protozoa counts and a reduction in DM, OM, NDF, and ADF digestibilities when compared with CON and NOP (4.43 × 104 vs. 4.24 × 103; mL rumen fluid; 60.7 vs. 66.8%; 62.0 vs. 68.7%; 47.6 vs. 61.0%; and 46.5 vs. 60.0%, respectively) (P < 0.01). The data demonstrated that the addition of OIL and NOP are effective enteric methane emissions.

Key Words: methane, ranking, dairy cow


The objective of this experiment was to evaluate the production effects of replacing corn silage (CS; serving as the control) with brown midrib dwarf pearl millet silage (PM) in the total mixed ration of lactating dairy cows. Sixteen Holstein cows (65 ± 21 DIM; BW 630 ± 71 kg) were used in a replicated 2 × 2 Latin square design experiment with two 28-d periods. Feeding was ad libitum for 5 to 10% refusals. The control diet consisted of (DM basis): 50% CS, 6% alfalfa haylage, 4% hay/straw mixture, and 40% concentrate feeds. For the PM diet, 20% of CS was replaced with PM (on DM basis). Control and PM diets were 16.7 and 17.2% CP, 30.3 and 32.4% NDF, and 28.0 and 24.1% starch, respectively. Metabolizable protein balance of control and PM diets was 27 and 208 g/d, respectively; NEb balance was −0.7 and −0.5 Mcal/d. Enteric methane emission was measured using the GreenFeed system. The PM diet resulted in equal DMI as the control (29.0 vs 29.1 kg/d; SEM = 0.65, P = 0.78, respectively) but lower milk yield (49.6 vs 51.3 kg/d; SEM = 2.02, P < 0.001) and lower feed efficiency (1.72 vs 1.77 kg/kg; SEM = 0.05, P = 0.01). Energy corrected milk (ECM) yield (46.7 kg/d; SEM = 1.92, P = 0.86), and ECM feed efficiency were not different between diets. The PM diet tended to increase milk fat content compared with the control diet (3.71 vs 3.47%; SEM = 0.118, P = 0.06, respectively) but true protein and lactose content were not affected. Yields of the individual milk components were not affected (P ≥ 0.23) by diet and averaged 1.81 kg/d fat, 1.45 kg/d true protein and 2.51 kg/d lactose. Enteric methane emission was increased by the PM diet over the control (454 vs 396 g/d; SEM = 18.4, P < 0.001) as was methane yield (15.7 vs 13.8 g/d; SEM = 0.54, P = 0.001) and methane intensity (9.6 vs 8.3 g/kg ECM; SEM = 0.39, P = 0.001). Brown midrib dwarf pearl millet silage has potential to partially replace CS in the diet of dairy cattle without affecting ECM yield and milk components. This replacement, however, is likely to increase enteric methane emission.

Key Words: pearl millet, methane, dairy cow

258 Assessing the potential of 3-nitrooxypropanol and canola oil alone and in combination to lower methane emissions from cattle and reduce their contribution to climate change. M. L. Smith*, S. M. Duval2, M. Kindermann3, K. A. Beauchemin4, and L. Kung Jr.1, 1University of Delaware, Newark, DE, 2DSM Nutritional Products France, Saint Louis Cedex, France, 3DSM Nutritional Products, Basel, Switzerland, 4Agriculture and Agri-Food Canada, Lethbridge, AB, Canada.

The objective of this study was to assess the potential of 3-nitroxypropanol, a novel methane inhibitor; and canola oil, a known methane mitigant; alone and in combination on methane emissions, rumen fermentation, and diet digestibility. Eight ruminally canulated beef heifers (Angus cross, 732 ± 43 kg) were used in a double 4 × 4 Latin square design with 4 28-d periods and assigned to one of 4 dietary treatments. The dietary treatments were: 1) control (CON) (no supplementation of 3-nitrooxypropanol or canola oil), 2) canola oil alone (OIL) (5% of diet DM), 3) 3-nitrooxypropanol alone (NOP; 200 mg/kg of diet DM; DSM Nutritional Products Ltd., Kaiseraugst, Switzerland), and 4) 3-nitrooxypropanol and canola oil combined (NOP+OIL). After a 14-d diet adaption, dry matter intake (DMI) was recorded daily. Rumen contents were collected on d 14 and 17 for volatile fatty acid (VFA) analysis and protozoal populations. Enteric methane emissions were measured on d 18 to 21 using open circuit chambers. Diet digestibility was measured on d 24 to 27. Methane production was reduced from 26.2 (CON) to 19.6, 17.9, and 12.7 g/kg of DM, for OIL, NOP, and NOP+OIL, respectively (P < 0.01). Total VFA concentrations (mM) were greatest for CON (101.3), similar between OIL (94.8) and NOP (94.8), and lowest for NOP+OIL (88.3) (P < 0.01). A decrease in acetate (A) and increase in propionate (P) proportions, and therefore a decrease in the A/P ratio was also observed with the OIL, NOP, and NOP+OIL treatments compared with CON (P < 0.01). The OIL and NOP+OIL treatments had a reduction in protozoa counts and a reduction in DM, OM, NDF, and ADF digestibilities when compared with CON and NOP (4.43 × 104 vs. 4.24 × 103; mL rumen fluid; 60.7 vs. 66.8%; 62.0 vs. 68.7%; 47.6 vs. 61.0%; and 46.5 vs. 60.0%, respectively) (P < 0.01). The data demonstrated that the addition of OIL and NOP are effective.
The objective of this in vitro study was to examine the trans-10 (r10) shift in relation to the ratio between hydrogenating bacteria capable of producing either cis-9,trans-11 conjugated linoleic acid (c9t11 CLA) or r10,cis-12 CLA (r10c12 CLA). The influence of the in vitro condition on this shift was also investigated. *Butyrivibrio fibrisolvens* D1 (BF) and *Propionibacterium acnes* DSM 1897 (PA) were chosen as model organisms for the production of c9t11 CLA and r10c12 CLA, respectively. Different ratios of these bacteria (100/0, 50/50, 10/90, 2/98, 0.4/99.6, 0/100) were incubated in different growth media containing 40 µg/mL 18:2n-6 (LA); (1) control, (2) low pH, (3) 22:6n-3 (DHA) enriched media. The low pH medium was prepared by adding 2 M HCl to the control to reduce the pH from 6.5 to 5.5. The DHA enriched medium was the control supplemented with 40 µg/mL DHA. Under control conditions, the residual amount of LA after 24 h of incubation increased with increasing amounts of PA at inoculation (P = 0.013), which implies a lower rate of LA metabolism by the latter as compared with BF. Increasing amounts of PA also increased r10c12 CLA accumulation (P = 0.002) at the expense of c9t11 CLA (P = 0.006), with a r10 shift, defined as r10/c11 ≥ 1, occurring when PA represented between 90% and 98% of the inoculum. The required residual amount of PA at inoculation to induce a r10 shift decreased to 50% and 90% in the low pH or DHA enriched medium, respectively. Low pH or DHA addition did not stimulate r10c12 CLA formation, but inhibited CLA formation by both bacteria whereby PA seemed to be more tolerant. The current results suggest that besides a specific balance between BF and PA, specific external factors might influence the r11 to r10 shift. A low pH or, to a lesser extent, addition of DHA gives some advantage to PA compared with BF. Nevertheless, required proportions of PA remained high under all conditions. Hence, it is unlikely that PA is the only or predominant species involved in the r11 to r10 shift under in vivo circumstances.

**Key Words:** bihydrogenation, linoleic acid, trans-11 to trans-10 shift

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**260 Are EPA, DPA, and DHA equally effective to modulate ruminal biohydrogenation in cows? A comparative in vitro study.**


Marine lipid supplements are rich in very long chain n-3 polyunsaturated fatty acids (PUFA) that inhibit the ruminal saturation of trans-11 18:1 and, consequently, may enhance the concentration of cis-9,trans-11 conjugated linoleic acid (CLA) in milk and meat. In this regard, docosahexaenoic acid (DHA, 22:6n-3) has been suggested to increase total trans-18:1 accumulation in the rumen to a greater extent than eicosapentaenoic acid (EPA, 20:5n-3), but information about changes in individual 18:1 isomers is very limited. Furthermore, although EPA and DHA are accepted to be the main responsible for this modulatory effect on ruminal biohydrogenation (BH), the contribution of docosapentaenoic acid (DPA, 22:5n-3), the third most abundant n-3 PUFA in marine lipids, remains unknown. The aim of this study was to compare the impact of EPA, DPA and DHA on the BH of dietary C18 fatty acids, using batch cultures of rumen microorganisms and cannulated cows as inocula donors. The 3 PUFA were added at a dose of 2% of incubated substrate (the TMR fed to the animals; 50:50 forage concentrate ratio) and effects were examined after 24 h of incubation. Data were subjected to ANOVA using the MIXED procedure of SAS 9.4. Overall, EPA and DHA were equally effective to increase the concentration of trans-11 18:1 (on average, +79% compared with the control; P < 0.01), suggesting that supplements containing differing EPA/DHA ratios (e.g., fish oils or marine algae) would have comparable effects at the same PUFA level. However, DHA further promoted alternative BH pathways that lead to trans-10 18:1 accumulation (+205% relative to the control; P < 0.01). The saturation of cis-18:1 and non-conjugated 18:2 isomers was also constrained, particularly by DHA in the former case and by EPA in the latter. Increases in trans-11 cis-15 + trans-10, cis-15 18:2 and in trans-9,trans-14 18:2 (P < 0.001) may indicate that EPA had specific effects on 18:3n-3 metabolism. Only minor variations in ruminal BH intermediates were observed in response to DPA (e.g., increments in trans-10,trans-13 and cis-15 18:1; P < 0.05), which suggests a low contribution of this PUFA to the action of marine lipids.

**Key Words:** rumen metabolism, trans fatty acid

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**259 Effect of pH and 22:6n-3 on in vitro biohydrogenation of 18:2n-6 by different ratios of *Butyrivibrio fibrisolvens* to *Propionibacterium acnes*.**

L. Dewanckele*, B. Vlaeminck, J. Jeyanathan, and V. Fievez. Laboratory for Animal Nutrition and Animal Product Quality, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium.

The objective of this in vitro study was to examine the trans-10 (r10) shift in relation to the ratio between hydrogenating bacteria capable of producing either cis-9,trans-11 conjugated linoleic acid (c9t11 CLA) or r10,cis-12 CLA (r10c12 CLA). The influence of the in vitro condition on this shift was also investigated. *Butyrivibrio fibrisolvens* D1 (BF) and *Propionibacterium acnes* DSM 1897 (PA) were chosen as model organisms for the production of c9t11 CLA and r10c12 CLA, respectively. Different ratios of these bacteria (100/0, 50/50, 10/90, 2/98, 0.4/99.6, 0/100) were incubated in different growth media containing 40 µg/mL 18:2n-6 (LA); (1) control, (2) low pH, (3) 22:6n-3 (DHA) enriched media. The low pH medium was prepared by adding 2 M HCl to the control to reduce the pH from 6.5 to 5.5. The DHA enriched medium was the control supplemented with 40 µg/mL DHA. Under control conditions, the residual amount of LA after 24 h of incubation increased with increasing amounts of PA at inoculation (P = 0.013), which implies a lower rate of LA metabolism by the latter as compared with BF. Increasing amounts of PA also increased r10c12 CLA accumulation (P = 0.002) at the expense of c9t11 CLA (P = 0.006), with a r10 shift, defined as r10/c11 ≥ 1, occurring when PA represented between 90% and 98% of the inoculum. The required residual amount of PA at inoculation to induce a r10 shift decreased to 50% and 90% in the low pH or DHA enriched medium, respectively. Low pH or DHA addition did not stimulate r10c12 CLA formation, but inhibited CLA formation by both bacteria whereby PA seemed to be more tolerant. The current results suggest that besides a specific balance between BF and PA, specific external factors might influence the r11 to r10 shift. A low pH or, to a lesser extent, addition of DHA gives some advantage to PA compared with BF. Nevertheless, required proportions of PA remained high under all conditions. Hence, it is unlikely that PA is the only or predominant species involved in the r11 to r10 shift under in vivo circumstances.

**Key Words:** biohydrogenation, linoleic acid, trans-11 to trans-10 shift

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**261 Altering the ratio of dietary C16:0 and cis-9 C18:1 interacts with production level in dairy cows: Effects on production responses and energy partitioning.**

J. de Souza* and A. L. Lock, Michigan State University, East Lansing, MI.

We evaluated the effects of altering the dietary ratio of C16:0 and cis-9 C18:1 on production responses and energy partitioning of lactating dairy cows. Cows were blocked by milk yield and assigned to 3 groups (12 cows per group) in a main plot. Production groups were: a) low (45.2 ± 1.7 kg/d); b) medium (53.0 ± 1.6 kg/d); and c) high (60.0 ± 1.9 kg/d). Within each production group, a truncated Latin square arrangement of FA treatments was used in 2 consecutive 35 d periods. The FA treatments supplemented at 1.5% diet DM were: 1) 80:10 (80% C16:0 + 10% cis-9 C18:1); 2) 73:17 (73% C16:0 + 17% cis-9 C18:1); 3) 66:24 (66% C16:0 + 24% cis-9 C18:1); and 4) 60:30 (60% C16:0 + 30% cis-9 C18:1). The statistical model included the random effect of cow within production group, and the fixed effect of treatment, production group, period, and their interactions. Treatment by production group interactions were observed for milk yield (P = 0.09), FCM (P = 0.05), ECM (P = 0.05), milk fat yield (P = 0.02), milk protein yield (P = 0.06), milk lactose yield (P = 0.08), and energy partitioned to milk (P = 0.02). Increasing cis-9 C18:1 in FA treatments reduced FCM, ECM, and milk energy output in low producing cows (linear, P < 0.05), but increased these in high producing cows (linear, P < 0.01). Increasing cis-9 C18:1 in FA treatments tended to reduce milk fat yield in low producing cows (linear, P < 0.10), but increased it in high producing cows (linear, P < 0.01). Increasing cis-9 C18:1 in FA treatments did not impact milk yield, milk protein yield, and milk lactose yield in low and medium producing cows, but increased these in high producing cows (linear, P < 0.01). Regardless of production level, increasing cis-9 C18:1 in FA treatments increased BW change (quadratic, P = 0.02) and BCS change (linear, P < 0.01); however, there was no effect of treatments on DMI (P = 0.98). Our results demonstrate that high producing dairy cows respond better
to fat supplements containing more cis-9 C18:1, while lower producing cows respond better to supplements containing more C16:0.

**Key Words:** energy partitioning, oleic acid, palmitic acid

### 262 Effects of timing of C16:0 supplementation on production and metabolic responses of early lactation dairy cows. J. de Souza* and A. L. Lock, *Michigan State University, East Lansing, MI.*

Fifty-two multiparous cows were used in a randomized complete block design experiment and assigned to either a control diet containing no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving to 24 DIM (fresh period) or from 25 to 67 DIM (peak period). Fresh diets were formulated to contain (% DM) 17% CP, 30% NDF, 24% forage NDF, and 24% starch. Peak diets were formulated to contain (% DM) 17% CP, 29% NDF, 19% forage NDF, and 26% starch. The C16:0 supplement (85% C16:0), added at 1.5% of diet DM, replaced soyhulls in the CON diets. During the fresh period, PA did not affect DMI (21.5 vs. 21.6 kg/d, P = 0.92) or milk yield (47.7 vs. 46.3 kg/d, P = 0.38) compared with CON. In contrast, compared with CON, PA increased milk fat content (4.89 vs. 4.48%, P = 0.01) and yield (2.29 vs. 2.01 kg/d, P < 0.01), milk protein yield (1.60 vs. 1.53 kg/d, P = 0.03), and ECM (56.6 vs. 51.9 kg/d, P = 0.02). PA decreased BW (668 vs. 709 kg, P = 0.05), BCS (3.25 vs. 3.34, P = 0.04), plasma insulin (0.21 vs. 0.24 μg/L, P = 0.05), and increased plasma NEFA concentration (0.35 vs. 0.59 mMg/L, P = 0.03) compared with CON. A treatment by day interaction was detected for BW (P = 0.05) and BCS (P = 0.07) due to PA only decreasing these variables after 10 DIM compared with CON. During the peak period, compared with CON, PA did not affect DMI (29.9 vs. 30.2 kg/d, P = 0.68), but increased milk yield (58.0 vs. 54.6 kg/d, P = 0.01), milk fat content (3.88 vs. 3.67%, P < 0.01) and yield (2.27 vs. 2.06 kg/d, P < 0.01), milk protein yield (1.80 vs. 1.66 kg/d, P = 0.04), and ECM (62.3 vs. 57.8 kg/d, P < 0.01). Compared with CON, PA reduced plasma insulin concentration (0.25 vs. 0.32 μg/L, P = 0.05), but did not affect plasma NEFA concentration (0.35 vs. 0.32 mMg/L, P = 0.41) or BW (673 vs. 684 kg, P = 0.93). There were no interactions between feeding PA and the time that supplementation started for production variables. Our results demonstrate that supplementing C16:0 during early lactation increases ECM without changes in DMI. Feeding PA may increase BW loss during the fresh period, but not during the peak period.

**Key Words:** fatty acid digestibility, emulsifier, milk fat

### 264 Milk fat depression in dairy ewes fed marine lipids: What are the reasons behind individual variation? P. G. Toral*1, L. Rodríguez-López1, G. Hervás1, A. K. K. Salama2, G. Caja2, and P. Frutos3

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Dairy ewes are less prone than cows to milk fat depression (MFD) but suffer this syndrome when marine lipids are added to their diet to improve milk fatty acid (FA) composition. This is very detrimental as most ovine milk is used for cheese manufacture. However, there are large individual differences in MFD severity; the reasons behind this variability being uncertain. This study was conducted in lactating sheep to test the hypothesis that differences in milk concentration of antilipogenic FA or in the transcriptional regulation of mammary lipogenesis may account for that individual variation. We used 15 ewes receiving, for 35 d, a total mixed ration supplemented with 0 (control; n = 5) or 20 g of fish-oil/kg DM [10 animals were selected out of 22 and divided into those showing a strong (RESPON+, n = 5) or slight (RESPON−, n = 5) MFD]. Milk production and composition, and milk FA profile were recorded for 3 consecutive days before and after treatments. Candidate gene expression was analyzed by quantitative reverse transcription-PCR (qPCR) on mRNA isolated from milk somatic cells collected before (d −2 or −1) and after (d 34 or 35) the dietary treatments. Data were analyzed with the MIXED procedure of SAS 9.4 using orthogonal contrasts. Milk production was not affected by the diets (P > 0.10) but milk fat concentration decreased by 25.4% in RESPON+ and 7.6% in RESPON− (P < 0.001). Supplementation with fish oil enhanced (P < 0.01) the milk content of both potentially healthy FA (e.g., cis-9,trans-11 CLA, trans-11 18:1, or very long chain n-3 FA) and antilipogenic FA (e.g., cis-9 16:1, trans-10 and cis-11 18:1, trans-9,cis-11, and trans-10,cis-12 CLA, and 10-oxo-18:0). Nevertheless, differences between RESPON− and RESPON+ were hardly detected. Consumption of the MFD-inducing diet was accompanied by reductions in the mRNA abundance of ACS2, FASN, LPIN1 and INSIG1 (P < 0.10), but only SCD and GPD1 tended to differ between RESPON+ and RESPON− (P < 0.10). These results oblige to reject the hypothesis and conduct a
265 Effects of supplementation of oleic acid and stearic acid in low fat and high fat diets on milk performance of early lactation cows. Y. T. Chen1, G. L. MA3, J. H. Harrison2, and E. Block3,  
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Two experiments were conducted to study the effects of supplementation of rumen inert C18:1 (Megalac) and C18:0 (Energy Booster) in diets containing low fat (3.3% DM, experiment 1) and high fat (6.6% DM, experiment 2) on milk performance in early lactation cows. The high fat diets were formulated by replacement of 2% barely grain in low fat diet with soybean oil, and the contents of other ingredients were similar. Each experiment utilized 30 cows blocked by parity and predicted transmitting ability, and randomly fed diets either supplemented with rumen inert C18:1 or C18:0 from 3 to 14 wk after calving. Milk yield and DMI were recorded daily, and milk samples were collected weekly. Data were analyzed using a randomly blocked design with repeated measurements. When cows were fed diets containing 3.3% fat, the cows fed C18:0 supplement had more (P < 0.05) DMI by 2.4 kg/d, milk fat percentage by 0.3% unit, milk fat yield by 0.2 kg/d, milk protein yield by 0.07% unit, milk protein percentage by 0.1% unit, milk lactose percentage by 0.1%. The milk production efficiency (milk yield/DMI) of cows fed C18:1 supplement was greater (P < 0.05) than cows fed C18:0 supplement (1.9 vs 1.8). The milk yield, other milk components percentages and yields, net energy balance and BCS were similar. In milk, cows fed C18:1 supplement had greater (P < 0.05) concentrations of C18:1 cis-9, and cis-6, and cows fed C18:0 supplement had greater concentration of C18:0. In blood, the concentration of β-hydroxybutyrate was greater (P < 0.05) in cows fed C18:0 supplement. In experiment 2, the milk protein percentage of cows fed C18:0 supplement was greater (P < 0.05) than cows fed C18:1 supplement, while the DMI, milk yield, percentage and yield of other milk components were not affected. The milk production efficiency (milk yield/DMI) of cows fed C18:1 supplement was greater (P < 0.05) than cows fed C18:0 supplement (2.0 vs 1.9). The results of these studies suggest that the milk performance of early lactation cows supplemented with rumen inert FA varies due to source of inert fat and amount of basal fat in the diet.

Key Words: oleic acid, linoleic acid, milk synthesis


The effects of nutritional strategies on body temperature of dairy cows are not well understood but could play an important role in adapting cows to changing climates. Cereal grains including wheat grain are rapidly degraded in the rumen while corn has a slower rate of fermentation. There is preliminary data that indicates there may be differences in body temperature between cows fed wheat or corn-grain. Twenty-four dairy cows were fed a diet that included either wheat grain (12 cows) or corn grain (12 cows) at 430 g/kg DM offered. Half the daily ration was offered at each of 06:00 and 15:00. At wk 4, 10 and 16 cows entered individual controlled-climate chambers at thermoneutral conditions for 48 h. Intravaginal temperature was recorded every 15 min using indwelling loggers. At wk 4, duration between afternoon feeding and maximum temperature tended (P < 0.10) to be longer for cows fed wheat (245 min) than cows fed corn (128 min). At wk 10, the maximum temperature after the morning feeding was lower (P < 0.05) in cows fed wheat (38.7°C) than cows fed corn (38.9°C). Cows fed wheat also had a lower minimum temperature than cows fed corn. After combining data across the entire experiment, the duration between feeding and maximum temperature was greater (P < 0.05) for cows fed wheat (230 min) than cows fed corn (155 min). Maximum temperature after the morning feeding was lower (P < 0.05) in cows fed wheat (38.6°C) than cows fed corn (38.8°C). Daily minimum temperature was lower (P < 0.05) in cows fed wheat (38.0°C) than those fed corn (38.1°C). Reasons for these differences, which are in contrast to the ruminal degradation rates of wheat and corn, are unclear. In our previous research, acetate concentrations in the ruminal fluid of corn-fed cows have been greater than in wheat-fed cows. Diets that induce acetate fermentation in the rumen have previously been reported to increase body temperatures in cattle, and we speculate that this may have been the cause for the higher body temperatures of the corn-fed cows in this experiment. Irrespective of the mechanisms for the differences in body temperature, the feeding of different types of grains constitutes a potential nutritional strategy for ameliorating heat stress in dairy cattle.

Key Words: diet-induced thermogenesis, thermoregulation, rumen fermentation

267 Heat stress decreases transcription of protein metabolism-related genes in mammary tissue of middle lactating cows. D. P. Bu1, L. Ma1, S. T. Gao1, L. H. Baumgard2, and M. Bionaz2,  
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Heat stress decreases milk production, compromises animal health, and increases mortality. Study objectives were to understand the biological adaptation of mammary tissue to heat stress via analysis of the transcriptome using RNA-seq. Four multiparous Holstein dairy cows (101 ± 10 DIM; 574 ± 36 kg BW, 38 ± 2 kg milk/d) were randomly assigned to 1 of 4 environment chambers with a crossover design. Following a 9d adaptation period, cows were either subjected to heat stress [HS: 36°C during the day and 32°C during the night; THI = 87.2 and 81.8] for 9d or kept in thermal neutral conditions [20°C; THI = 65.5] for 9d, but pair-fed (PF) with heat-stressed cows. There was a 30d washout period between periods. Mammary biopsies were obtained at the end of each period. HS decreased milk yield (17%) and protein content (4.1%). HiSeq2000 platform was used to measure the mRNA profile. Data were normalized by Lowess prior ANOVA analysis using JMP Genomic (SAS systems) with treatment as the main effect and cow as the random effect. HS had a minor effect on the transcriptome with only 198 differentially expressed genes (DEG; FDR < 0.05; 1.4% of annotated genes measured): 53 upregulated and 145 downregulated genes in HS vs. PF. No gene known to be related to milk or protein synthesis was affected by treatment. Functional analysis was performed using the Dynamic Impact Approach and Database for Annotation, Visualization and Integrated Discovery revealed general inhibition of pathways in HS vs. PF, in particular, pathways affected were protein export, proteasome,
basal transcription factors, and steroid biosynthesis. The inhibition of basal transcription factors and protein export may partly explain the observed decrease in milk and protein synthesis. The biological significance of inhibited mammary steroid biosynthesis remains unclear; however, decreased steroidogenesis has been previously reported in heat-stressed bovine granulosa cells. In summary, transcriptome analysis revealed that HS inhibited metabolic activity by decreasing transcripts associated with protein export that might affect lactation performance.

Key Words: transcriptomics, mammary tissue, heat stress
Impact of production animal scholars program on developing production veterinarians. E. L. Karcher*1 and D. Grooms2, 1Purdue University, West Lafayette, IN; 2Michigan State University, East Lansing, MI.

Animal science undergraduate programs have undergone a dramatic change with increasing numbers of students having limited experiences with agricultural animals. Between 2005 and 2008, several reports cited a reduced number of large animal veterinarians and the potential impact on national food security. Recognizing the need to educate and encourage undergraduate students to consider a career in production animal medicine, Michigan State University (MSU), in 2008, developed a cooperative program between the Department of Animal Science and the College of Veterinary Medicine (CVM). The Production Animal Scholars (PAS) program provides undergraduate students education in the areas of basic sciences and animal management, as well as provides a special admissions pathway to MSU CVM. The objective of this study was to determine the impact of the PAS program on developing production veterinarians. Three surveys were developed and each administered in Fall 2016 to one of each of the following populations: (1) 31 undergraduate Animal Science students currently enrolled in the PAS program, (2) 15 PAS students currently enrolled in CVM, (3) 14 veterinarians who completed PAS as undergraduates as well as graduated from MSU CVM. The response rate for undergraduates, current CVM students, and graduated veterinarians was 54.0%, 80.0%, and 92.8% respectively. 91.3% of current PAS CVM students and 58.3% of PAS veterinarians agreed or strongly agreed that participation in PAS benefited their veterinary program. A total of 14 veterinarians have graduated as alumni of PAS. Of these, 58.3% are currently working with production animals. Of those answering no, 80% reported hoping to find a position that involved production animals as circumstances allow. In conclusion, the PAS program offers a unique opportunity to engage undergraduate students interested in attending veterinary school with a focus on serving the food animal industry, in a curriculum that is based strongly in animal management and basic sciences. Participants in this program view this preparation positively in helping them to meet their career goals.

Key Words: production, undergraduate, veterinarian

The use of virtual farm tours in a dairy cattle management course. E. L. Karcher* and P. Reid, Purdue University, West Lafayette, IN.

On-farm visits in Dairy Cattle Management courses provide students the opportunity to utilize all of their senses when describing and evaluating management practices on farms. However, obstacles may exist that prohibit classes from visiting farms. Alternative instructional methods, such as virtual farm tours, must be considered. The objective of this study is to evaluate the use of virtual farm tours as an effective instructional tool in a Dairy Management course. Three farm videos were created and viewed by students throughout the semester. The 35 students enrolled in the course were divided into 8 groups (4 to 5 students/group). Immediately following each video, online technology was used for a question and answer period with the producer. Groups were then asked to evaluate the dairy and provide a written evaluation, including strengths, opportunities, and recommendations for the farm. At the end of the semester, groups did one on-farm evaluation and were asked to complete the same assignment. A survey was developed to better understand student perception of the videos' impact on the learning process and administered to the class (82.8% response rate). Additionally, group responses for each of the virtual farm assignment questions were compared with those from the on-farm visit and differences evaluated using a paired t-test. Enthusiasm for the course was high with 89.7% of students indicating they were very interested in the content area of the course. The majority of the students agreed or strongly agreed that the virtual tours stimulated interest in the subject matter (63.0%) and allowed them to apply subject matter to real-world settings (72.4%). No overall differences were observed for the strengths and opportunities questions between the 2 scenarios. There was a difference in the scores for the farm recommendation question between 2 of the 3 virtual farm tours (1: 71.9% ± 10.8%; 2: 75.0% ± 6.7%) and the on-farm visit (81.3% ± 7.3%)(P < 0.05). Students felt (69.0%) that virtual tours and live producer interviews were a worthwhile part of the course. Initial data suggest that virtual farm tours are a useful instructional tool in a dairy management course when on-site farm visits are not an option.

Key Words: management, undergraduate, video

Male seniors were the lowest performing students in an introductory to dairy cattle science course. C. G. Burgett*, J. A. Sterle, and J. M. Bundy, Iowa State University, Ames, IA.

With increased enrollment in animal sciences and limited class sizes, demographics of sophomore-level specie-specific courses vary by age and student background. Upperclassmen would be expected to outperform underclassmen due to more collegiate experience and more curriculum background. The effects of student demographics and year in school on class performance were evaluated in a sophomore-level introductory dairy cattle science course. In the fall of 2016 ANS 235 Dairy Cattle Science, student demographics of the 108 enrolled students were sophomores (n = 25), juniors (n = 53), and seniors (n = 30). Of those, 85 were female and 23 males with less than half (n = 32) of the class from a farm background. Each participant completed a 21-question survey pertaining to both demographic and agricultural involvement growing up (4-H, FFA and/or a high school agriculture course). Students also completed a 10 question pre-test on the first day of class and students answered the same 10 pre-test questions incorporated into their final cumulative exam. Progress between pre- and post-test questions and overall course grade were used to evaluate student performance. Least-squared means and the differences between least-squared means were calculated using the PROC MIXED procedure in SAS. Sophomore and junior status students out performed (P ≤ 0.01) seniors by more than 6.39%. Male seniors earned the lowest final grade (P < 0.01) and showed the least improvement (P ≤ 0.04) between pre- and post-test questions compared with all other gender/classification combinations. Surprisingly, no differences (P = 0.26) were found in overall course grade for those students who took an agriculture course in high school (n = 56) compared with those who did not (n = 52). Involvement in 4-H and FFA had no effect (P ≥ 0.51) on the students’ overall course grade. However, female students who grew up on a farm (n = 20) had an increased (P ≤ 0.01) overall performance in the course compared with females who grew up in an urban area. Additionally, female farm students had greater (P ≤ 0.02) performance than males (n = 12) who grew up on a farm at the end of the semester. New methods may need to...
be implemented in sophomore-level specie-specific production courses to engage certain demographics.

Key Words: dairy science, gender, teaching

271 An assessment of the impact of the U.S. Dairy Education and Training Consortium (USDETC) on dairy education. M. A. Tomaszewski1 and G. R. Hagevoort*2, 1Texas A&M University, College Station, TX, 2New Mexico State University, Clovis, NM.

With dairy courses and access to dairy cattle declining at many universities, the US Dairy Education and Training Consortium was established in 2008 to provide a unique educational opportunity through both classroom and experiential learning experiences. Since its inception, 342 students have completed the 6-wk program. A survey of the former students was conducted (62% response rate) to determine the impact the consortium had on their future and the impact the classes and hands-on experiences had on their professional career. Of the 213 respondents, 99 are currently still enrolled at a university, 111 are employed and 3 are not employed. Of those enrolled at a university, 37% are undergraduate students, 30% are working toward advanced degrees and 30% are obtaining a veterinary degree. Of the former students which have since entered the job market, 34% have found employment on a dairy, 33% are employed in a dairy related position (allied industry), 5% are in a non-dairy livestock position, 6% are in a non-dairy agricultural position and 21% are employed outside of agriculture. Of those employed, 87 students obtained a BS, while 11 completed their MS, 2 students are Ph.D.‘s and 9 students graduated with a DVM degree. When asked “What impact attending the consortium had on their current status,” 92% replied important, very important or extremely important. When asked about the impact the classes and experiential learning experiences had on their course work and subsequent careers, 44% replied extremely helpful, 35% very helpful and 15% helpful. When asked to rank the consortium classes as compared with other courses taken, 55% gave the consortium an A+ and 36% an A. When asked for general comments, the hands-on experiences and access to exceptional faculty were the student’s main remarks. The USDETC has proven to be a positive alternative or complementary education opportunity for students that do not or have limited access to dairy courses or the related experiential learning experiences at their home universities.

Key Words: dairy education, dairy training, experiential learning.
Animal Behavior and Well-Being II

T1  Assessment of two pain management strategies following scoop dehorning in dairy calves. A. A. Barragan*, S. Bas, and L. da Costa, Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH.

Dehorning of calves is routinely performed in dairy farms. Some advantages of dehorning include, safer handling, decreased injuries to other animals and/or humans, reduce the bruising of the carcass and the aggressive interactions between pen mates. However, dehorning requires pain management to minimize pain and improve welfare. Our objectives were to compare the effects of 2 consecutive treatments with 2 non-steroidal anti-inflammatory drugs (meloxicam [ME] and flunixin meglumine [FM]) on activity patterns (standing and lying time) and physiological parameters (rectal temperature and heart rate) in female Holstein calves after scoop dehorning. Holstein heifers (n = 36) aged 4 to 6 mo from 2 dairy farms were enrolled. On study d −7 HOBO accelerometers were placed on the rear leg of calves. On study d 0 (dehorning) calves were randomly allocated into 3 treatment groups: (1) ME (n = 13; 1.0 mg/kg P.O.); (2) FM (n = 13; 2.2 mg/kg I.V.); and (3) Control (CON; n = 13; no dehorning). A cornual nerve block was performed with 2% lidocaine (4 mL) before dehorning. The horns were removed using a Barnes dehorning instrument. Treatments (ME, FM) were administered immediately after dehorning. On study d1, all calves were restrained and second treatment dose was administered to ME and FM calves. Accelerometers were removed on study d6. Physiological parameters were measured on study d −7, 0, 1 and 6. The data were analyzed as a randomized block design with repeated measures using ANOVA by the MIXED procedure of SAS. Dehorned calves (ME and FM) spent less time lying (P < 0.05; ME = 760 ± 18.49 min/d; FM = 763 ± 18.42 min/d; CON = 830 ± 18.83 min/d) and more time standing (P < 0.05; ME = 680 ± 18.49 min/d; FM = 677 ± 18.42 min/d; CON = 610 ± 18.83 min/d) the day after dehorning (d 1); and on d 3 and d 4 (P < 0.05) compared with CON. However, no difference in activity patterns was observed between ME and FM groups. No difference was found on rectal temperature and heart rate between groups. Results of this study suggest that scoop dehorning affects activity patterns, and that the 2 pain management strategies had a similar effect on calf behavior. Dehorning is a painful procedure and pain management strategies should be implemented to minimize pain and improve well-being of animals.

Key Words: dehorning, dairy calf, activity

T2  Pair housing of dairy calves in modified individual calf hutches. L. Whalin* and M. A. G. von Keyserlingk, Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada.

The objective of this study was to determine if pair housing using a modified calf hutch on a commercial dairy farm would provide similar benefits to those previously described in research settings. Despite many benefits (e.g., weight gain, social behaviors, less fearfulness) of housing calves in small groups, individual calf hutches are still the predominant housing type in North America - perhaps because hutches are designed for one calf. Our modified hutch consisted of 2 individual hutches placed next to each other where both calves had access to both hutches and a common run. This study took place on a commercial farm in the Fraser Valley of British Columbia from May to November 2016. A week after birth, calves were randomly assigned to individual (n = 14) or paired (n = 8 pairs) hutches and fed milk 2/d using a nipple bottle. Calves were fed 6 L/d from d 1 to d 6, 10 L/d d 7 to d 35, and 6 L/d until d 56 (weaning). At each transition, milk volume changed over 48 h. Ad libitum access to calf starter, and water was offered beginning d 2. Feed intake was measured weekly until calves were 67 ± 5 d, while BW and health measures were recorded weekly until the calves were 85 ± 5 d. When the calves were 56 d a novel food test occurred where the amount of food consumed in 30 min was measured. Mixed model and independent t-tests were conducted in SAS (version 9.3, SAS Institute Inc., Cary, NC) to assess the effect of housing on the intake of starter and novel feed. Starter intake did not differ during high milk allowance (P > 0.5), but pair housed calves ate more starter after the first reduction in milk (pair: 1.72 ± 0.20 kg/d; individual: 1.02 ± 0.15 kg/d; P < 0.01), and during the post-weaning period (pair: 3.51 ± 0.27 kg/d; individual: 1.71 ± 0.21 kg/d; P < 0.01). Pair housed calves also ate nearly 3 times as much novel feed (pair: 170.92 ± 37.75 g; individual: 57.69 ± 10.55 g; P < 0.02). In conclusion, joining adjacent hutches is a viable option for pair housing; providing social contact during the milk feeding period increases calves’ willingness to consume different feeds resulting in increases in solid feed intake and potentially reducing weaning stress.

Key Words: novelty, feed intake, commercial farm

T3  Veal calf health on the day of arrival to growers in Ohio. J. Pempek*, D. Trearchis, M. Masterson, G. Habing, and K. Proudfoot, The Ohio State University, Columbus, OH.

Veal calves are at high risk for disease and mortality in early life. Calves face several stressors before arriving to the grower, including long transport times, which may contribute to poor health. Our objectives were to (1) estimate the prevalence of poor health outcomes in veal calves on arrival to growers in Ohio, (2) determine risk factors for health outcomes on arrival, including auction site (spread across 5 states in the Northeastern US), and (3) determine if health outcomes on arrival predict early mortality. A physical examination was conducted on approximately 30 calves from 12 cohorts (n = 383 calves). Exams included a blood sample to determine packed cell volume (an estimate of dehydration using a cut-off >46%) and total protein (an estimate failure of passive transfer using a cut-off <5.5 g/dL). Diarrhea, respiratory disease, depression, navel inflammation, and a skin tent test (a second indicator of dehydration) were also recorded. Mortality within 4 wk of age was recorded from farm records. Descriptive statistics were used to describe the prevalence of calves with poor health outcomes upon arrival. Generalized linear mixed models were used to identify risk factors for poor health on arrival and assess if poor health increased the incidence risk of mortality. Upon arrival, 6% (95% CI: 4.4–7.6) of calves had failure of passive transfer, 14% (9.1–18.8) presented with diarrhea, 0.5% (0–1.3%) had respiratory disease, 14% (8.5–19.3) were depressed, and 27% (22.7–30.7) had inflamed navels. In addition, 35.1% (23.5–46.6) of calves were dehydrated using a skin tent test, but only 1.3% (0.0–2.9) were dehydrated based on the packed cell volume threshold. Auction site of origin was significantly associated with depression on arrival (P < 0.001), and tended to be associated with skin tent on arrival (P = 0.08). None of the health variables were predictors of early mortality; however, mortality was generally low for all cohorts (4.3%; 1.7–6.8). These results provide evidence that veal calves experience dehydration, diarrhea, navel inflammation, and depression on arrival to growers, and some health outcomes are dependent on auction site.

Key Words: dehydration, failure of passive transfer
T4  Characterizing activity at social grouping in weaned dairy calves. K. C. Horvath* and E. K. Miller-Cushon, University of Florida, Gainesville, FL.

After weaning, dairy calves are commonly exposed to changing social environments, which are likely to influence activity patterns. We examined the activity of recently weaned dairy calves upon introduction to a social group. Holstein heifer calves (n = 26) were individually housed and provided milk (10 L/d) for 6 wk, and gradually weaned over 9 d. Calves were then introduced to group housing on pasture (15.2 × 45.7 m) in weekly cohorts (6 ± 2 new calves/wk; mean ± SD) at 60 ± 3 d of age. The group (9 ± 1 calves) always consisted of newly introduced calves and calves introduced the previous week. Calves were equipped with electronic data loggers (HOBO Pendant G data logger, Onset Computer Corp., Pocasset, MA) to measure daily standing time and bout frequency for a 2-wk observation period: the first week following grouping, and the second week, which began with addition of younger calves and removal of older calves. The effects of week and day on daily standing time and bout frequency were analyzed in a double repeated measures general linear mixed model. Additionally, standing time was summarized by hour to assess diurnal activity patterns. Activity data were subject to a week by day interaction (P < 0.003), with a peak on the day of introduction for standing time (8.8 vs. 7.1 min/d; d 1 of wk 1 vs. d 1 of wk 2; SE = 0.42; P = 0.0046) and bouts (20.5 vs 13.6 bouts/d; SE = 1.35; P < 0.001) but decreasing activity over wk 1 and stable activity during wk 2. Newly introduced calves also stood longer in the hour after introduction but spent less time standing later in the day, compared with their diurnal activity on the first day of the following week when younger calves were added (week by hour interaction; P < 0.001). These results suggest that the first few days of introduction to a social group have a marked influence on activity patterns, but that previously grouped calves are less responsive to the addition of new calves into the group.

Key Words: dairy calf, social housing, lying behavior

T5  Behavioral changes in group-housed dairy calves infected with Mannheimia haemolytica. C. L. Hixson*, P. D. Krawczel2, J. M. Caldwell3, and E. K. Miller-Cushon1, 1University of Florida, Gainesville, FL, 2University of Tennessee, Knoxville, TN.

Insight into sickness behavior may be the means to improving identification, management, and welfare of sick animals. The objective of this study was to identify components of sickness behavior in group-housed Holstein dairy calves infected with Mannheimia haemolytica. Calves (aged 3–7 weeks; 58.0 ± 12.0 kg BW) were group-housed based on age and BW in sand-bedded pens (6 calves/pen, 6.6 m²/calf) and provided pasteurized waste milk (8 L/d) 2 × /d. Within group, calves were randomly assigned to treatment. On d 0, treatment calves (MH; n = 12, 3/pen) were inoculated at the tracheal bifurcation with 3 × 10⁹ cfu of MH suspended in 5 mL of phosphate buffered saline (PBS) followed by a 120 mL wash PBS. Control calves (n = 12, 3/pen) were inoculated with 5 mL + 120 mL of PBS. Behavioral and health data were collected for 7 d post-challenge. Each calf was fitted with an accelerometer to record lying time and laterality. Rectal temperatures and respiratory disease scores (based on presence of cough, nasal or ocular discharge, and ear droop; 0 = normal; 5 = treat for illness) were obtained every 12 h. Data were analyzed in a general linear mixed model with day (or hour) treated as a repeated measure. Temperature was subject to a treatment by hour interaction (P = 0.015), peaking for MH calves at 12 h post-inoculation (40.1 vs 39.1°C; SE = 0.14, P < 0.001). Respiratory score was greater overall for MH calves (3.9 vs. 3.2; SE = 0.2; P = 0.024). A treatment by day interaction was evident in lying time (P < 0.001), with MH calves lying down for longer on d 0 (19.9 vs 18.4 h/d; SE = 0.33, P = 0.023). MH calves also spent more time lying on their left side than right in the week following inoculation (9.9 vs 7.5 h/d; SE = 0.58, P = 0.006), with control calves expressing no lateral preference (P = 0.74). These results show that this challenge model resulted in calves experiencing a mild disease state. Increased lying time and lying laterality may be useful indicators of early stages of disease.

Key Words: dairy calf, respiratory disease, behavior


There is evidence that the health and welfare of young dairy calves may be improved by increasing milk allowance and by providing milk through a teat. These aspects are easily incorporated into automatic milk feeding systems, which promote group housing that has been demonstrated to be important for social development of calves. Little is known about how calves interact with automatic milk feeders (AMF). We investigated the effect of stall design features on calves learning to use the AMF. Sixty-six male and 53 female Holstein calves were enrolled at 4 d of age and introduced to a group pen and trained on an AMF; calves were allowed to suck on a trainer’s fingers and guided to the teat. Calves were allocated to 1 of 2 stall designs: with gated (open) side walls (n = 59), or with solid side walls (n = 60). The hypothesis was that solid stalls would result in a longer latency to approach and feed from the AMF. For a 72-h period after training to the AMF, data from the AMF were collected and calf behavior was monitored by video. Main outcomes measured were latency to first voluntary visit to the feeder, latency to first feeding, time spent in feeder, amount of milk drank, and exploratory behavior such as sniffing and licking of the feeder. Data were analyzed using a mixed-effect linear regression model. The overall latency for calves to first voluntarily drink (without barn staff leading them) from the feeder was 5.3 h greater (P = 0.05; SE = 2.7) with a gated side compared with solid. This voluntary visit differs from if the calf was brought to the feeder for re-training at a 12-h check point to ensure at least 2 L every 12 h. The average time taken to first voluntarily drink was 23.7 h (min = 2.9, max = 69.1) with gated sides and 18.4 h (min = 3.2, max = 54.5) with solid sides. Male calves drank 2.0L more (SE = 1.0; P = 0.05) milk over the 72-h study period than females. Results from this experiment show that simple features of a stall can influence how quickly calves adapt to an AMF. Based on these results, calves adapted to using the AMF quicker when the stall walls were solid versus gated style.

Key Words: calf, feeding, behavior

T7  Feeding behavior of cows fed with oregano and green tea extracts during the transition period. V. Fischer*1, S. C. B. Stivain1, E. F. Vizzotto1, M. de Paris1, and M. B. Zanela1, 1Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, 2Empresa Brasileira de Pesquisa Agropecuaria, Pelotas, RS, Brazil.

This work aimed to investigate the effects on feeding behavior in dairy cows fed with Origanum vulgare or Camellia sinensis extracts during the transition period. Twenty-four Jersey cows with BW 430 ± 33 kg were randomly assigned to one of the following treatments: control (CO, no plant extract), green tea extract (GTE, 5 g/cow/day) and oregano extract (OE, 10 g/cow/day). Cows received the plant extracts mixed into the concentrate from ~21 d to +21 d relative to parturition, were fed with 4 kg of concentrate and 21 kg of corn silage offered separately
and were allowed to graze. Behavior was registered at 10-min intervals between milkings during 10 h on d −14, −7, −1, 7, 14, 21 relative to parturition. Data were submitted to variance analysis, using the Mixed procedure of SAS according to a completely randomized design, considering the effect of diet (n = 3), day of measurement (n = 6), diet by day interaction. There were no significant treatment by day interactions (P > 0.05). Cows fed GTE spent more time eating the concentrate, had more visits to the feeding trough with eating activity than cows fed CO or OE groups. Cows fed OE tended to have less visits to the feeding trough without eating and lower total time spent eating and time spent ruminating while lying than cows fed CO and GTE groups. Cows fed OE had higher time spent ruminating while standing than GTE group. Treatments did not influence time spent resting while lying or standing. Plant extracts affected distinctly feeding behavior, as GTE increased time spent eating and visits to the trough while OE decrease eating time and visits to the trough without feeding.

Table 1 (abstract T7).

<table>
<thead>
<tr>
<th>Behavior trait</th>
<th>Control</th>
<th>Oregano</th>
<th>Green tea</th>
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<th>SE</th>
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<tr>
<td>Time eating concentrate (min)</td>
<td>23.2b</td>
<td>21.6b</td>
<td>28.5a</td>
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<td>Total eating time (min)</td>
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<td>168.6b</td>
<td>179.3ab</td>
<td>&gt;0.100</td>
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<td>Visits to feeding trough with eating (no.)</td>
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<td>6.6b</td>
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<td>0.9</td>
</tr>
<tr>
<td>Visits to feeding trough without eating (no.)</td>
<td>6.1a</td>
<td>3.9a</td>
<td>6.2a</td>
<td>&lt;0.100</td>
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<tr>
<td>Resting and lying (min)</td>
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<td>30.8b</td>
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<td>Resting and standing (min)</td>
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<td>Ruminating and lying (min)</td>
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<td>Ruminating and standing (min)</td>
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<td>86.4a</td>
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</table>

Key Words: behavior, dairy cow, plant extract

T8 The effect of overstocking different resources within a freestall pen on the behavior and physiology of lactating Holstein cows. C. B. Kesterson*, R. A. Black, N. L. Eberhart, E. M. Edwards, and P. D. Kwawczel, Department of Animal Science, The University of Tennessee, Knoxville, TN.

The objective was to determine the effect of overstocking the headlock, the freestalls, or both at 160% on the physiology and resting behavior of dairy cattle. Holstein cows (n = 32) were housed in a freestall barn, fed a TMR at 0700 and 1530 h, and milked at 0830 and 1830 h daily. Groups (n = 4) were balanced by parity (1.8 ± 1.1) and DIM (224.3 ± 58.5 d). Treatments were 100% stocking density at freestalls (FS) and headlocks (HL), 100% stocking density at FS and 160% at HL, 160% stocking density at FS and 100% at HL, and 160% stocking density at FS and HL. Treatments were assigned using a 4 × 4 Latin square with 30-d periods. Cows were fed a partly mixed ration (PMR) supplemented with MCFA. MCFA composed of 80% capric acid and 20% caprylic acid was added to PMR at 0%, 0.5%, or 1.0% of the total dry matter intake. All cows received the same PMR (EE, 3.3%; NDF, 44.3%) ad libitum. A concentrate diet (EE, 3.5%) was offered at the AMS in proportion to individual milk yield. Data of milking activity and ruminating time were collected from the AMS system. Feeding behavior was analyzed using the records by the RIC system. The meal was determined by a log survivor curve for intervals between feedings for each cow. Data were analyzed by ANOVA to determine effects of MCFA. Correlation coefficients (r) were determined between feeding behavior and milking activity. The MCFA did not affect milking activity and feeding behavior. However, ruminating time was found to be positively correlated (P < 0.01) with milk yield and daily milking frequency (r = 0.77 and r = 0.64, respectively). An increased daily milking frequency was associated (P < 0.05) with longer meal duration and decreased visiting frequency to the feed box with RIC system (r = 0.58 and r = −0.49, respectively). In conclusion, these results indicated that bovine behavior of cows in AMS was not affected by the MCFA, and individual difference in feeding behavior among cows could relate to individual variation of milking activity.

Key Words: automatic milking system, feeding behavior, milking activity


Generally, the calcium salts of fatty acids affect feed intake, however, it is unclear whether these affect feeding behavior including concentrate provision and voluntary visits in an automatic milking system (AMS). This study investigated whether supplementation of calcium salts of medium-chain fatty acids (MCFA) modified the feeding behavior and milking activity in AMS. Six multiparous Holstein cows (initial days in milk, 202; initial BW, 707 kg; parity, 2.3) in a cow herd kept in a free cow traffic barn with AMS and the roughage intake control (RIC) system were used in a replicated 3 × 3 Latin square design with 21-d periods. Cows were fed a partly mixed ration (PMR) supplemented with MCFA. MCFA composed of 80% capric acid and 20% caprylic acid was added to PMR at 0%, 0.5%, or 1.0% of the total dry matter intake. All cows received the same PMR (EE, 3.3%; NDF, 44.3%) ad libitum. A concentrate diet (EE, 3.5%) was offered at the AMS in proportion to individual milk yield. Data of milking activity and ruminating time were collected from the AMS system. Feeding behavior was analyzed using the records by the RIC system. The meal was determined by a log survivor curve for intervals between feedings for each cow. Data were analyzed by ANOVA to determine effects of MCFA. Correlation coefficients (r) were determined between feeding behavior and milking activity. The MCFA did not affect milking activity and feeding behavior. However, ruminating time was found to be positively correlated (P < 0.01) with milk yield and daily milking frequency (r = 0.77 and r = 0.64, respectively). An increased daily milking frequency was associated (P < 0.05) with longer meal duration and decreased visiting frequency to the feed box with RIC system (r = 0.58 and r = −0.49, respectively). In conclusion, these results indicated that bovine behavior of cows in AMS was not affected by the MCFA, and individual difference in feeding behavior among cows could relate to individual variation of milking activity.

Key Words: overstocking, cow, welfare

T10 Feeding behavior of lactating dairy cows with genomic predisposition for residual feed intake fed at two levels of dietary neutral detergent fiber. F. Sun1, M. Aguerre2, J. Powell3,5, K. Weigel1, A. Pelletier3,5, P. Crump1, and M. Wattiaux1, 1Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, 2Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC, 3US Dairy Forage Research Center, Madison, WI, 4Department of Computing and Biometry, University of
Our objective was to determine if genomic predisposition for residual feed intake (RFI) influenced the expected change in feeding behavior associated with increasing neutral detergent fiber (NDF) concentration in diet. Multiparous Holstein cows (n = 24) in mid-lactation were used in a randomized complete block design. Genomic and phenotypic data from previous trials were used to group cows as low RFI (L-RFI, −0.42 ± 0.01 kg/d) or high RFI (H-RFI, 0.34 ± 0.03 kg/d). Following a 2-week covariate period during which cows were fed a diet with 31.7% NDF (dry matter basis), half of the cows in each RFI group were assigned to a diet with either 30.8 (L-NDF) or 34.0% (H-NDF) NDF for 4 weeks. Cows’ feeding behavior during the last 24 h of the experimental period was recorded with video camera. Videos were visually analyzed every 2.5 min and activity was assumed to persist the whole 2.5 min. Daily dry matter intake (DMI) and milk production were recorded individually for each cow. Covariate and experimental data were analyzed separately with SAS mixed procedure. During the covariate period L-RFI cows had lower DMI and higher feed efficiency (FE, milk-and-fat-corrected milk/DMI) than H-RFI cows (25.6 vs. 26.6 kg/d, and 1.61 vs. 1.46, \( P \leq 0.05 \)). After 4 weeks of dietary changes, L-RFI cows tended to maintain greater FE than the H-RFI cows (1.64 vs. 1.47, \( P = 0.08 \)), there was no dietary NDF by cow RFI interactions, and no RFI effects (\( P > 0.11 \)) on any feeding behavior responses. Dietary NDF did not influence daily time spent eating or ruminating (181 ± 16.4, \( P = 0.20 \), and 396 ± 42.0 min/d, \( P = 0.12 \)). However, cows fed H-NDF had lower DMI (25.4 vs. 23.6 kg/d, \( P = 0.03 \)), tended to spend more time chewing (539 vs. 601 min/d, \( P = 0.08 \)) and drinking (25 vs. 40 min/d, \( P = 0.07 \)), had smaller meal size (2.34 vs. 1.81 kg DM/meal, \( P = 0.02 \)) and spent more time eating, ruminating, and chewing per DMI (7.31 vs. 8.5, 15.2 vs. 18.3, and 22.5 vs. 26.8 min/kg DMI, \( P \leq 0.05 \)) than cows fed L-NDF. In this study, L-RFI cows maintained higher FE than H-RFI cows regardless of dietary NDF. Furthermore, most measured feeding behavior responses were influenced by dietary NDF but none of them were influenced by cow RFI or the dietary NDF by cow RFI interaction.

**Key Words:** chewing
The objective of this observational retrospective cohort study was to compare behavior and productive parameters of lactating dairy cows that developed metabolic and digestive disorders (MDD; displaced abomasum, ketosis, indigestion and abomasal ulcers) versus cows that did not develop health disorders (Healthy Control; HC) up to 30 DIM. Records were retrieved from 1,995 completed lactations from cows at a commercial farm in central NY collected by an automatic milking system (AMS) software (Lely T4C) from January 2014 to May 2016. Health event data were collected from DairyComp 305. Data collected up to 30 DIM by the milking unit of the AMS and neck-mounted electronic tags for automated rumination and activity monitoring was summarized daily and included: milk yield (MY), milk fat and protein percentage, milk fat:protein ratio (F:P ratio), body weight (BW), rumination time (RT), physical activity (ACT), and number of milkings per day (NM). Data were analyzed by ANOVA with repeated measurements using PROC MIXED of SAS. All parameters collected by the AMS (exploratory variables) were evaluated from 7 d before to 7 d after diagnosis of MDD (Day of diagnosis = D0). For cows in the HC group, average DIM at MDD diagnosis (9 DIM) was considered as D0. Cows with MDD (n = 275) had reduced RT (P < 0.01; greatest difference on D-1: 162 min/d), ACT (P < 0.01; greatest difference on D0: 163 units/d), MY (P < 0.01; greatest difference on D1: 13.3 kg/d), NM (<0.01; greatest difference on D-1 to D2: 0.8 visit/d), and BW from D-2 to D7 (P < 0.01; greatest difference on D7: 34 kg). Cows with MDD lost 72 kg from D-7 to D7 compared with 38 kg for HC cows (n = 789) in the same period of time. Furthermore, cows in the MDD group had increased milk fat percentage (P < 0.01; greatest difference on D1: 0.8%) and F:P ratio (P < 0.01; greatest difference on D1: 0.34). Milk protein percentage (P < 0.01) was greater from D-5 to D-2, and then lower from D0 to D7 for cows in the MDD than in the HC group. We conclude that cows which developed MDD exhibited changes in behavior and productive parameters around the time of clinical diagnosis. Thus, behavioral and productive parameters could be used to identify cows suffering metabolic and digestive disorders.

Key Words: mastitis, antimicrobial resistance, milk microbiome

T13 The effect of 2,4-thiazolidinedione on lipid-soluble vitamins in lactating goats induced with subclinical mastitis. C. Y. Tsai*,†, F. Rosa‡, M. Bionaz§, and P. Rezamand†, 1Department of Animal and Veterinary Science, University of Idaho, Moscow, ID, 2Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, OR.

2,4-Thiazolidinedione (TZD), a peroxisome proliferator-activated receptors agonist, modulates metabolism and inflammation. The objective of this study was to determine the effect of TZD on lipid soluble vitamins in goats with sub-clinical mastitis. The study included 2 experiments. In exp. I: 24 Saanen lactating dairy goats receiving low-energy diet without vitamins supplement. Six goats in each group received a daily IV injection of either 8 mg/kg BW of TZD or saline. A week later, goats were challenged with intramammary infusion (IMI) of saline (CTZD or CTRL) or Streptococcus uberis (MTZD or MCTR). Blood samples were obtained on d −7, −2, 1, 2 and 12 relative to IMI. In experiment II, 12 Saanen lactating dairy goats received supplemental lipid-soluble vitamins. Six goats in each group were injected with 8 mg/kg BW of TZD or saline daily, followed by an IMI of Streptococcus uberis 14 d later. Blood samples were obtained on d −15, 0, 1, 10 and 11 relative to IMI. Serum samples were analyzed for retinol, α-tocopherol, β-carotene, 13-cis retinoic acid and all-trans retinoic acid. Data were analyzed using the Proc Mixed of SAS with significance declared at P ≤ 0.05. In experiment I, MTZD on d 1, 2 and 12, showed lower retinol concentration compared with CTRL. On d 2, MTZD had a lower retinol concentration compared with MCTR. However, β-carotene in MTZD group was greater compared with CTRL and MCTR on d 12. No significant difference was observed among treatments in experiment II. In conclusion, during sub-clinic mastitis with low energy diets and inadequate vitamins, treatment with TZD may mobilize retinol toward CDCT receiving ceftiofur hydrochloride and teat sealant or selective dry cow therapy (SDCT) receiving only teat sealant. As expected at the time of enrollment (Dry off) no differences were observed for the somatic cell count (SCC) scores between treatments (P = 0.47). Selective dry cow therapy had no effects on the incidence of mastitis in the first 60 d postpartum (P = 0.46), percent of culture positive cows at d 7 postpartum (P = 0.68), and SCC scores at d 7 postpartum (P = 0.40). Firmicutes was the most abundant phylum, and Corynebacterium, Acinetobacter, and Staphylococcus, often involved in mastitis cases, were the most abundant genera across treatments and time. However, no effects of SDCT on milk microbiome and bacterial load were identified. Bacterial load was greater at 7 d postpartum than at dry off. The Chao 1 and Shannon indexes were not statistically different between CDCT and SDCT groups, regardless of time point. A negative correlation was detected between the total bacterial load, as assessed by the number of 16S rRNA gene copies, and the Shannon diversity index (r = −0.29, P < 0.01). Similar negative correlations were found when data were stratified by the occurrence of clinical mastitis, subclinical mastitis, and culture on d 7 postpartum. No correlation was found between the number of 16S rRNA gene copies and the Chao 1 richness index. Taken altogether, our findings suggest that the SDCT may help prevent rational use of antimicrobials without increasing presence of pathogens in milk’s microbiome and bacterial load.

Key Words: mastitis, antimicrobial resistance, milk microbiome

T11 Metabolic and digestive disorders affect behavioral and productive parameters of lactating Holstein cows milked with an automatic milking system. M. L. Stangaferro* and J. O. Giordano, Cornell University, Ithaca, NY.

The objective of this observational retrospective cohort study was to compare behavior and productive parameters of lactating dairy cows that developed metabolic and digestive disorders (MDD; displaced abomasum, ketosis, indigestion and abomasal ulcers) versus cows that did not develop health disorders (Healthy Control; HC) up to 30 DIM. Records were retrieved from 1,995 completed lactations from cows at a commercial farm in central NY collected by an automatic milking system (AMS) software (Lely T4C) from January 2014 to May 2016. Health event data were collected from DairyComp 305. Data collected up to 30 DIM by the milking unit of the AMS and neck-mounted electronic tags for automated rumination and activity monitoring was summarized daily and included: milk yield (MY), milk fat and protein percentage, milk fat:protein ratio (F:P ratio), body weight (BW), rumination time (RT), physical activity (ACT), and number of milkings per day (NM). Data were analyzed by ANOVA with repeated measurements using PROC MIXED of SAS. All parameters collected by the AMS (exploratory variables) were evaluated from 7 d before to 7 d after diagnosis of MDD (Day of diagnosis = D0). For cows in the HC group, average DIM at MDD diagnosis (9 DIM) was considered as D0. Cows with MDD (n = 275) had reduced RT (P < 0.01; greatest difference on D-1: 162 min/d), ACT (P < 0.01; greatest difference on D0: 163 units/d), MY (P < 0.01; greatest difference on D1: 13.3 kg/d), NM (<0.01; greatest difference on D-1 to D2: 0.8 visit/d), and BW from D-2 to D7 (P < 0.01; greatest difference on D7: 34 kg). Cows with MDD lost 72 kg from D-7 to D7 compared with 38 kg for HC cows (n = 789) in the same period of time. Furthermore, cows in the MDD group had increased milk fat percentage (P < 0.01; greatest difference on D1: 0.8%) and F:P ratio (P < 0.01; greatest difference on D1: 0.34). Milk protein percentage (P < 0.01) was greater from D-5 to D-2, and then lower from D0 to D7 for cows in the MDD than in the HC group. We conclude that cows which developed MDD exhibited changes in behavior and productive parameters around the time of clinical diagnosis. Thus, behavioral and productive parameters could be used to identify cows suffering metabolic and digestive disorders.

Key Words: automation, health monitoring, dairy cow

T12 Evaluation of milk microbiome provides evidence for selective dry cow therapy and rational use of antimicrobial in dairy cows. E. C. R. Bonsaglia1,2, M. S. Gomes1, I. F. Canisso1, Z. Zhou1, S. F. Lima3, V. L. M. Rall2, E. F. Garrett1, G. Oklahoma4, R. C. Bicalho3, and F. S. Lima*1, 1University of Illinois, Urbana-Champaign, IL, 2Sao Paulo State University, Botucatu, SP, Brazil, 3Cornell University, Ithaca, NY, 4University of Liverpool, Leahurst, Neston, UK.

Preventive infusion of antibiotics in the healthy mammary gland of cows might not be critical to prevent new infections, yet, 11 tons of medically relevant antibiotics are consumed yearly to support this practice. Here, we used next-generation sequencing and 16S rRNA gene quantitative real-time PCR to determine the effect of selective dry cow therapy on the milk microbiome and bacterial load in dairy cows. A cohort of 72 cows diagnosed negative for mastitis by an on-farm culture at dry off were randomly allocated to remain as conventional dry cow therapy (CDCT) receiving ceftiofur hydrochloride and teat sealant or selective dry cow therapy (SDCT) receiving only teat sealant. As expected at the time of enrollment (Dry off) no differences were observed for the somatic cell count (SCC) scores between treatments (P = 0.47). Selective dry cow therapy had no effects on the incidence of mastitis in the first 60 d postpartum (P = 0.46), percent of culture positive cows at d 7 postpartum (P = 0.68), and SCC scores at d 7 postpartum (P = 0.40). Firmicutes was the most abundant phylum, and Corynebacterium, Acinetobacter, and Staphylococcus, often involved in mastitis cases, were the most abundant genera across treatments and time. However, no effects of SDCT on milk microbiome and bacterial load were identified. Bacterial load was greater at 7 d postpartum than at dry off. The Chao 1 and Shannon indexes were not statistically different between CDCT and SDCT groups, regardless of time point. A negative correlation was detected between the total bacterial load, as assessed by the number of 16S rRNA gene copies, and the Shannon diversity index (r = −0.29, P < 0.01). Similar negative correlations were found when data were stratified by the occurrence of clinical mastitis, subclinical mastitis, and culture on d 7 postpartum. No correlation was found between the number of 16S rRNA gene copies and the Chao 1 richness index. Taken altogether, our findings suggest that the SDCT may help promote rational use of antimicrobials without increasing presence of pathogens in milk’s microbiome and bacterial load.

Key Words: mastitis, antimicrobial resistance, milk microbiome
inflammation. Treatment with TZD did not seem effective when goats were supplemented with lipid-soluble vitamins.

**Key Words:** thiazolidinedione, mastitis, goat

### T14 Bacterial ecosystem of the bovine mammary gland: Potential role of foundation taxa in shaping mammary gland microbiota and modulating udder homeostasis. H. Derakhshani*1, J. C. Plazier1, and E. Khafipour1,2, 1Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada, 2Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada.

Biotic interactions among commensal members of mammary gland (MG) microbiota play a crucial role in shaping the overall structure of the MG microbiome and determining its functional properties such as stability and resistance to pathogen invasion. The main objectives of the current study were to a) characterize bacterial communities that inhabit different ecological niches of the MG, b) determine their potential associations with the inflammatory status of the udder, and c) characterize niche-specific microbial relationships that are influential in shaping the biodiversity of the MG. To this end, aseptic quarter milk (n = 144) and teat canal swab (n = 144) samples were collected from dairy cows with a wide range of MG inflammatory status, as determined by somatic cell counts (SCC) of the milk, and subjected to genomic DNA extraction and high-throughput 16S rRNA gene sequencing of the V1-V2 hypervariable regions. The teat canal ecosystem was composed of a more diverse and compositionally distinct (P\(_{\text{PERMANOVA}}\) < 0.001) microbiota compared with the milk. This, coupled with identification of a large number of bacterial lineages that were exclusive to the teat canal microbiota suggested that milk ecosystem act as a potent selective force that precludes the growth of certain environmental bacterial lineages. Further, by utilizing correlation network analysis (CoNet), we identified candidate foundation taxa that showed disproportionately large impact on the structure and diversity of the MG microbiota. These foundation taxa consisted of 2 groups of phylogenetically distinct lineages within the phylum *Bacteroidetes* showing either positive (unclassified *Bacteroidaceae*) or negative (*Sphingobacterium*) correlation with biodiversity metrics of the MG. Certain species within the latter group also showed significant (P < 0.05) positive association with the SCC of the milk. Overall, our results provide novel insights into the biotic interactions that exist among the commensal microbiota of the MG and their potential role in modulating udder health status.

**Key Words:** mammary gland microbiota, foundation taxa, udder health

### T15 Quarter somatic cell count of culture negative and gram-negative cases of non-severe clinical mastitis enrolled in negatively controlled randomized clinical trials. M. J. Fuenzalida* and P. L. Ruegg, University of Wisconsin, Madison, WI.

The objective was to describe quarter-somatic cell count after occurrence of clinical mastitis (CM) in cows enrolled in negatively controlled randomized clinical trials. Cases of CM were enrolled in 2 separate randomized clinical trials conducted on 2 farms. In trial 1, cases confirmed as culture negative (NG) were randomly assigned to 5-d intramammary (IMM) cefitiofur therapy or non-treated control. In trial 2, cases confirmed as gram-negative (GN: *E. coli* or Klebsiella spp) were randomly assigned to 2 or 5-d of IMM cefitiofur therapy or non-treated control. Quarter milk samples were collected at detection of CM, 7, 14, 21, and 28 d after enrollment. Repeated measures analysis was performed to compare SCC trends among treatment groups. In trial 1, time (P < 0.01) had an effect on SCC compared with treatment (P = 0.11). In trial 2, pathogen (P < 0.01), treatment (P = 0.04), time (P < 0.01) and the interaction between pathogen-treatment and time (P < 0.01) had an effect on SCC. Quarter SCC at detection of CM was not different among pathogens. Quarter SCC decreased at different rates depending on the pathogen and treatment group. Proportion of cases with quarter < 200,000 cells/mL at 28 d after enrollment was not different within trials. For cases caused by Klebsiella spp, 8-d of IMM cefitiofur resulted in lower SCC but was not associated with proportion of cases with quarter-SCC < 200,000 cells/mL. Treatment was not associated with SCC for CM caused by *E. coli* or NG cases. We conclude that for non-severe CM, etiology has a greater influence on quarter SCC than IMM treatment using cefitiofur.

**Key Words:** clinical mastitis, randomized clinical trial

### T16 Macrophage activation during subclinical mastitis in dairy goats treated with 2,4-thiazolidinedione. F. Rosa*1,2, M. Moridi2, J. S. Osorio2, J. Lohakare3, C. Estill1, and M. Bionaz1, 1Oregon State University, Corvallis, OR, 2South Dakota State University, Brookings, SD, 3University of Arkansas, Fayetteville, AR.

Prevention of mastitis is a priority for the dairy industry. Once pathogens invade the mammary gland, resident macrophages are the first line of defense. Thus, they can be a target to aid in prevention of intramammary infection (IMI). Macrophages can be activated through 2 major pathways: classical activation (C) which releases pro-inflammatory cytokines and alternative activation (A) with the production of anti-inflammatory cytokines. TNFα and NOS2 are markers of the C while IL-10 and TGF-β are markers of the A activation of macrophages. Peroxisome proliferator-activated receptor γ (PPARγ) is expressed in human macrophages and it is known to activate the A pathway. The activation of A by PPARγ has not been tested in ruminants but may affect the response to IMI. This study aimed to evaluate the effects of PPARγ activation by the putative PPARγ agonist 2,4-thiazolidinedione (TZD) on the activation of macrophages during subclinical mastitis in dairy goats. To test this, 12 lactating goats received daily injections of either TZD (n = 6) or saline (n = 6, CTR). Following 14 d of treatment, all goats received an intramammary infusion of *Streptococcus uberis* to induce subclinical mastitis in the right half of the mammary gland while the left half served as control. Macrophages were isolated at 5 d post-IMI from 250 mL of milk using immunomagnetic sorting. Expression of genes markers of the C and A pathways on macrophages was assessed via RTqPCR. Data were analyzed by GLIMMIX of SAS. Significance was declared at P < 0.05. Abundance of transcripts related to the classical macrophage activation (NFκB1, IL8, and CCL2) was increased by mastitis but no effect was detected on TNFA. Expression of the markers for alternative activation of macrophages, IL10, was not affected whereas TGFβ1 tended to be increased by mastitis (P = 0.06). No effects were observed on expression of measured genes by TZD with the exception of a tendency (P = 0.09) for a lower IL4 expression. Thus, our findings do not support the alternative activation of macrophages in the mammary gland of dairy goats by PPARγ.

**Key Words:** macrophage, mastitis, PPARγ

### T17 Evaluation of the effects of metabolic diseases during the transition period on the culling risk of high-yielding dairy cows by survival analysis. M. Probo1, O. Bogado Pascottini*2, S. LeBlanc2, G. Opsomer3, and M. Hostens3, 1Central Laboratory, Veterinary Teaching Hospital, University of Milan, Lodì, Italy, 2Population Medicine, Ontario Veterinary College, University of Guelph, Guelph,
ON, Canada, 3Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.

The objective was to assess the effects of individual and multiple metabolic diseases (MD; MD+) in the transition period (+3 wk relative to calving) on the culling risk in the first 120 d in milk (DIM) in Holstein-Friesian dairy cows. We analyzed health records of all 1,946 calvings during a 1 year period from a transition management facility located in Germany (herd average 305-d milk 11,085 kg) using survival analysis. The overall culling risk to 120 DIM was 18%. The 120 DIM survival risk for healthy cows (72% of the total) was 87%, while it was 75% for MD (24%) and 67% for MD+ (4%) cows. The monitored MD were twinning (TWIN), milk fever (MF; diagnosed by clinical signs), retention of fetal membranes (RFM; placenta not expelled ≥24 h after parturition), metritis (MT; fetid uterine discharge and fever (≥39.5°C) < 21 DIM), ketosis (KT; urine ketone bodies ≥500 μmol/L), displaced abomasum (DA; based on clinical signs e.g., tympanic resonance on percussion of the left flank), and clinical mastitis (MAST; udder inflammation/abnormal milk within 30 DIM). The incidence risks and 120 DIM survival rates (SR) of MD and MD+ diagnosed during the transition period in 1,946 dairy cows in 1 herd in Germany are depicted in Table 1. Setting the retention of fetal membranes (RFM; placenta not expelled ≥24 h after parturition) as the primary event of interest, the probability of having SCM. Breed did not influence SCM. Our results suggest an association between lameness and SCM. Studies on the causal relationship between these 2 diseases, accounting for production, hygiene, and teat health, in grazing dairy cows should be considered to aid in the design of better prevention protocols for SCM.

Key Words: health, pasture, gait score

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**Table 1** (abstract T17). Incidence risk (IR) and 120 DIM survival rates (SR) of MD and MD+ diagnosed during the transition period in 1,946 dairy cows in 1 herd in Germany

Objectives were to evaluate chlorine (Cl) concentrations and stability, oxidation-reduction potential (ORP), and pH in 2 chlorine based disinfectants at teat dip concentrations. L. Timms*, Iowa State University, Ames, IA.

**Key Words:** dairy cow, metabolic disease, survival analysis

**T19** Evaluation of chlorine concentration and stability, oxidation-reduction potential (ORP), and pH in 2 chlorine based disinfectants at teat dip concentrations. L. Timms*, Iowa State University, Ames, IA.

Objectives were to evaluate chlorine (Cl) concentrations and stability, oxidation-reduction potential (ORP), and pH of 2 commercially available chlorine-based disinfectant tablets (EfferCept (EF) and EfferCeptSG (SG = EF + extra emollients) at teat dip concentrations for dairy cows. Appropriate dilutions of products based on company recommendations were made and tested in 2 L plastic bottles. Two EfferCept tablets were added to 1893 mL (0.5 gal) of water. One EfferCeptSG tablet was added to 1893 mL (0.5 gal) of water. Following initial measurements, both solutions were put into non-siphoning teat dip cups to mimic on-farm conditions. On Day 33, a new set of EfferCept and EfferCeptSG solutions were made in the 2 L plastic bottles (validation) and all measurements were made from plastic bottles. Products were tested for 90 and 60 d (set 2), respectively. ORP (mV) and pH were measured using a Combo pH/ORP and temperature meter (HI 98121, Hanna Instruments, Woonsocket, RI). Measurements were daily the first 2 d, every 2 d the first 2 wk, then every 3–4 d thereafter. Cl concentrations (ppm) were tested when pH and ORP were measured using Cl titration testing kit and compounds (AquaPhoenix, Hanover, PA). Initial pH values were 5.7–5.8 and similar among products. pH increased in the first 6 wk and was higher in EF compared with SG in set 1 (6.6 v. 6.2), then decreased to initial levels at 90 d (5.99 v. 5.65). Initial ORP values of the first solutions were 900 mV and increased to 980 mV the first week (EF and SG). ORP levels over the 90 d were 930–960 mV (EG) and 930–980 mV (SG). ORP levels of the 2nd set of EF and SG solutions were initially ~980 mV and increased to >1000 mV by 3 d (>1000 mV at 60 d). Overall

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ORP values show high oxidizing capacity and stability over time. CI concentrations of EF and SG initially were 2200–2300 and 3000–3100 ppm, respectively. CI concentrations remained higher in SG v. EF over the time period. CI concentration decreased over the 90-d period but were still 1000 and 1200 ppm, well above 200 ppm disinfectant strength. Overall, the 2 chlorine disinfectant solutions showed very high ORP levels and decreased but still very effective chlorine concentrations over time.

**Key Words:** chlorine, disinfectant, ORP

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**T20 Associations of dry-off management and somatic cell count in robotic milking systems.** F. H. Padua, M. T. M. King*, and T. J. DeVries, Dept. of Animal Biosciences, University of Guelph, Guelph, ON, Canada.

The objective of this study was to evaluate associations of dry-off management factors and production data with SCC before dry off and in the subsequent lactation of cows milked in robotic systems. Milk data were collected for a 2-yr period for 342 multiparous (2.9 ± 1.1 lactations) dairy cows from 5 farms in Ontario, Canada. Parity, DIM at dry off, dry period length, and daily milk yield were recorded for each cow. Producers were surveyed about dry off management protocols including products and gradual milking cessation (tapering) methods used. Two years of SCC data were collected from DHI tests for each farm, for 2 tests before dry off and 2 tests post-calving. On average, cows were dried off at 350 ± 67 (mean ± SD) DIM and were dry for 64 ± 50 d. Before dry off (mean of 2 d before), cows were producing 15.8 ± 0.4 (mean ± SE) kg/d, however before any tapering occurred, cows were producing 18.3 ± 0.4 kg/d. The median pre-dry off SCC was 66,500 and 93,000 cells/mL at the 2nd and 1st test before dry off. In the subsequent lactation, median SCC was 52,500 and 46,000 cells/mL at the 1st and 2nd test post-calving. In logistic regression models, milk yield before dry off was positively associated with the risk of being tapered; each 1 kg/d increase in milk yield was associated with a greater risk (OR = 1.14; 95% CI = 1.09–1.20) of being tapered (P < 0.001). In mixed linear regression models, 1st test post-calving SCC was associated with SCC at the test before dry off (P = 0.02). Post-calving SCC at the 2nd test was associated with whether cows were tapered before dry off (P = 0.05), and SCC at the test before dry off tended (P = 0.09) to be associated with tapering. Milk production before tapering was also associated with SCC at the test before dry off (P = 0.02), such that cows producing ≥20 kg/d before tapering also had lower SCC before dry off. There were no associations of post-calving SCC with parity, antibiotic use, sealant use, dry period length, or DIM at dry off. These results show that producers with robotic milking systems are using some form of gradual milking cessation when drying off their higher producing cows, and that the resultant reduction in milk and pre-dry off SCC are associated with SCC in the subsequent lactation.

**Key Words:** robotic milking, dry off management, somatic cell count

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**T21 Effect of minerals and vitamins supplementation during the non-lactating period on incidence of metritis in early lactating dairy cows.** G. A. Mattioli1, C. G. Sarramone2, E. Turic2, M. Sain-Martín2, and A. E. Relling3, 1Fc. Cs. Veterinarias, UNLP, La Plata, Buenos Aires, Argentina, 2Biogenesis Bagó, Garin, Buenos Aires, Argentina, 3Department of Animal Sciences, The Ohio State University, Wooster, OH.

Trace minerals and vitamins associated with antioxidant defense improve the immune response in dairy cattle during critical periods such as peri-partum. Metritis represents a severe consequence of immune failure. The objective of the current study was to evaluate the effect of prepartum trace mineral and vitamin supplementation on the incidence of metritis in early lactation dairy cows. The trial was realized in a commercial dairy herd in Buenos Aires, Argentina. The herd was comprised of 574 primiparous and multiparous Holstein cows that were randomly divided in 2 groups: supplemented (n = 301) and non-supplemented (n = 273). Supplementation consisted of 3 parenteral applications of trace minerals and vitamins (Cu: 50 mg, Zn: 200 mg, Mn: 50 mg, Se: 25 mg, Vit. A: 315000 IU, and Vit. E: 250 IU - Adaptador, Biogenesis Bagó SA). Treatment was applied at drying off (60 d before calving), 21 d before calving, and at calving. The effect of treatment on the incidence of metritis was assessed by logistic regression analysis. The model included the fixed effects of mineral and vitamin supplementation (supplemented vs. non-supplemented), parity (1 vs. 2+) and their interaction. For the main and interaction effects, the α level of significance was set at P < 0.05 and P < 0.1, respectively. The incidence for metritis shows a treatment by parity interaction (P = 0.09). The risk of metritis decreased in supplemented multiparous cows, but not in primiparous cows compared with non-supplemented. Supplemented multiparous cows had a lower prevalence of metritis than non-supplemented (14 vs 26%, respectively). Conversely, the percentage of affected primiparous cows was similar for those receiving or not receiving the supplementation (32 vs. 30%, respectively). In conclusion, supplementation with a mix of antioxidant trace minerals and vitamins reduces the odds for metritis in multiparous cows.

**Key Words:** antioxidant, mineral, vitamin

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**T22 Effect of somatic cell count around service on the fertility of grazing dairy cows.** N. Lorenti3, R. Rearte2,3, M. Giuliiodori3, and R. de la Sota4,5, 1Práctica Privada, Brandesn, Buenos Aires, Argentina, 2Cátedra de Higiene, Epidemiología y Salud Pública, Facultad de Ciencias Veterinarias- Universidad Nacional de La Plata (FCV-UNLP), La Plata, Argentina, 3Cátedra de Fisiología, FCV-UNLP, La Plata, Argentina, 4Cátedra y Servicio de Reproducción Animal, FCV-UNLP, La Plata, Argentina, 5Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

The objective of this study was to assess the effect of high somatic cell counts (SCC) in early lactation on the probability of cows to conceive at first artificial insemination (FAI). A total of 6642 lactations started from 2003 to 2015 from 5 dairy herds having SCC records 21 d before and 21 d after the first artificial insemination (FAI) were included in the study. Lactations were classified as: healthy (HEA) when both SCC were <150,000 cells/mL (<150K); as cured (CUR) when they had SCC >150K before and <150K after their FAI; as new cases (NEW) when they had SCC <150K before and >150K after their FAI; and as chronic (CHR) cases when they had SCC >150K before and after FAI. Logistic regression models were run to test the effect of SCC (healthy, cured, new, chronic) on the odds of conception at FAI (model 1); and to assess the effect of severity of chronic cases, classified according to their SCC after FAI as light (≥150K and <400K), mild (≥400K and <1000K) or severe (≥1000K) on the odds of conception (model 2). Both models also accounted for the effect of year (2003–2015), herd (1–5), parity (1 vs. 2 vs. 3+) and accumulated milk yield up to 150 DIM. In model 1, the odd of conception at FAI was 14.6% lower in chronic cases compared with healthy cows (P < 0.05, Table 1). In model 2, the odd of conception at FAI was 33% lower in severe chronic cases compared with healthy cows (P < 0.05, Table 1). In both models, the remaining groups had numerically lower non-significant odds of conception (Table 1). In conclusion,
cows having high SCC (>150,000 cells/mL) around FAI have reduced fertility, and this effect depends on the severity of the case.

<table>
<thead>
<tr>
<th>Model 1</th>
<th>N</th>
<th>CR (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>3067</td>
<td>41.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cured</td>
<td>1038</td>
<td>41.3</td>
<td>0.98</td>
<td>0.84–1.14</td>
</tr>
<tr>
<td>New case</td>
<td>1134</td>
<td>39.7</td>
<td>0.92</td>
<td>0.79–1.10</td>
</tr>
<tr>
<td>Chronic case</td>
<td>1403</td>
<td>38.0</td>
<td>0.85</td>
<td>0.74–0.99*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2</th>
<th>N</th>
<th>CR (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>3067</td>
<td>40.3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lightly chronic</td>
<td>728</td>
<td>38.9</td>
<td>0.94</td>
<td>0.78–1.13</td>
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<tr>
<td>Mildly chronic</td>
<td>376</td>
<td>35.7</td>
<td>0.82</td>
<td>0.64–1.05</td>
</tr>
<tr>
<td>Severely chronic</td>
<td>298</td>
<td>31.1</td>
<td>0.67</td>
<td>0.50–0.89*</td>
</tr>
</tbody>
</table>

*P < 0.05.

Key Words: somatic cell count, reproduction

T23 Assessing the validity of inline milk fat-to-protein ratio data as an indicator of subclinical ketosis in dairy cows in robotic milking herds. I. R. Salmazo, M. T. M. King*, and T. J. DeVries, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.

The objective of this study was to evaluate associations of inline milk fat-to-protein (F:P) data surrounding the detection of subclinical ketosis (SCK) in robotic milking herds. The ratio of fat-to-protein has been proposed as a tool for detecting SCK with moderate accuracy in past studies, however, some producers with robotic milking systems are trusting inline F:P data as their main source of SCK screening. To assess the validity of these data in commercial settings, we monitored 484 cows from 9 robotic milking herds for their first 3 wk of lactation, taking blood samples 1x/wk (n = 1427). Positive cases of SCK were defined by whole blood β-hydroxybutyrate (BHB) concentrations ≥ 1.2 mmol/L. Milk data were collected from the robotic systems on each farm for each cow and converted into 4 different F:P values: 1) value same day of BHB test; 2) 5-d centered moving average (CMA); 3) 5-d backward moving average (BMA); 4) 5-d forward moving average (FMA). In linear regression models, all 4 values were associated with BHB (P < 0.001), but slope estimates varied and R² was low: same day (slope = 0.9, R² = 0.07); CMA (slope = 1.0, R² = 0.07); BMA (slope = 0.7, R² = 0.04); FMA (slope = 1.2, R² = 0.09). In logistic regression models, the odds of having SCK increased with every 0.1 unit increase from the mean (1.16) using same day F:P (OR = 1.35, 95% CI = 1.25–1.47; P < 0.001) and CMA (OR = 1.39, 95% CI = 1.27–1.51; P < 0.001). The same increase in F:P from mean BMA (1.14) and FMA (1.17) were associated (P < 0.001) with 1.22 and 1.49 times the odds of SCK, respectively. For all 4 F:P variations, sensitivities and specificities of different F:P thresholds with SCK status were evaluated using chi-squared tests. As the F:P threshold was raised from 1.15 to 1.22, sensitivity decreased (range: 73 to 45%) while specificity increased (range: 53 to 71%). The F:P cut-offs at which a balance was reached between sensitivity and specificity were 1.17 to 1.20; however, even at these values there were high rates of false positives and negatives (range: 33 to 40%). These results suggest that inline milk F:P data should not be solely used to detect SCK in robotic milking herds.

Key Words: robotic milking, dairy cow, hyperketonemia

T24 Can the use of cefquinome be justified to treat Streptococcus agalactiae subclinical mastitis? R. Rossi, L. Correia, S. Guerra, A. Amarante, V. Rall, and J. Pantoja*, Universidade Estadual Paulista (UNESP), Botucatu, SP, Brazil.

Cefquinome is a 4th generation cephalosporin that is approved in Brazil to treat Streptococcus agalactiae subclinical mastitis (SM). Nonetheless, cefquinome is critically important for humans and its exposure to dairy cows could be reduced if traditional drugs, such as cloxacillin, were still effective to treat S. agalactiae SM. The objectives of this randomized clinical trial were to: 1) estimate the cure rate of S. agalactiae SM treated with intramammary cloxacillin (CLOXIMM), intramammary cefquinome (CEFIMM), or intramuscular cefquinome (CEFIM), as compared with a negative control group (CON); and 2) test the hypothesis that CLOXIMM is non-inferior to CEFIMM to treat S. agalactiae SM. Seven farms were visited for screening and milk samples were collected from all quarters of all lactating cows for microbiological examination. Streptococcus agalactiae-positive cows were randomized into 4 groups: CLOXIMM (n = 60), CEFIMM (n = 64), CEFIM (n = 31), and CON (n = 16 quarters). Treatments were administered per label directions (once a day for 3 d for CLOXIMM and CEFIMM, and every 12 h for 3 consecutive milkings for CEFIMM). Microbiological cure was assessed at 14 (D14) and 21 (D21) days after beginning of treatments. Outcomes were bacteriological cure at D14 (CURE14), D21 (CURE21), and D14 and D21 (CURE1421). Logistic regression was used to compare the cure rate between each treatment and CON. Non-inferiority analysis was performed considering a one-sided 95% confidence interval (CI) and a non-inferiority margin of 0.15. The cure rate for CLOXIMM, CEFIMM, CEFIM, and CON was 88, 100, 55, and 19% for CURE14; 85, 97, 52, and 7% for CURE21; and 85, 97, 52, and 6% for CURE1421, respectively (P < 0.05 for the comparisons between each treatment and CON). Although the cure rate difference between CLOXIMM and CEFIMM (0.12; 95% CI: 0.04–0.20) at D14 was < 0.15, non-inferiority was inconclusive because the required sample size has not been reached. Preliminary results suggest that cloxacillin can still be used to treat S. agalactiae SM, instead of drugs that should be prioritized for human use.

Key Words: mastitis, cefquinome, cloxacillin


Coagulase-negative staphylococcus (CNS) is the predominant group of bacteria in mastitis prevalence studies worldwide. An important virulence factor is the biofilm formation, once it assists in the persistence of the bacteria in the mammary epithelium. The aim of this study was to investigate the icaA, icaD, and bap genes in strains of CNS antimicrobial resistant isolated from bovine mastitis. Forty CNS (i.e., S. capitis, S. chromogenes, S. epidermidis, S. hominis, S. sciuri, S. similans, and S. warneri), exhibiting a resistance profile (ampicillin, clindamycin, florfenicol, gentamicin, kanamycin, linezolid, neomycin, oxacillin, penicillin, streptomycin, trimethoprim-sulfamethoxazole and tetracycline, and mecA gene negative), isolated from cows and heifers with mastitis, from commercial farms in São Paulo State, Brazil, were used. The extraction of genomic DNA was performed using DNA Kit RTP Bacterium - Invitek. The genes amplification was performed by PCR and the reaction product was visualized on 2% agarose gel electrophoresis in 1× TBE buffer (1 M Tris base, 0.9 M boric acid, 0.01 M EDTA) stained 0.5% red gel. For positive control, they were used to S. aureus ATCC BAA-977 and S. xylosus DSM-20266. Descriptive
statistical analysis was performed by calculating relative frequencies (PROC FREQ; SAS Institute, 2011) of resistant strains containing genes for biofilm production. Of the 40 isolates of CNS, 2.5% (n = 1) were positive for the icaA gene and 12.5% (n = 5) for the icaD gene, while were all negative for bap gene. The biofilm formation of staphylococcal is a complex process and involves the different mechanisms and gene regulation. The biofilms confer increased the capacity of adhesion and protection against the mechanisms of defense of the host and action of the antimicrobial agents, made difficult the elimination of the pathogen. Further studies are important for the understanding of the mechanisms of virulence and pathogenicity by strains of CNS, once these bacteria are frequently isolated in infections of the bovine mammary gland, representing the damages for the producers, the industry, and the public health.

**Key Words:** coagulase-negative staphylococcus, biofilm, mastitis

**T26** Validation of BHBCheck blood β-hydroxybutyrate meter as a diagnostic tool for hyperketonemia. K. J. Sailer*, R. S. Pralle, R. C. Oliveira, G. R. Oetzel, and H. M. White, University of Wisconsin-Madison, Madison, WI.

Accurate cow-side blood β-hydroxybutyrate (BHB) detection meters are valuable tools for minimally invasive, cow-side diagnosis of hyperketonemia. The objective of this study was to compare the blood BHB measured in whole blood by the BHBCheck meter (PortaCheck, Moorestown, NJ) to the gold standard of BHB measured in serum or plasma by colorimetric assay. Samples (n = 445) were collected from postpartum Holstein cows (n = 87 cows) enrolled in 1 of 2 experiments (exp) with different sampling schedules (exp 1: n = 47 cows, 72 samples; exp 2: n = 40 cows, 373 samples). In both exp, whole blood samples were collected from the coccygeal vessels after morning milking, before morning feeding, and used immediately for BHB quantification via the BHBCheck meter and the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL) as a comparison to another cow-side BHB meter that is widely accepted. Simultaneously, blood was collected into evacuated tubes containing no additive (exp 1) or potassium oxalate/sodium fluoride (exp 2) which were centrifuged for serum or plasma separation and stored at −20°C for subsequent analysis. Quantification of BHB concentration was via the BHB LiquiColor Assay (Stanbio; certified for serum and plasma) and analyzed with the BioTek spectrophotometer. Data were analyzed by Reg and Freq procedures of SAS 9.4. Average parity of the cows was 2.4 and average DIM at sampling was 14.8 d. The prevalence of hyperketonemia (BHB ≥1.2 mmol) was 23.1, 27.9, 30.4% as determined by the laboratory assay, BHBCheck meter, and Precision Xtra meter, respectively. Coefficient of determination for BHBCheck meter BHB concentration compared with the colorimetric assay concentrations was $R^2 = 0.88$ with a sensitivity of 91.2%, and specificity of 91.1%. Coefficient of determination, sensitivity, and specificity of the Precision Xtra meter concentrations were 0.90, 98.0%, and 89.9%, respectively. The BHBCheck meter exhibited reliable sensitivity and specificity for use as a valuable cow-side diagnostic test for hyperketonemia in dairy cows.

**Key Words:** ketosis, cow-side diagnostic tool, transition cow

**T27** Liver functionality index in periparturient dairy cows fed ethyl-cellulose rumen-protected methionine is associated with better performance and immunometabolic status. F. Batistel*,1, B. Sarem2, C. Parys3, E. Trevisi3, and J. J. Loor1, 1Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 2Department of Clinical Medical University of São Paulo, São Paulo, São Paulo, Brazil, 3Eastern Laboratory Service, Medina, OH.

The liver functionality index (LFI) represents an assessment of periparturient cow metabolic health by measuring changes in 3 blood biomarkers (albumin, cholesterol and bilirubin). The aim of this study was to determine the effect of peripartal ethyl-cellulose rumen-protected Met supplementation on LFI groupings, and to assess relationships with animal performance and oxidative stress status during the periparturient period. Thirty multiparous Holstein were used in a block design and assigned to either a control diet or Met-supplemented (Mepron, Evonik Nutrition and Care GmbH, Germany) diet. Mepron was supplied from −28 to 30 d relative to parturition at a rate of 0.09% and 0.10% of DM during the prepartum and postpartum period, respectively. That rate ensured that the ratio of Lys to Met in the metabolizable protein was close to 2.8:1. Blood samples from 15 clinically healthy cows per treatment were collected at 7 and 30 d relative to parturition for biomarkers analysis. LFI was calculated using an updated equation $[LFI = \{\text{Albumin (g/dL)} \times 2.87 + \text{Cholesterol (mg/dL)} \times 1.16 + \text{Bilirubin (mg/dL)} \}/0.49]$ and cows were ranked retrospectively into low LFI (LFI < 0; n = 10) and high LFI (LFI > 0; n = 20) groups regardless of Met supplementation. Most (67%) of the cows fell into the high LFI, which was composed mainly of Met-supplemented cows (P = 0.04; 13 Met vs 7 controls). Compared with cows in low LFI, postpartum BMI (P = 0.06; 17.7 vs 16.2 kg), milk yield (P = 0.05; 39.8 vs 34.8 kg), and ECM (P = 0.08; 47.1 vs 41.8 kg) were greater in high LFI cows. Furthermore, prepartum BMI (P = 0.12; 14.5 vs 13.3 kg) tended to be greater in high LFI compared with low LFI. The lower postpartum concentrations of reactive oxygen metabolites (P = 0.09; 17.7 vs 19.5 mg of H2O/100 mL) and greater paraaxonase (P < 0.01; 97.6 vs 75.8 U/mL) and tocopherol (P = 0.03; 6.64 vs 5.25 mg/mL) in high LFI cows indicated a state of reduced oxidative stress. B-carotene and retinol were not affected by LFI. Overall, feeding Met during the periparturient period results in higher LFI and better performance and oxidative stress status.

**Key Words:** liver function, LFI, milk production

**T28** Prevalence of Prototheca spp. in bulk tank milk from Ohio dairy farms. L. da Costa*1, A. Della Libera2, and H. Sullivan3, 1Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 2Department of Clinical Medical University of São Paulo, São Paulo, São Paulo, Brazil, 3Eastern Laboratory Service, Medina, OH.

Regardless of intense research on intramammary infection (IMI), IMI or mastitis continues to be the most important and costly disease of dairy cattle. For several years, research has been focusing on mastitis caused by gram-positive and gram-negative organisms. In the past, mastitis caused by Prototheca spp. was considered rare and frequently associated with poor environmental conditions or insufficient milking hygiene. Prototheca spp. is known today as an emergent pathogen that can cause both clinical and subclinical cases of mastitis that are usually chronic and progressive. Importantly, there is no treatment available, and all positive cows should be removed from the herd. Prototheca spp. also have zoonotic potential, which represents a human health risk due to the increase consumption of contaminated raw milk. The aim of the study was to investigate the presence of Prototheca spp. originated from Ohio bulk tank milk (BTM) samples. In total, 384 BTM samples were cultured to investigate the presence of Prototheca spp. in dairy farms. To enhance sensitivity, selective media Prototheca isolation medium (PIM) and increase volume (0.1 mL of milk instead of 0.01 mL) was used. Plates were incubated at 37°C and checked every 24 h for 72 h. Prototheca spp. was then presumptively identified by colony morphology, specific staining (Methylene Blue) and endospore visualization. Based on the results from this study, prevalence (number of positive
samples over population size) of *Prototheca* spp. was estimated to be 6% (22/384) in BTM from Ohio dairies. *Prototheca* spp. possess pathogenic potential for both humans and animals. Thus, it is crucial to investigate and describe its prevalence in bulk tank milk to develop improved prevention methods and control practices, to prevent further spreading and to protect both animal and human health.

**Key Words:** mastitis, *Prototheca* spp., bulk tank milk

### T29 Development and evaluation of hyperketonemia prediction models

R. S. Pralle*, K. A. Weigel, and H. M. White, University of Wisconsin-Madison, Madison, WI.

The objective of this experiment was to develop and evaluate models predicting hyperketonemia (HYK), utilizing several methods and variable inputs. Paired blood and milk samples were collected from multiparous cows 5 to 18 d in milk at 3 WI farms (3,629 observations from 1,013 cows). Blood β-hydroxybutyrate (BHB) concentration was determined by the Precision Xtra meter and milk samples were analyzed by a commercial lab (AgSource) for components and mid-infrared spectrum absorbance. Cow specific variables were extracted from DairyComp 305. A BHB ≥ 1.2 mM was considered HYK, resulting in a prevalence of 12.4%. The data set was divided into an external testing set (n = 609) and a training data set (n = 3020). Model fitting was completed with JMP 12. A 5-fold cross-validation (CV) was performed on the training data set for 3 methods with square root of BHB as the model dependent: multivariate linear regression (MLR), partial least squares regression (PLS) and artificial neural network (ANN). Each method was fitted utilizing 3 combinations of potential variables: milk spectrum, management variables (milk components and DairyComp 305 data), or all variables, resulting in 9 models. All models were evaluated based on r², RMSE, and the area under the curve (AUC) of a receiver data), or all variables, resulting in 9 models. All models were evaluated based on r², RMSE, and the area under the curve (AUC) of a receiver operating characteristic curve. Data are expressed as the mean ± SE of CV. Across modeling techniques, use of all data resulted in greater performing models than use of management variables (intermediary) or spectrum data. All MLR models were lower performing than other methods and MLR-spectrum was the lowest with an r², RMSE, and AUC of 0.23 ± 0.02, 0.45 ± 0.07, and 0.81 ± 0.01, respectively. PLS models performed intermediate with PLS-all values: 0.40 ± 0.01, 0.17 ± 0.002, 0.86 ± 0.01 for r², RMSE, and AUC. The ANN method performed the greatest, particularly the ANN-all with an r², RMSE, and AUC of 0.46 ± 0.01, 0.16 ± 0.002, and 0.86 ± 0.004. When the ANN-all model was validated against the test data set, r² = 0.56, RMSE = 0.16, sensitivity was 83%, and specificity was 80%. In summary, use of ANN methods with milk spectrum and management variables can achieve reasonable prediction of HYK.

**Key Words:** ketosis, predictive model, neural network

### T30 Quantifying milk leukocyte proportions in mastitic and healthy quarters

S. Paudyal1, G. Pena2, P. Melendez2, A. Villarroya2, N. Roman-Muniz1, and P. Pinedo1, 1Colorado State University, Fort Collins, CO, 2Advanced Animal Diagnostics, Morrisville, NC

Fluctuations in relative white cell proportions in milk from inflamed mammary glands can be determined using differential cell count. We hypothesize that infection of mammary tissue results in deviations of specific cell proportions, dependent upon the pathogen involved. Our objective was to evaluate the milk leukocyte differential (MLD) in mastitic and healthy quarters. Milk from 460 quarters in 115 Holstein cows was collected and lymphocytes, neutrophils, and macrophages were counted using QSCOUT milk analysis system (Advanced Animal Diagnostics, Morrisville, NC) which includes a fluorescence microscope. A sterile pooled milk sample was also collected from each cow for pathogen identification. Culture results were classified as no growth (NOG), gram-negative (GN), gram-positive (GP), or other (OTH), which included mycoplasma and prototheca. Quartiles with total leukocyte count (TLC) ≥ 200 were classified as affected. The MLD data were arcsine transformed whereas TLC was reciprocal transformed and back transformed after analyses. Models included quarter health status and position and pathogen category nested within cow. Average (95% CI) TLC for healthy and affected quarters was 52,000 (47,460–56,170) cells/mL vs 830,000 (678,530–1,031,860) cells/mL (P < 0.0001). Proportions of neutrophils (53.4% vs. 61.9%), lymphocytes (17.0% vs. 14.8%) and macrophages (28% vs. 21.4%) were different for affected vs. healthy quarters (P < 0.0001). Regarding quarter position in healthy quarters, TLC was greater in RF quarters, followed by L.R, RR, and LF (P = 0.05). MLD was associated with quarter position, with greater proportions of macrophages in the front quarters (P = 0.03). For affected quarters, TLC was greater when pathogen growth was detected; counts were greatest for OTH followed by GP, and GN (P = 0.01). MLD depended on pathogen: Neutrophil % was greatest in GP (67%) followed by NOG (59%), GN (58%) and OTH (57%; P = 0.009). Lymphocyte % was greatest in OTH (17%), followed by GP (15%), NOG (16%), and GN (7%; P = 0.00002). Macrophage % was greatest in GN (32%), followed by OTH (24%), NOG (23%) and GP (18%; P = 0.03). Differentiable patterns in the changes in relative leucocytes proportions may provide useful information for the identification of causal agent in mastitis cases.

**Key Words:** differential leucocyte, mastitis

### T31 Advancement of Dairying in Austria (ADDa): Antimicrobial dry cow therapy on conventional dairy farms—Farmers’ management decisions

C. L. Firth1, C. Schleicher2, A. Käsbohrer3, and W. Obristhauser1, 1University of Veterinary Medicine, Institute of Veterinary Public Health, Vienna, Austria, 2Austrian Agency for Health and Food Safety (AGES), Integrated Risk Assessment, Data and Statistics, Graz, Styria, Austria.

Advancement of Dairying in Austria (ADDa) is a 3-year research project encouraging cooperation between academic institutions and the dairy industry in Austria. The study presented here investigated how farmers dried their cows off and their use of antimicrobial substances at this time. Veterinary practices in Austria were asked to contact their dairy clients and inquire whether they would participate in the study. Livestock treatment records were collected and an online survey was sent via email to all participating farmers. A total of 251 farmers provided consent; of these, 211 responded to the survey (response rate: 84%). With respect to production system, 165 (78%) of respondents managed their farms conventionally and are included in this analysis. Herd size ranged from 8 to 94 cows (mean 29). One-third of conventional farmers kept their cows in tie-stalls, while the remaining farms had cubicle housing or straw/compost yards. The majority (69%) of farmers stated that they sent milk samples from symptomatic animals for bacteriology before drying off, while only 13% regularly ordered this precautionary investigation for all cows, regardless of history. Drying off was primarily carried out abruptly (74% of farms) and the most common drying off period was 56–60d antepartum (58%). When asked whether they used antibiotics at drying off, 55.8% of farmers responded “yes, in all cows” (i.e., blanket dry cow therapy [DCT]), whereas 27.9% used antimicrobial DCT only in cows with symptoms and 15.2% only in cows where a milk sample returned a positive bacterial culture. Preliminary results showed that the farmers’ level of agricultural education did not significantly affect...
whether they dried off cows with antibiotics ($P = 0.264$, Chi² test). The correlation between the use of antibiotics at dry off and whether the CMT was regularly used was not significant ($P = 0.807$, 2-sided Fisher’s exact test). More targeted antimicrobial use could lead to further reductions in blanket dry cow therapy and it is vital that vets and farmers work together to achieve this goal. The influence of farm management data regarding DCT (survey responses) on the incidence of mastitis on farm is to be analyzed using a generalized linear mixed model (GLMM).

**Key Words:** dry cow therapy, mastitis, antibiotics

**T32 Prevalence of subclinical ketosis in Chilean grazing dairy cattle calving during fall and spring.** P. Melendez*1, C. Chacon2, S. Poock1, and P. Pinedo3, 1College of Veterinary Medicine, University of Missouri, Columbia, MO, 2Agricola Pozo Brujo, Santiago, Chile, 3Department of Animal Sciences, Colorado State University, Fort Collins, CO.

Subclinical ketosis (SK) is a metabolic disease experienced by dairy cows during the transition period. It is characterized by blood concentrations of BHB ≥1.2 mmol/L. Few studies have reported the prevalence of SK in cattle under grazing conditions. In Chile, 70% of dairies are under grazing conditions with seasonal calving. The objective was to determine the prevalence of SK at 7 DIM in dairy cows calving in fall and spring. The study was conducted in a southern Chilean dairy, milking ~1,000 cows with a mature-equivalent 305-d milk yield of 7,500 kg. During prepartum, cows were housed without access to pasture and fed a TMR. After calving cows were moved to a group under grazing conditions. Cows were milked 2x, and fed a TMR (70% DM in fall, 30% DM in spring) before milking, complementing the grazing activity. During 2016, 866 cows were tested for blood BHB at 7 DIM using a hand-held device (Precision Xtra) with reported sensitivity and specificity of 94.8% and 97.5%, respectively. From this population, 234 cows calved in fall (27%; [10.7% primiparous, 89.3% multiparous]) and 632 in spring (73%; [30.9% primiparous, 69.1% multiparous]). Annual prevalence of SK at 7 DIM was 18.1%; however, the prevalence during fall was 8.1% and during spring 21.8% ($P ≤ 0.05$). Primiparous had a prevalence of 15.5% vs. 19.0% in multiparous ($P > 0.05$). Primiparous calving in fall had a prevalence of 12.0% and multiparous 7.65% ($P > 0.05$). Primiparous calving in spring had a prevalence of 15.9% vs. 24.5% in multiparous ($P ≤ 0.05$). Cows with BCS at calving ≤3.5 had a prevalence of 16.2%, vs. cows with BCS > 3.5 22.5% ($P ≤ 0.05$). During fall, cows with BCS at calving ≤3.5 had a prevalence of 6.5%, vs. cows with BCS > 3.5 18.8% ($P ≤ 0.05$). However, during spring, cows with low and high BCS had similar prevalence ($P > 0.05$; 20.3% vs 23.6%, respectively). In conclusion, the prevalence of SK at 7 DIM in Chilean grazing dairy cattle during 2016 was 18%. The prevalence was higher during spring and in multiparous. Cows calving with high BCS had a higher prevalence of SK than cows with BCS at calving ≤3.5.

**Key Words:** subclinical ketosis, grazing, prevalence

**T33 Nonesterified fatty acids induce proinflammatory macrophage phenotype.** G. A. Contreras*1 and W. Raphael, Department of Large Animal Clinical Sciences, East Lansing, MI.

Proinflammatory host responses contribute to disease incidence and severity in periparturient dairy cows. Classical phenotype, proinflammatory macrophages were recently described in adipose tissue of periparturient dairy cows undergoing lipolysis. Some nonesterified fatty acids (NEFA) activate macrophage proinflammatory pathways in studies of human disease. However, the impact of NEFA on bovine macrophage phenotype is unclear. The objectives were to model macrophage phenotypes in vitro and assess the effect of periparturient NEFA on macrophage phenotype. Peripheral monocytes isolated by Ficoll gradient and magnetic sorting were cultured with interferon-γ or interleukins 4 and 13 to induce classical or alternative macrophage phenotypes. Macrophage mRNA was quantified using qPCR. Surface protein expression was measured by flow cytometry ($n = 8$, $P < 0.05$). After 48 h in vitro, CD172α was 95.2% ± 0.4% and monocytes became undifferentiated macromphages with increased CD68. Classical phenotype macrophages showed increased CCL2, IL6, TNF, and CD16 expression relative to alternative and undifferentiated macrophages. Alternative phenotype macrophages showed decreased IL6 expression relative to classical and undifferentiated macrophages. Classical macrophages did not change phenotype with lipopolysaccharide stimulation, whereas alternative macrophages showed decreased expression of IL6 and TNF relative to classical and undifferentiated macrophages. A periparturient-like, NEFA mixture increased IL6 and TNF expression in undifferentiated macrophages to levels seen with lipopolysaccharide stimulation. These results demonstrate induction of the classical, proinflammatory macrophage phenotype with exposure to NEFA and suggest that adipose tissue macrophages of periparturient cows are likely polarized to classical phenotype by local NEFA released during lipolysis. Future studies will assess specific fatty acids and transport molecules, and explore the potential impact of classical macrophages on lipolysis in adipose tissue.

**Key Words:** macrophage, lipolysis, adipose tissue remodeling

**T34 Efficacy and clinical safety of pegbovigrastim against naturally occurring clinical mastitis in periparturient cows on US commercial dairies.** P. C. Canning*1, R. L. Hassfurther1, T. Ter-Hune2, K. Rogers3, S. Abbott4, and D. Kolb3, 1Elanco Animal Health, Greenfield, IN, 2HMS Veterinary Development Inc., Tulare, CA, 3Veterinary Research & Consulting Services, Greeley, CO, 4Dairy Vet Management, Sunnyside, WA, 5Lodi Veterinary Hospital, Lodi, WI.

Periparturient dairy cows exhibit impaired immune function including a decrease in neutrophil function, which is associated with an increased susceptibility to bacterial infections including mastitis in the early postpartum period. Treatment with granulocyte colony stimulating factor (G-CSF) has been shown to increase neutrophil count and enhances neutrophil function in the periparturient dairy cow. Administration of a PEGylated recombinant bovine G-CSF product (pegbovigrastim; IMR) around the time of calving has been shown to reduce the incidence of new clinical mastitis cases. The objective of this study was to investigate the efficacy and safety of IMR under herd management systems typical of those in the US dairy industry. Four trial sites located in CA, WI, WA, and CO were enrolled in this study and IMR or sterile saline (CON) was administered to primiparous (IMR n = 90; CON n = 97) and multiparous cows (IMR n = 230; CON n = 224) 7 d before anticipated calving and again within 24 h of calving. IMR cows exhibited 4–5 fold increases in circulating neutrophil numbers within 24 h of treatment initiation which persisted at least a week beyond the second dose relative to CON cows ($P < 0.0001$). Postpartum IMR treated animals exhibited a 35% decrease in the incidence of clinical mastitis associated with both gram-positive and negative bacteria relative to CON during the first 30 DIM ($P = 0.009$). Animals treated with IMR also exhibited a 52% reduction in the incidence of failure to return to estrus by 80 DIM ($P = 0.03$). There were no observed differences in milk yield ($P = 0.45$), milk composition ($P > 0.57$), or somatic cell ($P = 0.75$) count between IMR and CON cows. Similarly there were no differences in the duration of pregnancy ($P = 0.39$) or proportion of viable births ($P = 0.55$) between treatments. Overall, results of this study indicate...
that administration of IMR reduces the incidence of clinical mastitis during early lactation on US commercial dairy farms, and provides a novel management approach to assisting the cow during the period of periparturient immune dysfunction.

Key Words: transition cow, pegbovigrastim, clinical mastitis

T35 Reduction of the endotoxin concentration by a clay mineral-based product in a semi-continuous in vitro rumen model. N. Reisinger*1, C. Stoiber1, C. Emsenhuber1, I. Dohnal1, S. Schaumberger2, and G. Schatzmayr1, 1Biomin Research Center; Tulln, Austria, 2Biomin Holding GmbH, Getzersdorf, Austria.

Sub-acute rumen acidosis (SARA) can be induced by feeding high amounts of concentrates to dairy cows. During SARA, the pH value in the rumen decreases and can reach values below 5.6. These conditions lead to the release of high amounts of endotoxins (Gozho et al., 2006, 2007; Li et al., 2012). If endotoxins reach the blood flow, through an impaired rumen barrier, they can induce the release of pro-inflammatory mediators and the production of acute phase proteins (Gozho et al., 2006, Li et al., 2012). The aim of the presented study was to evaluate, if a clay-based product reduces the endotoxin concentration in a semi-continuous in vitro rumen model. For this purpose, rumen fluid was sampled at a slaughterhouse. For each trial, rumen fluid from 3 different dairy cows was pooled, and immediately transported to the lab. Rumen fluid was incubated at 39°C under anaerobic conditions for 360 h (4 reactors per treatment, 3 independent trials). Each reactor contained 1.25 L of the inoculation mixture (50% rumen fluid, 30% distilled water, 20% synthetic saliva). Turnover of rumen fluid was maintained by constant inflow of synthetic saliva. Feed was provided every day with a nylon bag (43% chopped hay, 57% concentrate). The clay-based product (0.3%) was added daily to the reactors. To evaluate endotoxin concentration in the rumen fluid, samples were taken at 48, 168, 240, and 360 h. The limulus amoebocyte lysate (LAL) assay was used for analysis. GraphPad Prism software was used for statistical evaluation of results. If data were normally distributed, Student’s t-test was used. If data were not normally distributed, the Mann Whitney test was used. The clay-based product was able to reduce the endotoxin concentration by 56% at 48 h (P = 0.0035), 76% at 168 h (P = 0.0002), 77% at 240 h (P < 0.0000), and 77% at 360 h (P = 0.0002). The results of the presented study revealed that the clay-based product was able to reduce the endotoxin concentration in a semi-continuous in vitro rumen model. However, in vivo trials are necessary to confirm these results.

Key Words: endotoxin, SARA, in vitro

T36 Dietary clay supplementation improves hepatic expression of inflammatory markers in Holstein cows challenged with aflatoxin. K. Ryan*1, S. Sulzberger1, M. Vailati-Riboni1, L. Guifen2, Y. Kihidoyatov3, J. Loo1, and F. Cardoso1, 1University of Illinois, Department of Animal Sciences, Urbana, IL, 2Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences, Jinan, China, 3United Minerals Group, Kiev, Ukraine.

Oral supplementation of clay to dairy cattle has been reported to reduce toxicity of aflatoxin (AF) in contaminated feed. The objective of this study was to determine the effects of 3 concentrations of dietary clay supplementation (EcoMix) after an AF challenge on hepatic gene expression of 7 different inflammation markers. Ten multiparous rumen-cannulated Holstein cows [BW (mean ± SD) = 669 ± 20 kg and 146 ± 69 DIM] were assigned to 1 of 5 treatments in a randomized replicated 5 × 5 Latin square design balanced to measure carryover effects. Periods (21 d) were divided into an adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). From d 15 to 17, cows received an AF challenge consisting of 100 μg of aflatoxin B1 (AFB1)/kg of dietary DMI. AFB1 was fitted into 10 mL gelatin capsules (TORPAC, Fairfield, NJ) and administered into the rumen through the cannula based on the average DMI obtained on d 12 to 14. Treatments were POS, no clay plus an AF challenge; 3 different concentrations of clay (0.5, 1, or 2% of dietary DMI) plus an AF challenge; and control (C), no clay and no AF challenge. Statistical analysis was performed using the MIXED procedure of SAS. Contrasts included CONT1 (POS vs. C), CONT2 (POS vs. the average of 0.5, 1, or 2%), and tests of linear and quadratic treatment effects of clay inclusion. When comparing POS with C, the AF challenge caused a 2.27-fold downregulation of haptoglobin (HP; P = 0.04) and tended to have a 1.06-fold downregulation of signal transducer and activator of transcription 3 (STAT3; P = 0.10). However, when supplemented with clay, cows had a linear increase in expression of nuclear factor kappa B subunit (NFKB1; P = 0.02) and a trend for linear increase of tumor necrosis factor (TNF; P = 0.10). In conclusion, liver gene expression profiling suggested that an AF challenge downregulated inflammation and there was a restorative effect when clay was supplemented orally that seemed to counteract the immunosuppression of AF.

Key Words: clay, aflatoxin, hepatic gene expression


Staphylococcus spp. are one of the main etiological agents of bovine mastitis, and exhibit high-level antimicrobial resistance. These microorganisms are reported as significant contaminants of raw milk and dairy products, being able to produce various toxins causing food poisoning outbreaks and toxic shock syndrome in humans. The aim of this study was to detect the presence of genes sea, sec, and tst, responsible for the production of staphylococcal enterotoxins A, C and TSST-1, respectively, in strains of Staphylococcus spp. antimicrobial resistant, isolated from bovine mastitis. Twenty-seven S. aureus and 40 CoNS (i.e., S. capitis, S. chromogenes, S. epidermidis, S. hominis, S. sciuri, S. simulans, and S. warneri), exhibiting a resistance profile (ampicillin, ciprofloxacin, clindamycin, enrofloxacin, erythromycin, florfenicol, gentamicin, kanamycin, linezolid, neomycin, oxacillin, penicillin, streptomycin and tetracycline, and mecA gene negative), isolated from heifers and cows with mastitis, were used. The samples were from commercial farms in São Paulo State, Brazil. The extraction of genomic DNA was performed using DNA Kit RTP Bacterium - Invitek. The genes amplification was performed by PCR and the reaction product was visualized on 2% agarose gel electrophoresis in 1× TBE buffer (1 M Tris base, 0.9 M boric acid, 0.01 M EDTA) stained 0.5% red gel. For positive control they were used to S. aureus sp. aureus ATCC 29213 and NRS111. Descriptive statistical analysis was performed by calculating relative frequencies (PROC FREQ; SAS Institute, 2011) of resistant strains containing enterotoxins genes. The presence of the sea and tst genes was not found in any of the 67 strains, and the sec gene was detected in 20 of S. aureus strains (29.85%). The bacterial resistance to antibiotics associated with the production of enterotoxins in strains of Staphylococcus spp. isolated from bovine mastitis, adds risk to public health. Once the enterotoxins are thermostable and remain active in the foods even after processing, the use of the good hygiene practices is
required to reduce the microbial load of milk and dairy products, and therefore the chance of causing food poisoning.

**Key Words**: coagulase negative staphylococcus, enterotoxins, mastitis

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**T38** Advancement of Dairying in Austria (ADDA): Preliminary results of an observational study into antimicrobial use on dairy farms in Austria, Europe. C. L. Firth*1, A. Käsbohrer1, C. Egger-Danner2, K. Fuchs3, and W. Obritzhauser1, 1University of Veterinary Medicine, Institute of Veterinary Public Health, Vienna, Austria, 2Zuchtdaten EDV-Dienstleistungen GmbH, Vienna, Austria, 3Austrian Agency for Health and Food Safety (AGES), Integrated Risk Assessment, Data and Statistics, Graz, Styria, Austria.

The Advancement of Dairying in Austria (ADDA) project is a 3-year research assignment encouraging cooperation between academical institutions and the local dairy industry. In this part of the study, veterinarians were asked to provide electronic treatment records to allow for an analysis of antimicrobial use on dairy farms. In Austria, antibiotics are always prescription-only medications, are never available over-the-counter and when antimicrobial substances are dispensed by veterinarians to farmers for use in food-producing animals then this must be reported annually to the relevant authorities. The preliminary analysis presented here covers 186 dairy farms, including treatment records for a total of 4,960 cows, 5,030 youngstock, and 2,271 calves. Data were collected on treatments carried out between October 1, 2015, and September 30, 2016. To date, 12 veterinary practices have provided their data via an online interface. Of 12,432 data sets received, 6,530 (52.5%) included antibiotic treatments. Antimicrobial treatments were analyzed by means of the following formula to calculate the number of treatment days per 100 production (prod) days (#TD100):

\[
\# \text{TD}_{100} = \sum_{i=0}^{n} \left( \frac{\text{amount of active substance (mg)}}{\text{DDD}_{\text{vet}} \times \text{prod days (d)} \times \text{std weight (kg)}} \right) \times 100
\]

A standardized liveweight (std weight) of 500 kg for a cow, 200 kg for youngstock and 80 kg for calves was used. The Defined Daily Dose for animals (DDDvet) unit was taken from official recommendations made for each active substance by the European Medicines Agency. Results are presented as descriptive statistics. Overall, the median TD100 for total antibiotic use was 0.48; that is, bovine animals were treated for 0.48 d per 100 d. When calculated according to diagnosis, the vast majority of antibiotic treatments were for udder disease (median TD100 = 0.33). With respect to the “highest priority critically important antibiotics” (HPCiAs: fluoroquinolones, third- and fourth-generation cephalosporins and macrolides), the median TD100 was 0.09. HPCiAs accounted for 21% of the total amount of antimicrobial doses used in bovines. This quantitative analysis will be used to develop guidelines to reduce antibiotic use, particularly HPCiAs, in livestock.

**Key Words**: antibiotics, antimicrobial resistance, veterinarian

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**T39** Explaining farmers’ adaptation of preventive measures against mastitis—An application of Theory of Planned Behavior. N. Lind*1, H. Hansson1, U. Emanuelsson2, and C.-J. Lagerkvist1, 1Department of Economics, Swedish University of Agricultural Sciences, Uppsala, Sweden, 2Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.

In dairy production, mastitis is one of the most challenging animal health problems. The objective of this study was to explain farmers’ adaptation of recommended management control options (MCOs) at the own herd using the psychological constructs from the Theory of Planned Behavior (TPB) as determinants. The TPB is a well-known model to explain human behavior as a function of 3 psychological concepts: attitude, perceived behavioral control (PBC), and subjective norm (SN). The study is based on a random sample of 356 Swedish full-time farmers specializing in dairy production. Data were collected from an online questionnaire in spring 2016. Measures of the TPB predictors were developed according to the research question to the target behavior of decision making in mastitis prevention. Based on recommendations by Swedish farm advisory company VAXA, 16 different management areas (representing MCOs) toward cow bound or infectious bacteria were used to describe behavior. A cluster analysis was used to group farmers based on adapted MCOs. This was done to test whether the TPB components could explain differences in adaptation across groups of farmers which used similar sets of MCOs. Statistical analyses were performed using hierarchical multinomial logistic regression, where herd size (number of cows) and farmers’ subjective estimation of the somatic cell count at the herd was used as base model and each of the TPB concepts where used as covariates. The results showed that farmers’ decisions about which set of MCOs to adapt as preventive actions was largely explained by farmers’ perceived control over the situation. Attitudes and SN did not, however, contribute to predict the adaptation of MCOs. These result suggest, as PBC relate to self-efficacy (one’s belief in ability to exert action or avoid the adverse outcome), that the work to implement MCOs should be complemented by programs specifically designed to include elements to foster ability of farmers to use and or combine MCOs to alleviate and prevent mastitis.

**Key Words**: Theory of Planned Behavior, mastitis, prevention

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**T40** Integration of phenotypic and transcriptomic data shows differences of metabolic response upon energy shortage in relation with genetic resistance to mastitis. J. Bouvier-Muller*1,2, G. Fournas2, and R. Rupp1, 1INRA Gen PhySE, Castanet-Tolosan, France, 2Université de Toulouse I MAPP INRA ENVT, Toulouse, France.

The transition from late gestation to early lactation is the most metabolically challenging physiological stage in dairy ruminants. During this period, ruminants experience indeed some degree of negative energy balance (NEB) which is considered to increase susceptibility to mammary infections. The aim of this study was to determine the effect of NEB on mastitis in a dairy sheep model. Accordingly, 48 early-lactation dairy ewes from genetic lines for high and low somatic cell score (SCS) were allocated in 2 homogeneous subgroups: a NEB group which was energy restricted to 60% of their energy requirements during 15 d and a control-fed group. Ewes were monitored for milk production, SCS, body condition and blood metabolites. Previous study revealed an interaction between genetic line and energy restriction on several metabolic parameters and body condition. Indeed high-SCS ewes showed higher weight loss and increase of plasmatic β-hydroxybutyrate (BHB) and nonesterified fatty acids (NEFA) concentrations than low-SCS ewes, when facing NEB. Blood transcriptome analysis by RNA-seq was performed in 24 ewes at 3 time points: before the diet change, after 10 d of energy restriction, and 8 h upon an inflammatory mastmary challenge. Transcriptomic and phenotypic data were integrated with a generalized partial least square discriminant analysis using mixOmics package framework (block PLS-DA). NEFA and BHB concentrations were the phenotypes that discriminated energy-restricted high-SCS ewes. The association between variables was computed using a similarity score, based on the coordinates of the variables on the axis defined by the principal components. The supervised analysis revealed a high
correlation between milk fat content, fat-to-protein ratio and BHB and NEFA concentration ($r > 0.8$). Moreover, BHB and NEFA concentrations were highly correlated with the level of PDK4 and CPT1A expression ($r > 0.9$), which encode 2 key regulatory enzymes involved in respectively glucose oxidation and fatty acids β-oxidation. These results strongly suggest a genetic link between susceptibility to mastitis and metabolic adaptation to energy shortage.

Key Words: mastitis, energy restriction, RNA-seq

T41 Impact of culling for SCC, milk revenue, and estimated breeding values on herd performance. K. Kaniyamattam*1, A. De Vries1, L. W. Tauer2, and Y. T. Grohn1, 1Section of Epidemiology, College of Veterinary Medicine, Cornell University, Ithaca, NY, 2Charles H. Dyson School of Applied Economics and Management, Cornell University, Ithaca, NY, 3Department of Animal Sciences, University of Florida, Gainesville, FL.

Our objective was to compare the economic, genetic and technical performance of a dairy herd implementing 6 different voluntary culling strategies for lowering bulk tank somatic cell count (BTSCC) with simultaneous maximization of milk revenues over a period of 15 yr. An existing stochastic dynamic dairy simulation model with 12 correlated genetic traits included in the 2014 lifetime net merit index (SNM) was used. The phenotypic performance of each animal’s 12 traits, (for example, daily SCC) was affected by their respective genetic and environmental component, along with a standard phenotypic function. Estimated breeding values (EBV) with genomic reliabilities were simulated for each animal, based on which selection and culling decisions were made. Genetic trends for sires in the model were similar to 15 yr projected trends for US Holsteins. In all 6 strategies simulated, surplus heifers born in the herd were culled based on lowest SNM to maintain a herd size of 1,000 milking cows. Whenever there was an incoming heifer, the lowest ranking cow was culled following 1 of these 6 strategies: I) daily SCC (highest phenotypic SCC), II) weighted average of SCC (highest moving average of SCC until day of culling), III) daily milk revenues (lowest milk revenues), IV) weighted average of milk revenues (lowest moving average of milk revenues until day of culling), V) EBV of SCS (highest SCS), and VI) EBV of SNM (lowest SNM), respectively. The 15 yr simulation results showed that the genetic performance of all the 6 strategies did not differ for the SNM trait. The true breeding value of the milk, fat and protein showed a difference of 120 kg, 3.9 kg and 3.6 kg, respectively, in year 15 between strategies IV and I. The phenotypic milk production, average BTSCC and profit per cow per yr differed by 108 kg, 10,920 cells/mL and $20$, respectively, in yr 15 between strategies IV and I. The cumulative 15-year net present value of return per cow was $-$190, $16$, $30$, $-$5736 and $52$ higher than strategy I for strategies II, III, IV, V and VI, respectively. Hence, we conclude that culling the cows with the lowest EBV of SNM is economically the best strategy to lower BTSCC, with simultaneous maximization of milk revenues.

Key Words: bulk tank SCC, modeling, profit


The primary objective of this study was to identify cow-level risk factors associated with the occurrence of subclinical and clinical mastitis, regardless of environmental or contagious pathogens responsible for the propagation of the disease. Mastitis prevalence was evaluated by parity across 6 key physiological time points in lactation: 0 to 1 in milk (DIM), 3 to 5 DIM, 10 to 14 DIM, 50 to 60 DIM, 90 to 110 DIM, and 210 to 230 DIM. Cows were scored for front and rear teat length, width, shape, and orientation, fore udder attachment, udder cleft, udder depth, rear udder height, and rear udder width. Two independent multivariable logistic regression models were used to generate odds ratios (OR) for farmer-diagnosed clinical mastitis and linear somatic cell score-based subclinical mastitis. We identified that loose fore udder attachment (OR = 1.5, 95% confidence interval (CI) = 1.1 to 2.2), and teat end shape by parity significantly increased the odds of a subclinical mastitis event, while centrally placed front teats decreased the odds of a subclinical event compared with medially pointing front teats (OR = 0.7, 95% CI = 0.5 – 0.9). Loose fore udder attachment (OR = 3.7, 95% CI = 1.2 to 11.9), flat teat end shape (OR = 1.6, 95% CI = 1.0 to 2.6), front teat width (OR = 1.8, 95% CI = 1.0 – 3.3), and rear teat width (OR = 2.1, 95% CI = 1.0 – 4.4) significantly increased the odds of a clinical mastitis event. In this study cohort, loose fore udder attachment and flat teat ends were identified having a significant role in elevated risk of both clinical and subclinical mastitis. In addition, front and rear teat width increased the risk of clinical mastitis. The identification of these cow-level risk factors for mastitis can provide farmers an effective and inexpensive tool to manage mastitis.

Key Words: mastitis, udder, teat

T43 Effects of feeding an extruded flaxseed supplement on fatty acids in milk and plasma and immune function in transition dairy cows. M. Fetter*1,2, J. Pate1,2, K. Harvatine1, J. Moats3, and T. Ott1,2, 1Department of Animal Science, Pennsylvania State University, 2Center for Reproductive Biology and Health, Pennsylvania State University, 3O&T Farms, Regina, SK, Canada.

During the transition period, cows exhibit reduced immune cell numbers and function, and elevated markers of inflammation. Compromised immune function is thought to be caused, in part, by metabolic stress and by changing hormone concentrations due to the transition from a pregnant state to a lactating state. If not properly managed, the transition period is accompanied by increased incidence of periparturient diseases. Feeding polyunsaturated fatty acids (PUFA) has been shown to affect immune function in dairy cattle. The objective of this study was to determine the effects of feeding a flaxseed supplement enriched in omega-3 PUFAs on immune function, milk yield and components, and FA composition of milk, plasma, and red blood cells. Multiparous Holstein dairy cows (n = 15) were randomly assigned to 2 treatments: control-fed cows (n = 8) received whole roasted soybeans at 4.8% DM, and flaxseed-fed cows (n = 7) received an extruded flaxseed product (LinPRO-R; O&T Farms) at 3.5% DM. The diets contained similar concentrations of crude protein and fat. Diets were fed for the first 21 d of lactation. Blood was collected on d 1, 7, 14, and 21 and milk on d 7, 14, and 21. Milk fat percentage tended to be greater (P = 0.07) in the flaxseed group (4.5%) compared with the control group (3.9%). Flaxseed-fed cows tended to have increased α-linolenic acid in milk (P = 0.06) and in plasma (P = 0.09) compared with controls. Neutrophil expression of reactive oxygen species was reduced in flaxseed-fed cows (P < 0.01) and phagocytosis also tended to be reduced (P = 0.08). There was a tendency for decreased mRNA abundance for tumor necrosis factor (P = 0.09) and interleukin 10 (P = 0.09) in peripheral blood mononuclear cells in flaxseed-fed cows. In summary, feeding an extruded flaxseed product increased ALA in plasma and milk without reducing milk fat or protein percentage or yield. Cows fed the omega-3 diet had reduced reactive oxygen species and phagocytosis activity ex vivo.

The allelic frequency of the CSN2 gene in Gyr and Guzerat pure breed animals was studied in an experiment conducted at the Rio Grande do Norte State, in Brazil, with 88 Guzerat and 68 Gyr animals of different categories. Hair samples from the cow tail tassel were collected and the DNA extraction was performed from the hair follicles, following the precipitation method with salt. Nucleotide sequence readings of the amplified fragment for the β-casein gene (A1 and A2) were aligned and edited. Allele frequencies (Xi) for β-casein alleles (1) and genotypic frequencies for the genotype (2) were obtained using the equations: $X_i = 2n_i + \sum n_j / 2n_i n_j = n_i / n$, in which $n_i$ and $n_j$ correspond to the number of homozygotes and heterozygotes observed in the i allele, respectively; and n corresponds to the number of individuals analyzed. Using the Hardy-Weinberg theorem, expected genotypic frequencies at equilibrium were estimated from expanding the binomial: $(x_i + x_j)^2 = x_i^2 + 2x_i x_j + x_j^2$, where $x_i^2$ is the expected frequency of homozygous for allele i, $2x_i x_j$ is the expected frequency for heterozygotes ij; and $x_j^2$ is the expected frequency of homozygous for allele j. It was found that Guzerat animals had a higher amount of heterozygous animals compared with Gyr of the population (Table 1). None of the evaluated animals presented homozygosity for A1. The allelic frequency of A2 allele and the genotypic frequency of A2A2 genotypes for β-casein gene in the assessed Zebu breeds indicate that these breeds may produce less allergenic milk for individuals who are sensitive to β-casein protein.

Table 1 (abstract T44). Allelic and genotypic frequencies for the A1 and A2 alleles of the CSN2 gene in Gyr and Guzerat breeds

<table>
<thead>
<tr>
<th>Breed</th>
<th>Allelic frequency</th>
<th>Genotypic frequency</th>
<th>HWE1</th>
<th>P-value2</th>
</tr>
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<td>0.98</td>
<td>0.04</td>
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<td>Guzerat</td>
<td>0.03</td>
<td>0.97</td>
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</tbody>
</table>

1HWE = Hardy-Weinberg equilibrium.
2Chi-squared test.

Key Words: allelic frequency, nucleotide sequence, Zebu breed

T45  Bull fertility evaluations for Angus service sires bred to Holstein cows. J. L. Hutchinson*1, P. M. VanRaden1, J. B. Cole1, G. C. Fok1, and H. D. Norman2, 1Animal Genomics and Improvement Laboratory, Agricultural Research Service, USDA, Beltsville, MD, 2Council on Dairy Cattle Breeding, Bowie, MD.

The purpose of this study was to investigate the use of beef service sires bred to Holstein (HO) cows and heifers and to provide a tool for dairy producers to evaluate Angus service-sires. Many US dairy cows are now being bred to Angus sires because beef prices are high and there is a surplus of dairy heifers in many herds. Sire conception rate (SCR), a phenotypic evaluation of service-sire fertility implemented in August 2008, is based on data from the most recent 4 years, conventional semen breedings up to 7 services, and cow parities 1 through 5. The SCR model and methodology was used in this study, with service-sire inbreeding and expected inbreeding of resulting embryo set to 0 because pedigree data were unavailable. Service-sire age was combined into 3 groups (1.8 to 4.5; 4.6 to 7.5; and >7.5 yr). A total of 97,987 breedings were available and included observations on 947 Angus service-sires and 64,601 HO cows (other beef breeds had too few records to evaluate). A mean conception rate of 30% was observed (46% standard deviation), compared with 32% for breedings with a HO cow mated to a HO sire. Publishable Angus bulls were required to have 100 total matings, 10 matings in the most recent 12 mo, and breedings in 5 or more herds. Mean SCR reliability was 56% for 95 publishable bulls, with a maximum reliability of 97% based on 8,840 breedings. Average SCR was near 0 (on an Angus base), with a range of −3.4 to 3.3. Breedings to HO heifers were also examined, which included 8,446 breedings (399 Angus service-sires and 6,570 HO heifers). A mean conception rate of 49% was observed (50% standard deviation), compared with 57% for breedings with a HO heifer mated to a HO sire. Angus sires were used more frequently for later services on problem breeders, which explains some of the difference. Mean service number was 1.77 and 2.90 for HO and Angus sires mated to HO heifers, respectively, and 2.21 and 3.41 for HO cows. Mating dairy cows to beef bulls may be profitable if the calf price is higher, fertility is better, or if practices such as sexed semen, genomics testing, and improved cow productive life allow herd owners to produce both higher quality dairy calves for replacement and beef calves for market.

Key Words: sire conception rate, beef breed

T46  Genetic and genomic analysis for oocyte number and embryo production traits in Holstein cattle using in vitro fertilization data. C. Sun*, D. Kendall, C. Heuer, J. Deeb, R. Vishwanath, M. Fosado, and J. Moreno, ST Genetics, Navasota, TX.

The modern reproduction technologies ovum-pickup (OPU) and in vitro fertilization (IVF), combined with genomic selection provide a rapid and sustainable route for genetic improvement in both efficiency and productivity in dairy cattle. The aim of this study was to estimate variance components and identify regions of the genome associated with traits related to oocyte number and embryo production in Holsteins. Data collected on a Holstein dairy farm in Wisconsin from 2013 to 2016 included 11346 OPU and in vitro fertilization records from 1505 unique elite females and 216 unique service bulls. Six traits were defined: number of oocytes collected (NOC), number of oocytes on drop (NOD), number of cleaved embryos (NCE), number of unfertilized oocytes (NUO), number of dead embryos (NDE) and number of transferable embryos (NTE). A univariate repeatability animal model analysis was performed for these traits. Because these are count variables following a Poisson distribution, generalized linear mixed models (GLMM) with a log link function were employed in ASREML. Of the 1505 unique females, 580 were genotyped using a variety of chips. All genotyped animals were imputed to include those markers used for official US genomic evaluations based on a large genotyped population. 58275 SNPs (after quality control) and EBVs from the GLMM models were used for genome-wide association studies by fitting all the SNPs as random effects using a linear mixed model in GCTA. NOC and NOD only depends on a donor’s maternal genetic effect, whereas paternal genetic effect must be considered for other embryo traits. Estimates of maternal heritability were 0.158 for NOC, 0.134 for NOD, 0.162 for NUO, 0.104 for NCE, 0.175 for NDE, and 0.139 for NTE, whereas the relative
genetic impact of the paternal component was small. Estimates of the genetic correlations between the maternal and the paternal component were slightly negative for NUO, NCE and NTE, indicating a genetic antagonism. The p-values of the genome wide association (GWAS) studies showed that several markers exceeded significance thresholds.

**Key Words:** in vitro fertilization, embryo, variance components

**T47** Accounting for potential bias due to the pre-selection of cows for hoof trimming using a multiple trait evaluation. F. Malchiodi*1,2, F. S. Schenkel1, A-M. Christen3, D. F. Kelton4, and F. Miglior1,4, 1Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada, 2Valacta, Sainte-Anne-De-Bellevue, QC, Canada, 3Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, 4Canadian Dairy Network, Guelph, ON, Canada.

A national genetic evaluation program for hoof health could be achievable by using hoof lesions collected directly by hoof trimmers. However, not all of the cows present in the herds during the trimming period are usually presented to the hoof trimmer and the pre-selection is rarely random, leading to potential bias and, consequently, inaccuracies in the genetic evaluation. The objective of this study was to investigate a multiple trait evaluation to account for the potential bias due to pre-selection of cows for hoof trimming. Hoof lesions from 70,305 animals were recorded by 23 hoof trimmers in 521 Canadian herds from 2009 to 2012. The lesions included in the analysis were digital dermatitis, interdigital hyperplasia, and sole ulcer. Multiple trait evaluation was performed to account for the cow pre-selection bias. In addition of the presence of the lesion, locomotion (LOC) and the overall score for feet and legs (FL) were considered. The differences between average EBV of the lesions estimated with univariate or multiple trait model increased as the percentage of non-trimmed daughters increased, suggesting that including LOC and FL might have an effect on the estimations. However, correlations between the EBV estimated with single or multiple trait models by percentage of not-trimmed daughters were very high (0.97 to 0.99), showing that the sire ranking was very similar.

**Key Words:** hoof lesions, multiple-trait evaluation

**T48** Genomic prediction of lactation curves for milk, fat, protein, and somatic cell score in Canadian Jersey cattle. H. R. Oliveira*1,2,3, L. F. Brito1, J. Jamrozik1,3, F. F. Silva2, and F. S. Schenkel1, 1University of Guelph, Guelph, ON, Canada, 2Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, 3Canadian Dairy Network, Guelph, ON, Canada.

Application of random regression models (RRM) in 2-step genome-wide selection (GWS) may provide opportunities for selecting young animals based on the pattern of the lactation curve, without changing the traditional genetic evaluation system used in several countries. In this context, the prediction accuracy of direct genomic values (DGVs) for milk (MY), fat (FY) and protein (PY) yields, and somatic cell score (SCS) over days-in-milk in a 2-step genomic evaluation approach was investigated. Estimated breeding values for each test-day (from 5 to 305 d) from the first 3 lactations of Jersey cows (referred as 1, 2 or 3 beside trait acronyms), derived from estimates of the lactation curve coefficients (Legendre polynomials of order 4), were de-regressed (dEBVs) and used as pseudo-phenotypes in the second step of GWS. Genotyped individuals included in the official Canadian Jersey genetic evaluation in December, 2012, by the Canadian Dairy Network (CDN; Guelph, ON, Canada) were used as training population (n = 1,463 animals). The validation population included 315 individuals born after 2012, which had an official genetic evaluation in December, 2016. Individual additive genetic random regression coefficients for each trait were predicted using Genomic Best Linear Unbiased Prediction (GBLUP) and further used to derive DGV for each day of the 305d lactation. Prediction accuracy for each trait was evaluated based on Pearson correlation between DGVs and dEBVs (r_DGV,dEBV) adjusted for the average reliability of dEBVs in the validation population. The average estimated r_DGV,dEBV over the lactation curve was 0.64, 0.73, and 0.75 for MY1, MY2, and MY3; 0.53, 0.50, and 0.54 for FY1, FY2, and FY3; 0.85, 0.74 and 0.56 for PY1, PY2, and PY3; and 0.26, 0.54, and 0.37 for SCS1, SCS2, and SCS3, respectively. Therefore, the use of RRM in 2-step GWS produced moderately accurate DGVs for milk production traits and SCS over the lactation in Canadian Jersey cattle. Strategies to optimally blend DGVs and traditional RRM EBVs will be investigated next.

**Key Words:** GBLUP, genome-wide selection, random regression

**T49** Identifying, analyzing, and comparing runs of homozygosity in Canadian dairy populations using next-generation sequencing data. C. Vogelzang*1, F. Miglior1,2, N. Melzer3, M. Sargolzaei4, C. Maltecca3, B. Makanjuola1, A. Fleming1, F. Schenkel1, and F. Baes1, 1CGIL, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, 2Canadian Dairy Network, Guelph, ON, Canada, 3Leibniz Institute for Farm Animal Biology, Institute of Genetics and Biometry, Dummerstorf, Germany, 4Semex Alliance, Guelph, ON, Canada, 5Department of Animal Sciences, North Carolina State University, Raleigh, NC.

Inbreeding coefficients in dairy cattle are typically estimated by calculating the degree of parental relatedness through use of pedigree data. More recently, genomic data in the form of single nucleotide polymorphisms (SNPs) have been used, which provide increased accuracy in calculating individual inbreeding coefficients. The use of SNPs allows more accurate estimation of the realized proportion of the genome that 2 individuals share, as opposed to using the expected proportion obtained from pedigree information. There has been an increase in the rate of inbreeding over the past few decades, possibly leading to a reduced level of fitness in individuals due to the accumulation of deleterious homozygous alleles. Runs of homozygosity (ROH), or regions of homozygous loci in a genome, occur more often in animals whose ancestors are closely related, where regions of an individual’s genome have inherited identical haplotypes. Length of ROH varies from individual to individual, and has been seen to accumulate in subsequent generations, strongly suggesting an increased level of genomic inbreeding over time. There is a need to assess and implement new tools that use genomic information, such as array and sequence information, to better understand ROH and the underlying mechanics of genomic inbreeding in dairy breeds. Here we will present a comprehensive analysis, with a focus on rate and functional severity of deleterious and neutral variants. Preliminary work using Next-Generation Sequencing data and SNP1101 software to identify ROH in Holsteins will be explored further. Genomic data from the Canadian Dairy Network along with next-generation sequence data made available through the 1,000 Bull Genomes project will be used to identify and visualize ROH in the bovine genome. A comparative analysis of ROH will be conducted among Canadian dairy breeds. The results of this study will help us better understand genotype diversity in Canadian dairy populations.

**Key Words:** dairy, genetics, genomics
The increasing use of genomic selection has resulted in a shorter generation interval, reduced effective population size, increased selection intensity and consequently an increased annual rate of inbreeding. Inbreeding accumulation is a growing concern for the dairy cattle industry, mainly due to strong negative correlations that exist between inbreeding and fitness traits. On an animal level, increased homozygosity is associated with increased risks of disease susceptibility, defects, or death based on the presence of deleterious alleles, and loss of genetic diversity on a population level. Identification of regions containing deleterious alleles are, therefore, pertinent for genetic improvement purposes. Conventionally, estimation of inbreeding coefficient has been done using pedigree information, with SNP data more recently included when available. The availability of genomic information and the increasing number of Canadian dairy animals with genotypic records has led to the use of runs of homozygosity (ROH) in predicting or estimating inbreeding. ROH are unbroken homozygous SNP regions present on homologous chromosomes of a specific animal. In a previous internal study, PLINK, SNP101 and BCFTools were used to identify and characterize ROH in Holstein animals using >150,000 50K genotypes and >3,500 HD genotypes provided by the Canadian Dairy Network (CDN), and 402 whole-genome sequence genotypes made available by the 1,000 Bull Genomes project. In the current analysis, these ROH regions will be annotated using Kyoto Encyclopedia of Genes and Genomes (KEGG), an online bioinformatics resource and Variant Effect Predictor (VEP), a freely distributed software. Positions of annotated ROH will be determined and further investigated to evaluate their effect on genes, transcripts, and proteins. Additionally, analysis to determine whether amino acid substitution changes the protein structure will be performed. The Canadian dairy industry will benefit from these results as the inclusion of annotated ROH regions in selection strategies may help manage deleterious alleles, control inbreeding and ultimately improve fitness performance of the Canadian dairy Holstein population.

**Key Words:** runs of homozygosity, inbreeding, cattle

<table>
<thead>
<tr>
<th>Population size</th>
<th>TRD(^1)</th>
<th>Sire-specific TRD(^2)</th>
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<td>0.00</td>
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<tr>
<td>125</td>
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<tr>
<td>500</td>
<td>0.46</td>
<td>0.65</td>
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\(^1\)Random minor allele frequency \(\geq 0.05\).

\(^2\)All sires were heterozygous; the same results were observed for dam-specific TRD (heterozygous dams).

**Key Words:** transmission ratio distortion, homozygous parents, Bayes factor
mucins development and plug formation were identified. A total of 3,566 genes were DE between milk from healthy and mastitic cows (SC). Some of these genes impact the immune response of the bovine (IL18, IL10, MUC20, PCNA), while others effect plug formation (MUC1, MUC15) and epithelial renewal and differentiation (MUC4). The prevalence of some bacteria also depended on the health status of the milk. Further analysis will be performed to study the microbiome by 16S ribosomal sequencing. In conclusion, the identification of genes and biomarkers associated with mucins and teat plug formation will aid in improving the sustainability of agricultural practices, by facilitating the selection of cows with improved immune systems and resistance to infection.

Key Words: mastitis, transcriptomics, metagenomics

T53 Genetic susceptibility of Canadian dairy heifers to mastitis. S. G. Narayana*1,2, F. Miglior2,3, A. Naqvi1, P. Martin2, and H. W. Barkema1, 1University of Calgary, Calgary, AB, Canada, 2University of Guelph, Guelph, ON, Canada, 3Canadian Dairy Network, Guelph, ON, Canada.

Mastitis is the most common, expensive and detrimental disease of dairy cattle. Mastitis in heifers around calving threatens udder health in the first and consecutive lactation, increases the risk of premature culling, and leads to economic losses. Together with enhanced preventive management practices, genetic selection for heifer mastitis resistance could aid in a more efficient and sustainable way. Although good progress has been made in comprehension of genetics of mastitis resistance, knowledge of genetic variation of pathogen-specific heifer mastitis is still very limited. Moreover, little genetic research has been conducted on heifer mastitis focusing on the period around first calving and also on pathogen-specific occurrence. Despite the low heritability of clinical mastitis, studies have shown that there is exploitable large genetic variation among bulls. The objective of this study is to investigate genetic variation of overall and pathogen-specific heifer mastitis in Canadian dairy herds. Data collected over a 2-year period as part of National Cohort of Dairy Farms of Canadian Bovine Mastitis and Milk Quality Research Network (CBMQRN) from 91 Canadian dairy herds spread over 6 provinces will be used for the study. Incidence of overall and pathogen-specific clinical mastitis will be treated as 0, 1 and > 1 cases of clinical mastitis. A generalized linear mixed model will be used for the estimation of variance components. Estimated genetic parameters from this research will provide insight into genetic variation of heifers associated with mastitis in Canadian dairy herds. Results will be ready to be presented at the conference.

Key Words: heifer, mastitis, heritability
The Farmer to Farmer Program (F2F) was authorized by Congress in the 1985 Farm Bill. Land O’Lakes International Development is one of the implementers and is currently managing this USAID program for the Middle East and North Africa (MENA), which includes the Lebanese Republic (Lebanon), Egypt and Morocco. US citizens and Green Card holders volunteer as short-term technical assistants to work with farmers, processors, agribusinesses, and universities in-country on specific, demand driven, projects. F2F MENA implements country projects focusing in the areas of enhancing food quality and safety, increasing access to agricultural finance. Additionally, in Lebanon there is also a country project focusing on environmental conservation. There are a large number of entrepreneurs who run small and medium-sized dairy plants where they manufacture Lebanese style cheeses and yogurts and desire technical assistance. While there are universities in Lebanon training food scientists, there is no comprehensive extension system to work with the farmers and processors directly. The F2F program allows for the ability to conduct extension activities internationally. This poster will describe 2 2-week projects working with 8 cheese processors to develop new varieties of cheese, and transfer processing and food safety knowledge. All the cheese makers visited pasteurized either their cheese milk, or their cheese. However, several studies have shown the presence of *Listeria monocytogenes* (26% of the baladi samples examined), pathogenic *E. coli*, and *Brucella abortus* in market samples of cheese in Lebanon. While the projects described had the expressed goal of developing new varieties of cheese with the cheese makers, the main results were technology transfer in terms of processing safety recommendations, and recommendations for improving curd firmness and cheese yield. The F2F in-country staff provide support to the processors after the volunteers leave so that recommendations can be implemented. The documentation of the results and outcomes are also important metrics collected by the staff and will be presented. Mostly undocumented are the benefits volunteers receive from learning new skills and meeting potential university collaborators.

**Key Words:** USAID, cheese, Lebanon

**T55**  
Impact of milk hauling practices on microbiological quality. E. Kuhn*, L. Goddik, and J. Waite-Cusic, Oregon State University, Corvallis, OR.

The Pasteurized Milk Ordinance (PMO) allows for milk tanker trucks to be used repeatedly for 24 h before mandatory clean-in-place (CIP) cleaning. There are no specifications for length of time a tanker can be empty between loads. We partnered with a Pacific Northwest dairy company to investigate if extended idle time between loads influences microbiological populations in subsequent loads of milk. This processor does not allow tanker trucks to sit idle between loads for more than 6 h. Two farms were selected to participate in the study based historical microbiological data from January 2014 through December 2015, quantified using Foss Bactoscan and reported as individual bacteria count (IBC) and preliminary incubation count (PIC). Historically, Farm A IBC and PIC (n = 729) averaged 47.8 and 432.3, and Farm B (n = 982) had substantially lower average IBC and PIC (8.8 and 13.2). The study occurred over 6 consecutive days; for 3 d Farm B milk was collected immediately after unloading farm A, and the other 3 d Farm B milk was collected 6 h after unloading. For each day milk samples were obtained each farm bulk tank and from the tanker before unloading. Each sample was microbiologically assessed in duplicate for standard plate count (SPC), lactic acid bacteria (LAB), and coliforms. Colony isolates were assessed for lipolytic and proteolytic activity using spirit blue agar (SBA) and skim milk agar (SMA), respectively. There was not a significant difference in microbiological counts and enzyme activity in farm B’s tanker sample where comparing 0 and 6 h between hauling. We have demonstrated that 6 h between loads does not negatively impact subsequent loads of milk, and that the processors parameters are adequate.

**Key Words:** hauling, cleaning, quality

**T56**  
Influence of somatic cell count on sensorial acceptance of bovine milk and cheese in the semi-arid region of Brazil. E. R. Lima1, M. F. Bezerra1, J. G. B. Galvao Jr.2, S. A. Urbano1, and A. H. N. Rangel1, 1Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil, 2Instituto Federal de Educaçao, Ciencia e Tecnologia do Rio Grande do Norte, Ipanguaçu, RN, Brazil.

The objective of this study was to investigate the influence of somatic cell count (SCC) on the sensorial acceptance of bovine milk and Coalho cheese. Milk samples from different bulk milk tanks were classified into low SCC (less than 200,000 cells/mL) and high SCC (ranged from 200,000 cells/mL to 500,000 cells/mL). Pasteurized milk was evaluated after 2 d of storage, and cheeses at 20 and 40 d of shelf life under refrigeration (4°C to 7°C). Appearance, color, odor and flavor were analyzed by a sensorial acceptance test with a 9-point hedonic scale ranging from 1 (I disliked it very much) to 9 (I liked it very much) by panels of untrained tasters composed of 92, 84 and 78 volunteers, who respectively evaluated milk and cheese samples at 20 and 40 d of shelf life. The differences between the groups were determined by ANOVA, complemented by the Tukey test (P < 0.05). SCC levels did not affect the sensorial acceptance of milk; the average scores varied between 6.8 (I liked it slightly) and 7.2 (I liked it regularly). Cheese samples presented similar scores for appearance and odor attributes. For flavor, only the cheese with low SCC at 40 d shelf life showed significantly lower sensory scores 6.1 (I liked it slightly) than cheese samples with high SCC at 20 and 40 d shelf life. The results show no sensorial changes between pasteurized milks with different levels of SCC consumed at up to 2 d of storage under good refrigeration conditions; moreover, they indicate the possibility of obtaining good sensorial acceptance for Coalho cheese from milk with a high level of SCC and up to 40 d shelf life.

**Key Words:** milk quality, sensory evaluation, shelf life

**T57**  
Prevalence of sporeformers in raw milk in Nebraska: A year in perspective. B. Martínez, R. Crespo*, J. Stratton, and A. Bianchini, University of Nebraska-Lincoln, Lincoln, NE.

The quality of dairy products is limited because of the ability of sporeformer bacteria to survive pasteurization and grow in these products. This growth causes changes in quality due to oxidation and proteolysis, leading to off odors and texture defects. Additionally, special sectors, such as the milk-powder industry, require low spore counts in their products to compete in a global market. To improve the quality of dairy products related to sporeformers, a better understanding of the factors associated with their entrance into the milk chain is needed. Previous
research has indicated a seasonal variation associated with levels of sporeformers in dairy farms. However, it is not clear if this also occurs in Nebraska. Therefore, the objectives of this research were (1) to determine if the prevalence of different groups of sporeformers varies throughout the year in raw milk collected at farm level, (2) to observe the prevalence of sporeformers that are able to produce spoilage during refrigeration (7°C) and their dependence on seasonal variations, (3) to determine if environmental samples at farm level show a seasonal variation regarding different groups of sporeformers, and (4) to implement interventions at farm level that can potentially reduce sporeformers in raw milk. Results suggest that the prevalence of all tested groups of sporeformers are constant, regardless of the season in which samples were collected. Mesophilic spore counts ranged from 0.81 to 1.0 log cfu/mL, while thermophilic spore counts varied between 0.76 and 0.98 log cfu/mL in raw milk samples. The prevalence of psychrotrophic sporeformers was as high as 65% of the samples tested, with 45% of the samples showing counts > 6 log cfu/mL at 21 d of storage. A change in the sanitation protocols showed a statistically significant reduction in sporeformers in raw milk; whereas a change in sanitizing tea dips showed no difference. Overall, individual farm practices seem to exert a significant effect on the level of sporeformers in raw milk. This observation suggests that careful implementation of better farm practices would have more impact on the milk quality than any perceived seasonal variation.

**T58 Population dynamics of a common dairy sporeformer, Bacillus licheniformis, in spiked raw milk samples stored at low temperatures.** N. Awasti1,2, R. Suliman3, S. Anand1,2, and G. Djira1, 1Midwest Dairy Food Research Center, Brookings, SD, 2Department of Dairy and Food Science, South Dakota State University, Brookings, SD, 3Department of Mathematics and Statistics, South Dakota State University, Brookings, SD.

Bacillus licheniformis is a widely reported sporeformer in raw milk, and milk powders. The organism, being thermotolerant, is considered a challenge during milk processing. It would be of interest to understand its growth dynamics during raw milk storage at low temperature in plant silos. The current study was conducted to observe the changes in population of vegetative cells and spores of B. licheniformis, spiked in raw milk samples at about 4.0 log cfu/mL. The spiked milk samples were stored at 4°C, 6°C and 8°C, for durations of 0, 36 and 72 h. Standard protocols were followed for microbial analysis. Spore enumeration was done by heating the spiked milk samples at 80°C for 12 min before platting on Brain Heart Infusion agar. Three trials, in replicates of 3 were conducted, and the data were analyzed using 2 sample t-test, ANOVA, and first order regression model. While log vegetative counts increased to 4.09 after 72h at 4°C, the counts were 4.42 logs at 8°C. A significant difference (P < 0.02) was thus observed in the mean counts after 72 h of holding for 4 and 8°C. On the other hand, the spore counts mainly remained unchanged during 72 h at different storage temperatures. The results thus suggest that B. licheniformis may multiply to a greater extent, when milk is held at the higher temperature of 8°C. Moving forward, to accurately approximate the true response surface for vegetative cells, the fitted first order model suggests using the second degree model including additional design points. Whereas, no lack of fit (P = 0.294) was observed for spore values, and the entire regression surface was not significant. A higher degree model with additional design points will thus give us the optimum temperature and time combinations where no significant change or a minimum shift in vegetative cell numbers is observed, which may reduce the chance of sporeformer build up during low temperature storage of raw milk.

**Key Words:** spore, sporeformer, dairy silos

**T59 The role of Bacillus cereus and their enzymes in gelation of UHT milk.** R. S. Obaid1, 1University of Sharjah, Sharjah, United Arab Emirates, 2Jordan Food and Drug Administration, Amman, Jordan, 3United Arab Emirates University, Al Ain, United Arab Emirates.

Gelation is one of the major defects in UHT milk products as it limits the shelf life of the products. The role of Bacillus cereus and bacterial proteinases in gelation of UHT milk during storage was investigated. Samples of UHT milk were inoculated with B. cereus and stored at 4, 25, and 37°C for 7 d. Gelation and pH of milk were monitored during storage. Proteinolysis was assessed by electrophoresis, soluble nitrogen and liquid chromatography. Gelation was detected in samples inoculated by B. cereus stored at 37°C for 12 h when count reached 8.87 to 10.3 log cfu/mL. At 25°C, the gelation was observed after 48 h at a count of 8.6 to 9.3 log cfu/mL. The pH values of inoculated samples during storage at 25 and 37°C decreased from 6.65 to 5.3 and 5.8, respectively. The samples stored at 4°C did not show any increase in count and pH changes thus, there was no gelation. The gel electrophoresis of casein breakdown showed that proteinases enzymes secreted by B. cereus degraded κ-casein extensively after 6 h, where after 9 h storage, β-casein and much of the α-casein was degraded after 12 h (time of gelation). The levels of soluble nitrogen were high due to the enzymatic activity that caused gelation after storage. The peptide profiles showed that the peaks eluted after 1, 3, 6, 9, 12, and 24 h of storage. The peaks represented the largest, least acid-soluble as well as the most hydrophobic peptides and represent the peptides produced from κ-casein hydrolysis. The profiles were different in 12 and 24 h of storage. Further, new peptides were eluted due to continuing extensive proteolysis that led to the release of more hydrophilic and hydrophobic peptides. The results indicate that gelation of UHT milk correlated well with the growth of B. cereus and changes in pH during storage. Bacterial proteinase enzymes hydrolyzed casein and cause gelation during storage of UHT milk.

**Key Words:** Bacillus cereus, UHT milk, gelation

**T60 New insights into post-pasteurization contamination of fluid milk—Detection, effects, and environmental persistence.** S. Reichler1,2, A. Alles1, A. Trmcic2, N. Martin3, K. Boor4, and M. Wiedmann1, 1Cornell University, Ithaca, NY, 2University of British Columbia, Vancouver, BC, Canada.

In spite of decades of continuous improvement in dairy sanitation and dairy quality, post-pasteurization contamination (PPC) of fluid milk by gram-negative organisms remains a burden to many processors. PPC may cause quality issues to arise during cold storage, including off-flavors, off-odors, changes in texture, and changes in color. PPC reduces the shelf-life of milk and results in increased waste. We collected finished product samples of pasteurized milk from 10 northeastern United States fluid milk plants 4 times over the course of 10 mo. To assess the prevalence and diversity of PPC, 280 samples were analyzed for total bacterial counts, coliforms, Enterobacteriaceae, and total gram-negative bacteria over 21 d of shelf life. Predominant organisms from all tests were identified and subtyped using 16s RNA sequencing. A trained sensory panel evaluated each sample at 21 of shelf life. As has been demonstrated previously, PPC is highly prevalent in fluid milk, and 49% of samples contained heat-labile gram-negative bacteria that were most likely introduced post-pasteurization. Samples predominated by gram-negative organisms had more severe sensory defects than those contaminated with gram-positives. Coliform and Enterobacteriaceae tests, commonly used for PPC monitoring in the United States and the European Union, respectively, were unable to detect Pseudomonas, Acinetobacter, and several other gram-negative genera, which were
the predominant organisms isolated in 38% of samples. Crystal violet tetrazolium agar, the standard for total gram-negative detection in dairy products, detected these organisms in 99% of cases. Using 16s sequence typing, we found evidence that certain bacterial strains responsible for PPC may persist in milk processing plants for several months. The distribution of 16s sequence types within a single plant provided a tool for pinpointing the processing steps at which contamination may have occurred. Subtyping methods are a potentially useful tool for fluid milk processors, as they may assist in the tracking of spoilage issues and the investigation and elimination of contamination sources within the plant.

Key Words: spoilage, gram-negative, subtyping

T61 Effect of lutein and antioxidant feed supplementation on milk quality and lutein content under different heat processes and storage times. D. Ren¹, C. Wang², Z. Wei*¹, J. Liu¹, and Z. Duan³. ¹Institute of Dairy Science, College of Animal Science, Zhejiang University, Hangzhou, Zhejiang, China; ²College of Animal Science and Technology, Zhejiang A & F University, Lin'an, Zhejiang, China; ³Kemin Industries (Zhuhai) Co. Ltd., Zhuhai, Guangdong, China.

The present study was conducted to investigate the effects of dietary addition of lutein and antioxidant (vitamin E, VE; tea polyphenols, TP; ethoxyquin, EQ) on the lutein content in raw, pasteurized and UHT milk and milk flavor. Milk samples were collected from 5 groups of dairy cows after 2 mo of feeding. The 5 groups included a control without any addition, and groups supplemented with lutein (200 g/cow·day), lutein and VE (1.2 g/cow·day), lutein and TP (1.2 g/cow·day), and lutein and EQ (2 g/cow·day), respectively. Milk samples were treated under pasteurization (72°C, 15s) and the UHT (135°C, 4s) process, and the milk flavor was evaluated via electric nose and sensory indices. Milk lutein content of the milk for different heat processes and storage times was analyzed by RP-HPLC. Data were analyzed by SAS using the one-way ANOVA model.

Key Words: milk, digestibility

T62 Impact of processing on in vitro digestion of milk from grazing organic and confined conventional herds. D. L. Van Hekken*¹, M. H. Tunick¹, D. X. Ren², and P. M. Tomasula¹. ¹USDA, ARS, DFFRU, Wyndmoor, PA; ²Zhejiang University, Hangzhou, China.

Debate on differences between milk from grazing and non-grazing cows has not addressed the effects that standard processing may have on milk digestibility. In this study, raw milk from grazing organic (ORG) and non-grazing conventional (CONV) herds was adjusted to 0 and 3.25% fat and processed as follows: raw skim milk (Sr) was HTST (Sp) or UHT (Su) pasteurized, and raw whole milk (Wr) was homogenized (Whr), HTST pasteurized (Wp), homogenized and HTST pasteurized (Whp), or homogenized and UHT pasteurized (Whu). Milk then underwent 60 min of gastric digestion (NaCl, pepsin, and HCl; pH 1.5) and 120 min of intestinal digestion (ID) (K₂HPO₄ buffer, bile salts, pancreatin, and NaOH; pH 7.0). Samples were evaluated using a particle size analyzer, SDS-PAGE, nitrogen analysis, FFA titration, and GC-FID. Adjusting milk to gastric conditions resulted in large clots; Sr and Sp formed the largest clots, ORG > CONV (P < 0.05), while Whu had the smallest. Within 15 min, clots had decreased in size (nonfat > whole milk) and the caseins hydrolyzed to large and medium-sized peptides. Transition to intestinal conditions further decreased particle sizes (whole > skim) and only medium and small peptides remained at 15 min ID. Skim and ORG Whu samples contained only small peptides as early as 15 to 60 min ID. Proteins were 85 to 94% digested at 120 min ID. Addition of lipase in the ID phase resulted in rapid release of free fatty acids (FFA) during the first 15 min and then slowed as FFA accumulated. Homogenized whole milk released the most FFA and contained highest levels of saturated FA (8:0, 10:0, 12:0, and 14:0). Compared with ORG milk, CONV whole milk released more FFA and contained higher levels of C16:0 and 18:0. ORG whole milk contained higher levels of 6:0 14:1, 16:1, 18:1 trans, 18:2 isomers (conjugated linoleic acid, CLA), and 18:3 (P < 0.05) before and after processing than CONV milk. Although some minor differences existed between the milk from grazing ORG and confined CONV cows during in vitro digestion, milk from both sources responded similarly to standard processing treatments and were highly digestible, important information for health conscious consumers.

Key Words: high-pressure processing, whole milk, casein

T63 Effect of high-pressure jet processing on casein-fat interaction. M. Tran* and F. M. Harte, The Pennsylvania State University, State College, PA.

Homogenization has been traditionally used in the dairy industry to reduce the particle size of milk fat globules and prevent cream separation. Pressures of 3–20 MPa have historically been applied to a wide variety of dairy products to improve texture, stability, flavor, and shelf-life. High-pressure jet (HPJ) technology is a novel process that can achieve processing pressures of up to 600 MPa. The HPJ contains a diamond nozzle (75 to 400 µm diameter) that forces liquid into a jet stream, differing from high-pressure homogenization that uses a valve (HPH). Previous studies on pasteurized skim milk displayed an increase in viscosity, foaming, and emulsifying properties. The objective of this study was to evaluate the changes in casein-fat interaction of pasteurized and conventionally homogenized whole milk processed through HPJ-processing at 0 to 500 MPa (125 MPa increments), centrifuged, and freeze-dried. After centrifugation at 100,000 x g for 30 min, 3 distinct layers: cream (top), whey (middle), casein (bottom) was observed. The dry weight of the cream fraction decreased (0.67 ± 0.03 g to 0.11 ± 0.04 g) and the casein fraction increased (0.44 ± 0.01 g to 1.21 ± 0.05 g), while the whey fraction slightly decreased (1.46 ± 0.04 g to 1.21 ± 0.03 g) with increasing pressure and compared with the control (0 – 500 MPa). Fat content was also measured on the dry fractions to confirm migration of fat to the casein fraction. Fat content of the cream fraction decreased from 0.59 ± 0.06 g to 0.08 ± 0.04 g, while the fat content of the casein fraction increased from 0.02 ± 0.02 g to 0.44 ± 0.05 g. Casein-fat stability and aggregation was highly affected by HPJ processing. It is suggested that with increasing pressure, casein micelle and fat globule dissociation occurs and individual caseins interact with triglycerides to form stable casein-triglyceride aggregates. The results from this study will provide further applications for high-pressure jet processing in dairy foods.

Key Words: high-pressure processing, whole milk, casein
T64  
Quantitative analysis of Lactobacillus rhamnosus GR-1 in fermented probiotic milk products over refrigerated storage. S. Hekmat*, M. Soltani, and L. Ahmadi, Brescia University College at Western University, London, ON, Canada.

Lactobacillus rhamnosus GR-1 is considered to be an effective probiotic agent with therapeutic properties. Probiotic products containing L. rhamnosus GR-1 have been proven to help maintain a favorable microbial balance in the intestine and can survive in intestinal tract without induction of systemic immune or inflammatory responses. The objective of this study was to measure survival of L. rhamnosus GR-1 in fermented dairy products supplemented with various functional components that may be considered as probiotic agents over storage period. Five formulations of milk (1% fat) with 4% (wt/vol) honey (H), 0.05% (wt/vol) stevia (S), 2% (wt/vol) inulin (I), 1.5% (wt/vol) ginseng extract (G), 2.5% (wt/vol) moringa (M) powder and one with no additives (C) were prepared. The mixtures were autoclaved for 15 min, cooled to 37°C, and inoculated with 2% of L. rhamnosus GR-1 mixture and then were incubated anerobically at 37°C overnight. Selective MRS agar containing 0.015g/L fusicid acid was used to enumerate L. rhamnosus GR-1 after 1, 14, and 28 d of storage at 4°C. There were no other lactic acid bacteria in the samples. L. rhamnosus GR-1 remained viable (10^7 cfu/mL) in all samples over 28 d of storage. There was no significant difference (P > 0.05) in colony counts among different treatments during storage period. This study demonstrates that fermented dairy products combined with various functional components could be considered as suitable vehicles to deliver L. rhamnosus GR-1 to consumers.

Key Words: probiotic, prebiotic, yogurt

T65  
The role of heat treatment, fat content, and storage time on mechanical and sensory behaviors of fluid milk. H. S. Joyner (Melito)*1, Y. Li1, B. G. Carter2, and M. A. Drake2,1 School of Food Science, University of Idaho, Moscow, ID; 2Department of Food Bioprocessing and Nutrition Sciences, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC.

Fluid milk may be pasteurized by high-temperature short-time pasteurization (HTST) or ultrapasteurization (UP). Literature suggests that UP increases milk astringency, but definitive studies have not demonstrated this effect. Thus, the objective of this study was to determine the impacts of pasteurization method, storage time, and fat content on milk sensory and mechanical behaviors. Raw skim (<0.5% fat), 2% fat, and 5% fat milks were pasteurized in duplicate by indirect UP (140°C, 2.3 s) or by HTST pasteurization (78°C, 15 s), homogenized at 20.7 MPa, and stored at 4°C for 8 weeks. Sensory properties, instrumental viscosity, and friction profiles were evaluated at 25°C at 1, 4, and 8 weeks. SDS-PAGE and confocal microscopy were used to determine protein structural changes in milk at these time points. Fresh milks were processed by HTST at wk 7 for wk 8 evaluations. UP increased milk sensory and instrumental viscosity compared with HTST pasteurization (P < 0.05). Increased fat content increased sensory and instrumental viscosity, but decreased astringency and friction profiles (P < 0.05). Astringency increased for UP versus HTST (P < 0.05); however, UP did not significantly affect friction profiles (P > 0.05). Increased storage time increased instrumental viscosity and friction profiles (P < 0.05) but did not affect sensory viscosity or astringency (P > 0.05). SDS-PAGE and confocal microscopy showed more denatured whey proteins in UP processed milks compared with HTST processed milks. The network formed by these proteins likely caused the increase in viscosity during storage. Astringency and increased friction were likely due to the presence of denatured proteins, which formed large molecules. Overall, fat content had a greater impact on milk mechanical and sensory behaviors than storage time or heat treatment. Mechanical–sensory relationships were not straightforward; however, instrumental testing may still be used to evaluate milk behavior and enhance the understanding of sensory behaviors.

Key Words: milk, rheology, tribology

T66  
Detection of microorganisms responsible for a musty off-odor in nonfat chocolate milk. D. Batty*, E. Kuhn, L. Goddik, and J. Waite-Cusic, Oregon State University, Corvallis, OR.

Producers of nonfat chocolate milk have reported shelf-life failures of a musty off odor. The objective of this study was to determine when the failure takes place in shelf-life. Nonfat chocolate milk products with and without off-odors were acquired from regional processors. A shelf-life study was performed to detect when the musty off odor occurs. Fresh product was stored at 7°C and evaluated daily for the presence of the odor. It was determined that product failure occurred between d 15 and 17 with 100% product failure. Microbiological analyses were performed using standard serial dilution and spread-plating methods on tryptic soy agar for standard plate count (SPC), pseudomonas isolation agar (PIA), spirit blue agar (SBA), skim milk agar (SMA), MRS Agar, eosin methylene blue (EMB) and MacConkey, and chocolate milk agar. Chocolate milk agar was prepared using 2 formulations: one agar was prepared by adding chocolate milk to standard methods agar, while the other was prepared by adding chocolate syrup to skim milk agar. These media were selected to help differentiate the colonies that grow in a chocolate environment. All plates were incubated at 25°C before enumeration. The chocolate milk samples analyzed at 6 d had counts of <10 cfu/mL on all media types. At 15 d the samples had a SPC of 10^4 to 10^6 cfu/mL. Coliforms and lactic acid bacteria were <10 cfu/mL for all samples. Bacteria producing proteolytic and lipolytic enzymes were detected on SMA and SBA in all samples. The 2 chocolate milk agars performed equally well, and had growth of similar morphology from all samples. A musty odor was observed during incubation on the chocolate milk agars, and it increased with growth. This indicates that chocolate milk agars may be effective as early warning for product failure.

T67  
Rheological and tribological characterization of saliva interaction with acid milk gels. M. Bantiasadiekohdi* and H. S. Joyner (Melito), University of Idaho, Moscow, ID.

Fat replacers in food products can significantly affect their texture and rheological behavior. One reason for this effect is changes to food–saliva interaction. These changes are not always reflected in standard rheometry. Therefore, the objective of this study was to determine the impact of saliva addition on the rheological and tribological behaviors of acid milk gels. Samples (24 total) were prepared by mixing skim milk with various amount of fat (0–3.5% wt/wt), skim milk powder (SMP, 0–2.8% wt/wt), sweet whey protein isolate (WPI, 0–2.8% wt/wt), and hydrocolloids (locust bean gum (LBG), cellulose gum, potato and corn starch, 0–1.55% wt/wt). SMP and WPI were added to adjust protein and solid nonfat. The mix was pasteurized at 85°C for 30 min, then homogenized at 5000 RPM for 1 min. The mix was cooled to 42.2°C, then glucono-delta-lactone (GDL, 1.2–1.6% wt/wt) was added to slowly acidify the mix over a 4 h incubation at 42°C (final pH of 4.55–4.60). The gel was broken at the appropriate pH and refrigerated overnight. Shear rate sweeps, strain sweeps, and frequency sweeps were done at 8°C and 25°C to evaluate flow and viscoelastic properties. Tribological testing (0.01–600 mm s^{-1} sliding speed, 1 N normal force) was also performed at 25°C. All samples were tested with and without human whole saliva (1:6 ratio of saliva: sample). Two-tailed t-tests and a 2-way ANOVA were performed to assess the temperature, saliva and
hydrocolloids effects. Most samples showed viscoelastic solid behavior and all samples showed shear-thinning behavior. Sample viscosity and viscoelastic moduli decreased with added saliva, increased temperature, or both but changes were not significant for samples with added saliva. Most samples tested with saliva showed lower friction coefficients than samples tested without saliva. Hydrocolloid type and amount highly affected acid milk gel strength and stability. LBG and potato starch made stronger gels than corn starch and cellulose gum. This information on the impact of saliva on acid milk gels with various components will allow targeted formulation of yogurt with desirable textures.

**Key Words:** rheology, tribology, saliva

**T68 Preparation of polymerized whey protein directly from cheese whey and its application as a gelation agent for yogurt making.** T. Fang1, X. Shen1, J. Zheng1, Y. Wang1, and M. Guo*1,2, 1Department of Food Science, College of Food Science and Engineering, Jilin University, Changchun, Jilin, China, 2Department of Nutrition and Food Sciences, College of Agriculture and Life Sciences, University of Vermont, Burlington, VT.

Whey protein is a valuable by-product of cheese making because of its highly nutritional value and some desirable functional properties. The objectives of this study were to prepare liquid whey protein (LWP) concentrate solution using membrane technology from Cheddar cheese whey, to polymerize whey proteins in the solution, and to use the polymerized whey protein (PWP) as a thickening agent for yogurt formulation. Fresh whey collected from cheese vat was pasteurized (60°C for 30 min) and pre-filtrated using screen mesh. The treated whey was subjected to microfiltration (0.1 μm, at 45°C for 1 h). The microfiltrated whey was ultrafiltered using a 10-kDa cut-off membrane to about 20-fold. The UF treated whey was electrodealyzed (ED) to remove 90% of salt. The final protein content of the liquid whey protein (LWP) concentrate was 8.0%. The obtained liquid whey protein concentrate (8% protein) and WPC solution of same protein content were heated at 85°C for 30 min at pH 7.0. The particle size and zeta potential of PWP prepared from LWP was similar to those of PWP prepared from commercial whey protein concentrate (WPC80). Addition of PWP significantly (P < 0.05) increased the viscosity of the yogurt samples, whereas addition of polymerized LWP did not significantly affect the viscosity compared with the control. There were no differences in pH, TS, ash, fat, protein, and lactose contents among the yogurt samples (P > 0.05). This new technology of preparing PWP directly from liquid whey protein concentrate through membrane separation technology can be used as gelation agent for yogurt making. This product (PWP) could be also used as a natural and functional ingredient for formulation of milk based fermented foods.

**Key Words:** whey membrane processing, whey protein polymerization, yogurt

**T69 Chemical, physiochemical and microstructural properties, and probiotic survivability of goat milk kefir using polymerized whey protein as co-thickening agent.** H. Wang1, C. Wang1, M. Wang1, X. Zhou1, and M. Guo*1,2, 1Jilin University, Changchun, Jilin, China, 2University of Vermont, Burlington, VT.

Fermented goat milk products are considered as specialties in the United States. A probiotic goat milk kefir using polymerized whey protein (PWP) as co-thickening agent was developed. The goat milk kefir (GK) with PWP and pectin had desirable syneresis (5.44 ± 0.92%), viscosity (952.86 ± 61.52 mPa·s) and hardness (112.57 ± 3.23 g), which were comparable to cow milk kefir (CK). Sensory evaluation data (5-point hedonic scale) showed that GK with PWP had a higher scores of both flavor (4.41 ± 0.39) and taste (3.72 ± 0.34) than the sample without PWP. The new product contains 13.02 ± 0.02% total solids, 3.50 ± 0.12% protein, 2.90 ± 0.09% fat, 5.97 ± 0.24% carbohydrates, 0.75 ± 0.02% ash, 0.33 ± 0.02 mg 100 g⁻¹ zinc, 11.50 ± 0.54 mg 100 g⁻¹ magnesium, 140.90 ± 12.24 mg 100 g⁻¹ calcium, 42.20 ± 1.23 mg 100 g⁻¹ sodium and 172.40 ± 11.26 mg 100 g⁻¹ potassium. No significant difference in viscosity and hardness was observed between GK and CK during the 8-week study (P > 0.05). L. Acidophilus population in both GK and CK remained above 10⁶ cfu g⁻¹ during the first 4 weeks. Sodium dodecyl sulfate PAGE photographs showed some changes in the protein profile for both GK and CK during storage at 4°C. Scanning electron micrographs displayed a compact and homogeneous protein network of goat milk kefir with PWP and pectin. Polymerized whey protein may be a novel protein-based thickening agent for formulation of goat milk kefir.

**Key Words:** goat milk, kefir, polymerized whey protein

**T70 Oxidative stability of Iranian ghee (butter oil) and soybean oil: A comparative study.** M. Enteshari*1,2, K. Nayebsazdeh1, and S. Martinez-Monteaudo2, 1Faculty of Nutrition and Food Science and Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran, 2Dairy and Food Science Department, South Dakota State University, Brookings, SD.

The oxidative stability of Iranian ghee (butter oil) and soybean oil was studied over one-month storage at 4, 25, 45, and 60°C. Throughout the storage period, the oxidative stability of both samples was evaluated in terms of changes in the acid value (AV), iodine value (IV), peroxide value (PV), p-anisidine value (p-AV), thiobarbituric acid value (TBA), fatty acids profile, and oxidative stability index (OSI). In general, the values of PV, p-AV and TBA gradually increased while the values of OSI decreased. Ghee samples showed significantly (P < 0.05) lower amount of PV, p-AV and TBA and higher OSI than soybean oil samples. However, higher values of AV were observed for ghee samples than that of soybean oil. This observation is in agreement with the amount of free fatty acids (FFA %). Moreover, the obtained data suggest that temperatures within the range of 45–60°C significantly affect the oxidative stability. Results confirmed that ghee displayed higher oxidative stability as evidenced by lower PV, p-AV and TBA with higher values of OSI at accelerated storage conditions.

**Key Words:** oxidative stability, Iranian ghee, soybean oil

**T71 Trans-isomers in cultured butter under the cream fermentation of Flora Danica in combination with Lactobacillus acidophilus La-5 at different temperatures.** O. Tsisaryk*, L. Musiy, and I. Slyka, Lviv National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine.

We investigated microbial count, acidity in cream and fatty acid composition in cultured butter under the cream fermentation of Flora Danica (Lactococcus lactis ssp. cremoris, Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. diacetylactis and Leuconostoc mesenteroides ssp. cremoris) – FD in combination with L. acidophilus La-5 – La-5 (Chr. Hansen commercial starters) at different temperatures. We evaluated flavor and aroma of butter also. Four samples of butter were made: CB1 – FD; fermentation at temperature 20°C; CB2 – FD in combination with La-5 (1:1); fermentation at temperature 20°C; CB3 – FD in combination with La-5; fermentation of creams at temperature 30°C; SB – sweet butter (control). The initial concentration of starter cultures in
creams was 5 log cfu/mL. The experiment was repeated 3 times. Cultured butter was packed in polystyrene cups with the capacity of 200 mL and stored at temperatures 0...-5°C. Titrated acidity, pH, cfu in cream and fatty acids composition in butter were determined. Counts (cfu) of cells FD was determined in M17 Agar CM-0785 (Himedia), cfu of cells La-5 was determined in MRS Agar M 641–500G (Himedia). The fatty acids methyl esters were separated on a column (100 m × 0.25 mm × 0.2 µm [HP-88] 88%-cyanopropyl aryl-polysilixane, Agilent Technologies) in the chromatograph (Hewlett Packard 6890). It was established that creams titrated acidity was the highest and pH was the lowest in CB3 (P < 0.05). The cfu of FD and La-5 was the largest in CB3 (7.2 lg and 7.4 respectively versus 6.8 and 7.0 lg cfu/cm³ in CB2, P < 0.05). The results showed that the content of CLA cis-9, trans-11 was 1.84% in SB and 1.92, 1.87, 1.93% in CB1, CB2, CB3, respectively. The sum of all isomers CLA in the CB1, CB2, CB3 ranged from 2.08 to 2.13% and was 2.06% in SB. The content of trans-9 isomers in a CB3 was 0.24 versus 0.26 in SB. The results demonstrate that the temperature 30°C of cream fermentation provides best conditions for starter growth; however, the temperature of fermentation did not affect a possible trans-11 isomerization. CB3 had clean, with pleasant yogurt flavor and aroma. CB2 and CB3 were characterized by indistinct flavor and aroma. SB was characterized by pasteurized cream flavor.

**Key Words:** cream, La-5, CLA
Ultrafiltration (UF) is a commonly used membrane process in dairy industries, especially for cheese milk concentration. Little attention has been given to other processes such as reverse osmosis (RO) and nanofiltration (NF) for milk concentration and the cheesemaking properties of the concentrates are unknown. The objective of this work was to compare the rennet-induced coagulation kinetics as well as cheesemaking properties of UF, NF, and RO concentrated milks. Batch lots of pasteurized skim milk (SM) were concentrated by means of a pilot-scale filtration system (GEA NIRO) operated at 50°C until a volume concentration factor of 3× using 3 different spiral-wound membranes (Synder Filtration). Cheesemaking kinetics of concentrates was characterized by dynamic rheology and model cheeses were made to further study the cheesemaking properties. All experiments were performed in triplicate. SM concentrated using UF showed similar rennet coagulation time (RCT) and time to reach maximal firming rate (MFR) than SM (P > 0.05). However, RO and NF milks had longer RCT and MFR (P > 0.05). All concentrates presented higher firming rates than SM (P > 0.05). Model cheeses experiments showed that all concentrates had higher moisture adjusted curd yield as well as higher protein retention (P > 0.05). However, RO and NF curds had higher moisture than UF (P > 0.05). Membrane concentration process and its selectivity deeply modify the composition of milk and affect cheesemaking properties of the concentrate. This study has shown that RO and NF milks have impaired cheesemaking properties, probably because of their higher salts content. Further studies are needed to find proper ways to limit the impact of their high salts content to use these concentrates for cheesemaking.

Key Words: cheesemaking, milk concentrate, reverse osmosis
impact of these 2 processes will need to be evaluated through a life-cycle assessment before comparing their efficiency.

**T75  Milk fatty acid composition and long-seasoning cheese-making qualities of milk from dairy cows given algae in pelleted or meal concentrate form.** M. Morlachchini1, F. Giorgio1, C. Moran2, D. Graugnard2 and K. Jaques3, 1CERZOO, Piacenza, Italy, 2Alltech Inc., Nicholasville, KY.

Milk containing higher amounts of unsaturated long-chain fatty acids (LCFA), including docosahexaenoic acid (DHA) can provide an added value stream for producers. However, it is important to understand how milk fatty acid (FA) profiles are affected and the impact of these changes on dairy foods, particularly cheese. This experiment compared milk profiles of cows fed a high-DHA algae added in meal or pelleted concentrate. In addition, cheese-making properties were measured. Italian Friesian mid-lactation cows (36) were blocked by parity and assigned to 3 treatment groups of 12 cows in an 85-d study. Cows were given a TMR that included 0 or 150 g algae, the latter in meal or pelleted concentrate. The algae source was Aurantiocytrium limacinum CCA 40872/2 algae (FORPLUS, Alltech Inc.). Milk samples were taken at d0, 28, 56, and 84 on 2 consecutive milking days, combining 4 milkings into 1 pooled sample made with 5% of the milk production of each milking for component analysis and FA profile. Coagulant properties, titratable acidity, and natural creaming for production of long seasoning cheese were evaluated. Data were subjected to ANOVA with means separated (P < 0.05) using Student t and Tukey tests. The C20:3n-3 acids, total LCFA and saturated FAs were higher in control vs meal (P < 0.05), with pellets intermediate. Oleic, stearic, α-linolenic, and eicosatrienoic acids were lower in diets with algae (P < 0.05). C18:1 trans, rumenic, and behenic acids were lower in controls than in diets containing algae (P < 0.05). DHA and the n-3:n-6 ratio were lowest in control and highest in meal (P < 0.05). DHA was not detected in controls. Milk titratable acidity was numerically reduced over the study when cows received algae in concentrate meal. No statistical differences were found in milk rennet coagulation properties or natural creaming. The evaluated cheesemaking properties were unaffected. It was concluded that algae effects on milk FA profile were more evident in meal than pelleted concentrate and that cheese-making qualities remained in normal ranges.

**Key Words:** algae, docosahexaenoic acid (DHA), cheese

**T76  Multivariate analysis in the study of association between Mozzarella cheese yield and processing factors.** D. C. Sales1, A. H. N. Rangel1, A. R. Freitas1, J. G. B. Galvão Jr.*2, S. A. Urbano1, E. P. E. Silva1, and H. Tonhati3, 1Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil, 2Instituto Federal de Educaçao, Ciencia e Tecnologia do Rio Grande do Norte, Ipanuacu, RN, Brazil, 3Empresa Brasileira de Pesquisa Agropecuaria (Retired), Sao Paulo, SP, Brazil, 4Universidade Estadual Paulista Julio de Mesquita Filho, Jaboticabal, SP, Brazil.

The aim of this study was to investigate the association between Mozzarella cheese yield (MCY) and variables of milk composition, processing and the recovery of whey constituents by multivariate analysis. The study involved tracking the processing of 30 lots of buffalo Mozzarella cheese in a dairy industry of northeast Brazil. The variables milk fat (MF), true protein (TPRO), casein (CAS), lactose, total solids (TS), solids-not-fat content (SNF), density (DS), cryoprocycy, pH, titratable acidity (ACID), and somatic cell score (SCS) of raw milk were measured before processing. The variables pH, acidity, age of starter culture (ASC), volume of calcium chloride, volume of rennet, average pH of curd during stretching, coagulation time, time between cuts, fermentation time (FIT), stretching time for the whole curd, whole fermentation time, fat (FREC), protein, casein, lactose, total solids, and solids-not-fat recovery were measured during the processing and in the whey. MCY association to the variables was verified by PROC PRINCOMP procedure of SAS. The explained variability of PC1, PC2 and PC3 was 26.37%, 17.38% and 12.44% respectively, totaling 56.18%. A direct association between milk characteristics TS, SNF, CAS, TPRO, MF, ACID and FREC was observed, as well as an antagonistic association between them vs. MCY, pH vs. DS, and FIT vs. ASC. This means that low kg milk per kg cheese ratio is lower when using buffalo milk with higher concentrations of TS, SNF, CAS, TPRO and MF, and when there is greater loss of MF in whey. A direct association can be found between those representing the loss of non-fatty constituents in whey and SCS. Thus, the volume of cheese obtained may be lower when milk with higher SCS is used. The main components of Mozzarella indicated that the yield has more relevant associations with pH, DS, ASC, time elapsed between curd cuttings, and curd stretching time, indicating that these elements must be well controlled to achieve optimal efficiency in the manufacturing of this cheese.

**Key Words:** cheese, dairy food, industry

**T77  Tuning meltability and stretchability of pizza cheese using modified starch.** X. Yang*, J. Hirsch, A. Speranza, and S. Ganesh, Ingredion Incorporated, Bridgewater, NJ.

Important functional properties of pizza cheese, such as meltability and stretchability, depend on the structural formation and interaction of casein gel and fat globules. The aim of this study was to understand the effects of modified starch on pizza cheese microstructure, and the resulting cheese functional properties. Pizza cheeses containing modified starches from various plant sources, and 9–22% rennet casein, were prepared. Starches were chosen based on their ability to form a gel, including gel rate, meting, hardness. Cheese texture and meltability were evaluated using Texture Profile Analysis (TPA) test and modified Schreiber melt test. Stretchability was scored using a pizza bake method. Cheese microstructure was observed under light microscopy using 3 dyes (iodine, fast green and Nile red) to specifically stain starch, protein and fat phases. Results show that microstructure and functionality of pizza cheese are changed by the addition of modified starch. Microscope images show that upon heating during pizza cheese process, modified starches completely cook out, and form a separate gel phase in the matrix. The modified starches enable formation of a continuous casein network, which improves stretching texture of melted cheese. Modified starch with reduced gelling rate (slower increase of elastic modulus G’ over time) enabled more phase separation, and greater stretching. Modified starch with more melted structure (greater loss of G’ during heating) contributed to larger cheese spread area in Schreiber test, and more fusing of cheese shreds in pizza bake test. Native starch, however, tended to form small gel pieces, interfering with the casein gel network, which restricted cheese stretchability after baking. This study indicates that modified starches and their blends alter cheese microstructure, leading to improved functionality. Starches, based on their functional properties, such as gelling rate and melting, can be used to improve cheese meltability and stretchability for specific formulations and applications, by enabling creation of a continuous casein gel network.

**Key Words:** cheese microstructure, meltability, stretchability

**T78  Utilization of konjac glucomannan as a fat replacer in low-fat and skimmed Mozzarella cheese.** S. Dai*, H. Corke1,2, and
T80 Physicochemical and texture analysis of camembert cheese variants. D. Batty*, J. Waite-Cusic, and L. Goddik, Oregon State University, Corvallis, OR.

Camembert is a bloomy rind cheese that can be produced by several different processes that involve altering starter culture, fermentation time and temperature, and curd handling to attain multiple varieties including the traditional lactic curd, rennet curd, and extended shelf life stabilized curd. The objective of this research was to compare different varieties of Camembert cheese and measure physicochemical characteristics of the cheeses. Multiple varieties of Camembert cheese were manufactured and analyzed for key compositional components including calcium, fat, protein, moisture, pH, sodium and color. Firmness of the paste was also analyzed using a TA.XT2i Texture Analyzer. The 2 most common varieties are rennet curd and stabilized curd. Rennet curd cheese is made using traditional mesophilic cultures and fermenting at 35°C for 180 min to a set pH of 6.20, while stabilized curd is made using thermophilic cultures fermenting at 40°C for 120 min to a set pH of 6.45. For these cheeses there were differences in both pH during ripening and firmness at the end of the initial ripening (d 14). Due to the lower initial pH, pH of the rennet curd variety (4.81 on d 1 to 7.37 on d 10) increased more than pH of the stabilized curd variety (5.20 on d 1 to 7.26 on d 10). The difference in firmness from the center of the paste (7.053 N) to the edge of the rennet curd curd variety (7.053 N) to the edge of the stabilized curd variety (7.053 N) was significant (P = 0.032), while the firmness from the center of the paste (2.876 N) to the edge of the rennet curd curd variety (2.876 N) to the edge of the stabilized curd variety (2.876 N) was significant (P = 0.281). Comparing the 2 cheese varieties, the difference in firmness of the paste center (P = 0.015) and rim (P = 0.016) were both significant. A characteristic with an insignificant difference (P = 0.126) was moisture (dry matter basis) for the rennet curd (60.4%) and stabilized curd (61.3%). It is interesting to note that although the cheeses were made by different methods, they ended up with same moisture content and final pH while having a significant difference in firmness. These findings allow us to compare the difference in Camembert cheese varieties based on the methods of manufacture.

Key Words: cheese, physicochemistry

T79 Behavior of starches with different amylose content in mixtures with casein for replacing fat in cheese. V. R. Diamantino, M. S. Costa, C. M. L. Franco, and A. L. B. Penna*, São Paulo State University, São José do Rio Preto, SP, Brazil.

The production of reduced-fat foods has been a preoccupation of scientists and industry. Konjac glucomannan (KGM) is a natural polysaccharide with several desirable nutritional characteristics, and has the potential functional properties as a fat replacer in dairy products. In our study, physicochemical, textural, pizza baking properties and structural characteristics of low-fat and skimmed Mozzarella cheese with KGM (LFKGM and SKKGM) were compared with those of full-fat, low-fat and skimmed Mozzarella cheese control (FFC, LFC and SKC) during storage, respectively. FFC and LFC showed long protein channels, while SKC, LFKGM and SKKGM showed densest protein with LKC and SKC. Additionally, FFC and LFC showed long protein properties may considerably vary due to their amylose content. Thus, WMS could be considered a promising fat replacer in cheese and may have the potential to help industries to improve the characteristics of dairy reduced-fat products.

Key Words: Mozzarella cheese, konjac glucomannan, fat replacer.

T81 Compositional and proteolytic study of Danish Blue cheese during ripening. A. Mane*2,1, F. Ciocia2,1, T. K. Beck4, S. Lillevang2, and P. McSweeney2,3. 1Food for Health Ireland, Dublin, Ireland, 2University College Cork, Cork, Ireland, 3Arla Foods, Vojens, Denmark.

Danish Blue cheese is a semi-soft blue veined cheese, made from cow’s milk. In addition to proteolytic enzymes, present during normal
cheese ripening, the mold *Penicillium roqueforti* produces aspartyl and metalloproteases that cause considerable changes leading to the unique aroma, flavors and texture of Blue cheese. A study was carried out to investigate the compositional and proteolytic changes occurring in this cheese during 28 weeks of ripening. Moisture levels generally decreased during ripening with concomitant increases in NaCl (~45 to 42% and 3.0 to 3.5%, respectively). Levels of pH 4.6 - soluble N as a percentage of total N increased from 4.2% to 46%, indicating extensive proteolysis during ripening. Urea-PAGE was performed. Before 23 d of ripening, patterns of proteolysis could be explained through the action of chymosin from the coagulant and plasmin from the milk. The action of enzymes from *P. roqueforti* was apparent in samples ripened for longer periods up to 28 weeks. pH 4.6-Soluble fractions were analyzed by ultra-performance liquid chromatography and showed complex peptide profiles, particularly after 2 weeks of ripening. Extensive proteolysis was associated with the action of the fungal proteolytic enzymes in the cheese. Free amino acid profiling showed an increase in content as ripening proceeded. In an attempt to identify peptides in the cheese produced by mold enzymes, a commercial strain of *P. roqueforti* PRG-3 was cultured in Potato Dextrose Broth for 7 d at 25°C. Cell-free supernatants were obtained from the culture medium and the action of enzymes on \( \alpha_{\mathrm{S1}} \) and \( \beta \)-casein was determined with resulting peptides identified by ultra-performance liquid chromatography and mass spectrometry. Several peptides found in cheese were thus proven to be produced by the action of fungal enzymes. The results of this study show the extensive proteolysis in Blue cheese later in ripening is mediated mainly by the action of *P. roqueforti* enzymes.

**Key Words:** Danish Blue cheese, proteolysis, proteolytic cleavage

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**T82** Withdrawn

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**T83** *Quantification of starch through an enzymatic starch assay to quantify flow aid concentrations in shredded cheeses.* A. Zumbusch and T. Schoenfuss*, University of Minnesota, St. Paul, MN.

Starch is a common ingredient in flow aids used in the production of shredded cheese. It serves as an anticalking agent as well as a carrier for antimycotics and oxygen scavengers to increase shelf-life and quality. There is no current standard method of analysis to confirm the amount of flow aid in shredded cheese. The objective of this research was to develop a total starch assay method to quantify the starch in shredded cheese blends to quantify the total amount of flow aid present. The Megazyme Total Starch HK kit K-TSHK 09/15, based on AOAC method 996.11, AACC method 76.13, and ICC standard method No. 168 was chosen as this kit does not contain glucose oxidase as a reagent. Glucose oxidase is present in many flow aids for shredded cheese to act as an oxygen scavenger. An initial extraction step was added to remove the \( \delta \)-glucose present in the flow aid. There was also an issue after a centrifugation step with breaking up the pellet. Glass beads were added to the test tubes to alleviate this problem. Finally, a gravity fed filtration step with grade 1 filter paper was added to remove interference from the food matrix not removed from the final centrifugation. The method was tested on 6 cheese samples consisting of 3% flow aid (wt/wt) that was hand blended. The flow aid itself was analyzed to determine the percent starch. It was determined that the flow aid contained 61.3% (±4.52%) starch. Analysis of cheese samples produced an average of 1.81% (±0.011%) starch. With analysis of flow aid resulting in 61.3%, the total calculated flow aid in cheese samples was 2.95% (±0.017), resulting in a percent relative error of 1.79%. The development of this method provides a valuable tool for the cheese industry and regulators. This method allows for manufacturers to accurately determine the amount of flow aid added to shredded cheese blends to ensure their manufacturing and regulatory specifications are being met. The total analysis time for this method is approximately 3 h. Ultimately, accurate determination of flow aid addition will improve product quality, safety, and consumer confidence for the shredded cheese industry.

**Key Words:** cheese, shredded, starch
Comparative environmental impact analysis of distilled whey spirit and white whiskey production. D. Risner, A. Shayevitz, L. Goddilk, and P. Hughes, Oregon State University, Corvallis, OR.

Whey disposal can be an environmental and economic challenge for artisanal creameries. The biochemical demand of whey can be reduced via an ethanol producing fermentation. This fermentation creates a 2.5% alcohol by volume (ABV) wash which can be distilled to produce a potable spirit; Distilled Whey Spirit (DWS). The environmental impact of the distillation process for DWS and another novel spirit, white whiskey, was compared. This was done using a process-based life cycle analysis (LCA). The functional unit of 750 mL of 45% ABV spirit was chosen to compare the environmental impact of DWS and white whiskey. The LCA compared the differences in the production processes. To compare these differences a model 2 pot distilled was created. These differences were quantified via mass of CO₂ produced and water inputs and outputs. The differences measured included energy inputs, mass of water used and output, production byproducts, and CO₂ produced during the fermentation. The energy usage was quantified using thermodynamic calculations and converted to kilograms of CO₂e based upon the burning of natural gas. Conversion of waste material to mass of CO₂e was done through the use of the EPA tool, the waste reduction upon the burning of natural gas. Conversion of waste material to mass of CO₂e was done through the use of the EPA tool, the waste reduction model (WARM). This tool was used to quantify the environmental impact of the spent grain in white whiskey production and to quantify the removal of whey from the waste stream. Production of DWS, instead of white whiskey, emitted 8–9 fewer kilograms of CO₂e per 750 mL of 45% ABV spirit. Water input for the DWS production was 0.4 kg per 750 mL of 45% ABV spirit less than the white whiskey production. The difference in output water of DWS production was 3.4 kg greater per 750 mL of 45% ABV spirit than white whiskey production. The difference in water inputs and outputs can be attributed to differences in the initial amount of alcohol present in washes and the inclusion of a mashing step in white whiskey production. The production of DWS instead of white whiskey was found to reduce the CO₂e emissions and water usage of the spirit. Converting whey to DWS is effective in LCA terms as both a valorization of whey and the fact that DWS production performs well relative to typical spirits such as white whiskey.

Key Words: whey, distilled, sustainability

Production of whey protein-maltodextrin conjugates at a pilot plant scale. Y. Lu, Y. Gong, S. Khanal, M. Molitor, and J. Lucey, Center for Dairy Research, University of Wisconsin-Madison, Madison, WI, Department of Food Science, University of Wisconsin-Madison, Madison, WI.

Conjugation of whey proteins with dextran has been previously studied at a bench top level, and these conjugates had greatly improved functionality. We wanted to develop a process to produce whey protein conjugates at a pilot plant scale so that potential applications of these ingredients could be explored. For scale-up process, we switched from dextran to food grade maltodextrins (MD). We evaluated the impact of the different molecular weight (dextrose equivalent, DE) of MD. We studied the ratio of carbohydrate to protein and concentration of MD for conjugation reaction. The novel “wet” conjugation process developed at University of Wisconsin was used for conjugation. A mixture containing 20% total solids with a ratio of MD to protein = 3:1 was selected, and MD with DE values of 4, 10, 15, and 18 were tested. Mixtures were held at 62°C for 24 h to promote conjugation. The average molecular weight of the conjugates was around 22–96 kDa. We observed bacterial growth during the conjugation reaction, and the source of bacteria was identified as the heat stable spore former, Geobacillus stearothermophilus. Bacterial growth caused a significant decrease in pH, which negatively impacted the conjugation reaction. Microfiltration of the reaction mixture eliminated this bacteria from the raw material before conjugation. No further pH drop was observed in conjugation process. Nanofiltration was used to remove small sugars from MD before conjugation. A full scale up pilot plant trial that was completed that produced a spray dried conjugate powder. This powder had a protein content of ~12% and was tested to confirm presence of conjugates. We also confirmed that partially hydrolyzed whey proteins could react with MD to form conjugates. We are exploring options to produce conjugate powders with higher protein levels and enhanced functionality.

Key Words: maltodextrin, conjugation, whey proteins


Acid whey, a byproduct of Greek yogurt, is a significant disposal challenge for the dairy industry. Current acid whey utilization schemes include ethanol production. Since Saccharomyces cerevisiae cannot utilize lactose, the main sugar of acid whey, enzymes or non-traditional yeast strains need to be used. These methods are expensive, and therefore, an alternative approach is needed. A β-galactosidase (β-gal) with activity for lactose has been isolated from barley. Hydrolysis of lactose into glucose and galactose by β-gal would allow for the incorporation of acid whey as a fermentable sugar source in beer production. The objective of this study was to evaluate whether a barley mash at β-gal’s optimum temperature of 40°C, would result in detectable hydrolysis of lactose in acid whey. A mash containing 250 mL of acid whey and 65.9 g of barley meal was shaken constantly at 40°C for 3 h. A control mash consisting of barley meal and water, with no source of lactose added, was used to determine the amount of free glucose in the grain or released from barley amylase activity. Samples were taken at 0 and 180 min and heated to 70°C for 5 min to stop further enzyme activity. Levels of glucose in the samples were analyzed via an enzymatic assay to indicate lactose hydrolysis. Triplicate samples were taken at each time point and the experiment was repeated 3 times. A student’s t-test was conducted to determine significant differences between mean glucose levels in the treatment and the control. At 0 min the control contained 0.03 ± 0.01 g/L glucose and increased to 0.63 ± 0.01 g/L glucose after 180 min. The treatment started at 0.14 ± 0.02 g/L glucose and increased to 4.65 ± 0.17 g/L glucose after 180 min. The level of glucose in the treatment after 180 min was significantly different (P < 0.05) from the control. These results indicate that indigenous enzymes in a barley mash can sufficiently hydrolyze lactose in acid whey. This gives opportunity for utilizing the yogurt byproduct as a raw material in the brewing industry. Further research will look into process development for optimal enzyme activity.

Key Words: acid whey, β-galactosidase, brewing


We studied the ratio of carbohydrate to protein and concentration of MD for conjugation reaction. The novel “wet” conjugation process developed at University of Wisconsin was used for conjugation. A mixture containing 20% total solids with a ratio of MD to protein = 3:1 was selected, and MD with DE values of 4, 10, 15, and 18 were tested. Mixtures were held at 62°C for 24 h to promote conjugation. The average molecular weight of the conjugates was around 22–96 kDa. We observed bacterial growth during the conjugation reaction, and the source of bacteria was identified as the heat stable spore former, Geobacillus stearothermophilus. Bacterial growth caused a significant decrease in pH, which negatively impacted the conjugation reaction. Microfiltration of the reaction mixture eliminated this bacteria from the raw material before conjugation. No further pH drop was observed in conjugation process. Nanofiltration was used to remove small sugars from MD before conjugation. A full scale up pilot plant trial that was completed that produced a spray dried conjugate powder. This powder had a protein content of ~12% and was tested to confirm presence of conjugates. We also confirmed that partially hydrolyzed whey proteins could react with MD to form conjugates. We are exploring options to produce conjugate powders with higher protein levels and enhanced functionality.

Key Words: maltodextrin, conjugation, whey proteins
The actions and supervision of the Brazilian government to enforce the regulations to adapt to the new requirements, should raise the quality standards of the entire dairy chain. The objective of this study was to analyze the microbiota and natural incidence of aflatoxin M1 (AFM1) in milk based dietary supplements. For the analysis were used the standards described by the normative instruction n. 62 by Brazilian Ministry of Agriculture, Livestock and Supply (MAPA); the normative instruction no. 7 by National Health Surveillance Agency (ANVISA); Standard Methods of the Examination of Water and Wastewater (APHA); the Merck manual and the modified method ISO/TS 22964; Pitt; Hocking in Fungi and food; mycotoxins handbooks of FAO/WHO Expert Committee on Food Additives (JECFA). The analyzes were carried out in the laboratories of the State Center for Food Research at PESAGRO-RJ.

Ten brands were collected from 8 different lots. Classification with a bimetallic difference at different market stablesmen’s, with intention to make an average per brands (total of 80 samples). It was observed the following minimum and maximum variations in the counts for the proposed incubation schemes: DRBC (2.00 to 4.86); DG18 (1.88 to 4.40); YPD (2.00 to 4.90); DCPA (2.0 to 3.90). Trials of the natural incidence of mycotoxins demonstrated detectable levels of AFM1 (0.023 - 0.050 ug kg⁻¹) in the samples evaluated (Table 1). All brands of milks supplements analyzed by AFM1 incidence agree Brazilian legislation standards, but some trials exceed international legislation. The fungi count exceeded the stipulated by APHA and FDA. Such contamination confers potential risk to consumers.

**Table 1 (abstract T87).** Fungi count (log₁₀ cfu g⁻¹) in the Dichloran Rose Bengal Chloramphenicol agar (DRBC) and aflatoxin M₁ concentration (µg kg⁻¹), in milk food supplement samples

<table>
<thead>
<tr>
<th>Brand</th>
<th>DRBC</th>
<th>AFM₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>4.06⁺</td>
<td>0.0415⁵</td>
</tr>
<tr>
<td>Y</td>
<td>3.41⁻</td>
<td>0.0365⁵</td>
</tr>
<tr>
<td>Z</td>
<td>3.30⁻</td>
<td>0.0345⁵</td>
</tr>
<tr>
<td>W</td>
<td>3.29⁻</td>
<td>0.0353⁵</td>
</tr>
<tr>
<td>A</td>
<td>3.06⁻</td>
<td>0.0425⁵</td>
</tr>
<tr>
<td>B</td>
<td>4.41⁺</td>
<td>0.0505⁵</td>
</tr>
<tr>
<td>C</td>
<td>4.30⁻</td>
<td>0.0245⁵</td>
</tr>
<tr>
<td>D</td>
<td>3.69⁻</td>
<td>0.0233⁵</td>
</tr>
<tr>
<td>E</td>
<td>3.16⁻</td>
<td>0.0345⁵</td>
</tr>
<tr>
<td>F</td>
<td>3.41⁻</td>
<td>0.0365⁵</td>
</tr>
<tr>
<td>Mean</td>
<td>3.60</td>
<td>0.3596</td>
</tr>
</tbody>
</table>

ᵃᵇ⁻Means with the same letter in column are equivalent in accordance with Duncan test (P ≤ 0.005).

**Key Words:** Aspergillus flavus, mycotoxin production, aflatoxin M₁


Significant volumes of skim milk are concentrated in the Dairy Industry, primarily as an intermediate step in the production of milk powder. When concentration is conducted by thermal evaporation, detrimental changes to product quality occur. Additionally, mesophilic and thermophilic spores can develop and form biofilms within milk evaporators. These spores are extremely difficult to remove and ultimately affect the quality and shelf life of products made from the concentrated milk. In this work, the process of concentrating milk using forward osmosis (FO) was evaluated for its ability to concentrate skim milk at refrigerated to sub ambient temperatures and maintain product quality unchanged. Pasteurized skim milk (Cornell Dairy, Ithaca, NY) was concentrated at 4°C and 15°C using a pilot-scale FO unit (Ederna, France), equipped with a polymeric membrane. Batches of 8L of skim milk were concentrated in triplicate, and the physico-chemical properties of the concentrates were evaluated. The water flux for the FO process decreased exponentially with time, while sample concentration increased exponentially. At 4°C, flux decreased from 3.02 ± 1.32 L/(m²h) at 5min (initial sample °Brix: 9.83 ± 0.15°) to 0.96 ± 0.21 L/(m²h) after 7h (sample °Brix: 28.50 ± 0.78°). The flux was higher for the 15°C runs, ranging from 3.13 ± 0.57 L/(m²h) at 5min (initial sample °Brix: 9.83 ± 0.15°) to 0.87 ± 0.18 L/(m²h) at 7h (sample °Brix: 33.17 ± 2.39°). Because of the lower viscosity at 15°C, a higher concentration factor was achieved at this temperature (4.17 ± 0.65) as compared with 4°C (3.37 ± 0.43). FO concentrates were diluted to their original total solids (TS) content with deionized (DI) water and subjected to color measurements, in triplicate, using a CR-400 chromameter (Konica Minolta, Japan). Luminosity (L*) values of concentrated and re-diluted FO concentrates were not significantly different (P>0.05) compared with the original skim milk. These results demonstrate that FO can achieve a high concentration factor for skim milk, with no impact on the product color or its chemical components. The process requires further optimization to maximize concentration rate, but the data obtained so far suggests that FO can be a very attractive alternative to thermal concentration of milk.

**Key Words:** forward osmosis, skim milk concentrate

**T89 Withdrawn**

**T90 Edible electrospun nanofibers from caseinate and pullulan blends.** S. Akkurt*¹,², K. L. Yam¹, L. Liu², R. Kwoczak², and P. M. Tomasula², ¹Food Science Department, Rutgers University, New Brunswick, NJ, ²Dairy & Functional Foods Research Unit Department of Agriculture, Agricultural Research Unit Service, Eastern Regional Research Center, Wyndmoor, PA.

Electrospinning is a technique that applies an external voltage to a polymer solution to produce micro- or nano-scale fibers. This technique has been used to electrospin synthetic polymers from organic solvents and more recently to create edible fibers from aqueous calcium (CaCAS) or sodium caseinate (NaCAS) solutions. Previous studies showed that electrospinning of pure CaCAS or NaCAS from aqueous solutions was not possible. To overcome this challenge, pullulan (PUL), which creates homogeneous nanofibers, was used as a spinning aid. The objective of this study was to examine the effect of PUL addition on the entanglement of PUL and CAS molecular chains, compared with the pure CAS and PUL solutions, and on the morphology and size of the resultant electrospin nanofibers. Stock solutions of 15 wt% CaCAS, NaCAS, and PUL solutions, and on the morphology and size of the resultant electrospin nanofibers. Stock solutions of 15 wt% CaCAS, NaCAS, and PUL (controls) were prepared separately, and stirred for 2h at 20°C. Blends of the CAS and PUL solutions were prepared in a 1:1 weight ratio at various concentrations. 3mL of each solution was then loaded into a syringe to feed a nanofiber electrospinning unit at flow rate of 1mL/h, and at 11 or 20kV, respectively. Each run was observed for fiber deposition on the rotating drum. Electrospaying was observed for pure PUL, CaCAS or NaCAS solutions at concentrations below 6.0, 9.0, or 7.0 wt% because the low solution viscosities did not promote molecular entanglement. Fibers were observed for CaCAS: and NaCAS:PUL above 9.0 and 9.5wt% showing entanglement with the added PUL. Fiber sizes were determined using ImageJ software to sample the fibers and calculate mean diameters from scanning electron microscopy images. More
uniform fibers were observed at 11kV than at 20kV for the PUL and NaCAS:PUL solutions, but CaCAS:PUL fibers were of similar sizes at both kV possibly because of reported Ca\(^2\)\(^+\) ion interactions with the CAS. For the 15 wt% solutions, fibers with diameters of 298 ± 25nm, 255 ± 22nm, and 170 ± 34nm were obtained, respectively. This study showed that it is possible to create edible CAS:PUL fibers with potential use for protecting food, improving food quality, and preserving bioactive agents.

Key Words: fibers, nanoscale preservation

T91 Delactosed milk powder: Determination of the optimal drying parameters. T. L. Fialho\(^1\), E. Martins\(^1\), A. C. P. Silveira\(^2\), C. R. J. Silva\(^1\), I. T. Perrone\(^1\), P. Schuck\(^3\), and A. F. Carvalho\(^*\)\(^1\), \(^1\)Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, \(^2\)GEA, Campinas, São Paulo, Brazil, \(^3\)Institut National de la Recherche Agronomique, Rennes, Bretagne, France.

Delactosed milk powders (DMP) are produced from enzymatic lactose hydrolysis and, due to presence of galactose and glucose in their formulation, these powders have higher tendency of stickiness, caking and browning during the drying process. For this reason, the production of delactosed powders is yet a challenge for the dairy industry. This work aimed to evaluated the effect of operational drying parameters (\(\theta_{\text{air,in}} = \) inlet air temperature and \(M_{\text{CM}} = \) concentrated milk flow rate) on the physicochemical and technofunctional properties of DMP. Furthermore, the expenditure of energy during the drying process was evaluated from mass and energy balances. DMP was produced by both variations of \(\theta_{\text{air,in}}\) (from 115 to 160°C) and \(M_{\text{CM}}\) (from 0.3 to 1.5 kg h\(^{-1}\)) in a pilot single stage spray dryer. Powder produced at lower temperatures \(\theta_{\text{air,in}} < 145°C\) and higher milk flow rates \(M_{\text{CM}} > 1.3 \text{ kg h}^{-1}\) presented elevated mass loss (~30%). Under these conditions, water was not efficiently removed from the product resulting in powders with high humidity (~11% w·w\(^{-1}\)), \(a_w > 0.2\) and strong agglomeration to equipment. The combination of higher temperatures \(\theta_{\text{air,in}} > 130°C\) and lower milk flow rates \(M_{\text{CM}} = 0.3 \text{ kg h}^{-1}\) resulted in powder with high temperature favoring the Maillard reaction in which were confirmed by presence of products as 5-hydroxymetylfurfural and brown color. In general, by working with \(M_{\text{CM}}\) values between 0.5 and 1.0 kg h\(^{-1}\) for any tested temperature, it was possible produce DMP with color, rehydration, humidity, \(a_w\) and particle morphology closer to milk powder containing lactose (control). Within this group, best results were observed in the humidity, \(a_w\) and particle morphology closer to milk powder containing temperature, it was possible produce DMP with color, rehydration, hydrolysis and, due to presence of galactose and glucose in their formulation, these powders have higher tendency of stickiness, caking and browning during the drying process.

Key Words: delactosed milk powder, mass and energetic balances, caking

T92 The physical and chemical effect of thermal processing on high- and low-heat nonfat dry milk set yogurt. S. Brooks*, Kansas State University, Manhattan, KS.

The physical and microstructural properties of yogurt are often a function of the whey protein denaturation that occurs during mix processing. Nonfat dry milk (NDM) is manufactured to have high amounts of denatured whey proteins (high-heat) or low amounts of denatured whey proteins (low-heat). When used in yogurt, these denatured whey proteins influence the texture due to increased formation of large, denatured whey aggregates that leads to decreased casein micelle saturation. Thus, this study was undertaken to determine if a subsequent thermal process step in yogurt mixes made of high-heat (HH) NDM or low-heat (LH) NDM could improve physical properties or chemical characteristics in set-style yogurts. Yogurt mixes were formulated to 3.5% protein from either HH- or LH-NDM. Half of each mix received an additional thermal process (85°C for 30 min) before cooling, inoculation incubation (until pH of 4.6), storage at 4°C overnight, and followed by yogurt assessment. Data were analyzed with SAS \(^a\) statistical software as a 2-way ANOVA, followed by a Tukey’s pair-wise (99% confidence level) at \(p \leq 0.05\). Statistically, syneresis was significantly lower in low-heat processed (LHP) yogurt and low-heat non-processed (LHPN) yogurt than high-heat processed (HHP) yogurt and high-heat non-processed (HHPN) yogurt. Cohesiveness and firmness were significantly greater in LHP yogurts followed by HHNP, HHP, and then LHPN yogurts. Additional thermal processing in yogurt reveals an association between improved yogurt texture and initial denatured whey protein in yogurt mixes. The thermal processing on whey protein-casein micelle interactions during a subsequent heat treatment of LH-NDM in a yogurt mix may result in better coalescence of casein micelles leading to cross-linking in a gel rather than a possible spatial interference occurring from yogurt mixes made with HH-NDM.

Key Words: yogurt, nonfat dry milk, thermal processing

T93 Preliminary studies on heat stability of high protein dairy beverages containing modified milk protein concentrate. K. Pandalaneni\(^*\)\(^1\), J. Amamcharla\(^1\), C. Marella\(^2\), and L. Metzger\(^2\), \(^1\)Kansas State University, Manhattan, Kansas, \(^2\)Midwest Dairy Foods Research Center, Brookings, South Dakota.

Milk protein concentrates (MPC) are becoming a preferred source of protein in ready-to-drink dairy beverages. Calcium-mediated aggregation of proteins during storage is one of the main reasons for the failure of these beverages. In the current study, 2 batches of each MPC85 (control), 20%-calcium reduced (MPC-20%), and 30%-calcium reduced (MPC-30%) were evaluated in 2 phases and in duplicate. In both the phases, 6 MPC powders were reconstituted to 8% protein solutions, added with 0, 0.15, and 0.25% concentrations of sodium hexametaphosphate (SHMP), and analyzed for heat stability by measuring heat coagulation time (HCT) at 140°C. In phase I, MPCs were reconstituted in distilled water and pH was adjusted to 7 before 3 concentrations of SHMP were added. MPC-30% and MPC-20% exhibited the highest HCT of ~32 min at all levels of SHMP addition while MPC85-Control has the least HCT time of ~21–25 min at 0 and 0.15% SHMP. HCT of control (28.06 min) at 0.25% SHMP and HCT of MPC-30% (32.79 min) and MPC-20% (30.96 min) at 0% SHMP were not significantly different (\(P > 0.05\)). In phase II, MPCs were reconstituted in a model dairy beverage formulation consisting, 10.26% of a mixture of gums (gellan gum, carrageenan, cellulose gel, and microcrystalline cellulose), maltodextrin, and sugar along with, 0.12% potassium citrate. Formulations were homogenized and treated with 3 concentrations of SHMP after adjusting pH to 7. It was found that the presence gums and sugar adversely affected the HCT of formulated model beverage. Control at 0% SHMP and MPC-20% at 0% SHMP exhibited the highest HCT of 8.86 and 8.37 min, respectively and the HCT is not statistically different (\(P > 0.05\)). This study shows the possibility of reduced levels of phosphate addition by using calcium reduced MPCs.

Key Words: calcium-reduced MPC, sodium hexametaphosphate, high protein beverage
T94 Development of the method for the determination of the undenatured whey proteins in milk powder products. Z. Zhao*1, Z. Gaygadzhiev2, and M. Corredig1,2, 1University of Guelph, Guelph, ON, Canada, 2Gay Lea Foods, Guelph, ON, Canada.

The whey protein nitrogen index (WPNI) is an established method for grading skim milk powder (SMP) products depending on their heating history. This method is based on the principle of salting out of denatured soluble protein (whey proteins) and then an acid-induced aggregation of the remaining native protein, which causes an increase in turbidity. The WPNI index is derived from a standard curve. The objective of this research was to evaluate if WPNI number is also applicable to milk protein concentrates (MPC), as in these systems, the type of soluble proteins and their aggregation state may be different than in skim milk powders, after reconstitution. WPNI numbers were derived, and the composition of the serum phase as well as the level of denaturation for various milk concentrates and isolates were measured using nitrogen analysis, as well as electrophoresis and cation exchange chromatography. To test the method, milk powder products were reconstituted to a final protein content of 3.2% and skim milk was used as standard. The results show that WPNI numbers obtained from the standard method were higher than the cation exchange chromatography. The denaturation of whey proteins, especially the β-lactoglobulin, was inhibited in MPC compared with SMP. In MPC 70, the WPNI number obtained from cation exchange chromatography was 5.24 ± 0.12 mg/g, while the WPNI number for low heat SMP was only 3.98 ± 0.11 mg/g. Therefore, the WPNI method is not an appropriate method to determine the undenatured whey proteins for MPC as their turbidity values are out of the range of the standard curve. Alternatively, the method of cation exchange chromatography exhibits great accuracy and reproducibility and can be used for determining the undenatured whey proteins in both liquid and powder milk products.

Key Words: whey protein, skim milk powder, cation-exchange chromatography

T95 Effect of sonication on viscosity of reconstituted SMP and MPC as influenced by solids content. V. Deshpande* and M. Walsh, Utah State University, Logan, UT.

Skim milk powder (SMP) and milk protein concentrate (MPC) are evaporated before spray drying. It would be an economical advantage to obtain a solution of higher % total solids (TS) before spray drying. This is problematic because it leads to an increase in the viscosity. Ultrasound or sonication has been shown to decrease the viscosity of solutions, therefore, this research studied the effects of sonication on the viscosity of reconstituted MPC (rMPC) and SMP (rSMP) as influenced by %TS at 60°C in a continuous operation. MPC and SMP were reconstituted to 30–34% TS and 46–54% TS, respectively and circulated in a continuous operation at a flow rate of 1.8 L/min for a total of 60 min and 15 min respectively before being sonicated (Hielscher UIP500 sonicator with flow cell). Samples were sonicated (70% amplitude) for a total of 6 min (samples collected after every 2 min). The viscosity was measured at 60°C using a viscometer. Statistical analysis was performed on triplicates using t-tests (α = 0.05). Overall, there was an increase in viscosity with an increase in solids content and a decrease in viscosity upon sonication for both rSMP and rMPC. For rSMP, as compared with presonication, the decrease in viscosity after 2, 4, and 6 min of sonication was 25.3, 29.8, and 33.0% (for 46% TS); 16.0, 37.9, and 42.0% (for 50% TS); 5.7, 9.6, and 13.3% (for 52% TS); 12.0, 16.2, and 22.6% (for 54% TS), respectively. For rMPC, as compared with presonication, the decrease in viscosity after 2, 4, and 6 min of sonication was 30.6, 36.6, and 46.8% (for 30% TS), 19.5, 30.3, and 36.0% (for 32% TS), 24.4, 19.2, and 25.0% (for 34% TS), respectively. Sonication significantly decreased the viscosity of rMPC and rSMP at 2, 4, and 6 min as compared with presonication. For rMPC, the mean viscosity of the 34% TS sample after 6 min of sonication was lower than the mean viscosity of 30% TS sample before sonication. Thus, allowing for an increase in TS by 4% to be spray dried without increasing the viscosity of the solution. For rSMP, sonication did not allow for an increase in %TS without increasing the viscosity of the sample, which can be attributed to the age thickening of the samples.

Key Words: milk protein concentrate, spray drying, skim milk powder

T96 Determination of the appropriate emulsion formulation for microencapsulated milk fat powder production. A. B. Himmetaagolu1, Z. Erbay*2, and M. Cam1, 1Department of Gastronomy and Culinary Arts, Faculty of Tourism, Alanya Hammadhullah Emin Pasa University, Antalya, Turkey, 2Department of Food Engineering, Faculty of Engineering and Natural Sciences, Adana Science and Technology University, Adana, Turkey, 3Department of Food Engineering, Faculty of Engineering, Erciyes University, Kayseri, Turkey.

Microencapsulation technology provides a great protection for perishable food materials, which degrade in the presence of heat, moisture and light, and it’s highly preferable to minimize handling, transportation, and storage costs. Emulsion properties (stability and viscosity) directly affect the microencapsulation process and thus stability of microencapsulated product. In the spray-dried encapsulation process, it’s important to obtain a low-viscosity feed emulsion to achieve a successful microencapsulation. Combinations of carbohydrates and proteins are primary choice as wall materials since they provide low viscosity and improved solubility. In this study, 5 different carbohydrates: 6-DE maltodextrin (LM), 18-DE maltodextrin (HM), lactose (L), sucrose (S), oxidized starch (OS), and 2 different proteins: sodium caseinate, fat-free whey protein concentrate powder (W) used in 5 different proportion (ratio of protein/wall material in between 10 and 50%) and 50 types of emulsions were prepared. Oil-in-water emulsions with 25% solid and 30% wall material content were prepared from cream with 72.5% milk fat content. To evaluate emulsion stability, creaming index and viscosity analyses were conducted. The viscosity of the emulsion at 35°C and 45°C was measured by Brookfield DV-II+ Pro Viscometer (Brookfield Engineering). To calculate creaming index, emulsions were placed in test tubes and stored at room temperature for 24 h. Separation of cream and serum phases was observed after 24 h storage. The results of creaming index analyses showed that the most stable emulsion wall materials were HM+C (10%, 20%), LM+C (10%), HM+W (30%, 40%, 50%), L+W (30%, 40%, 50%), LM+W (10%, 20%, 30%, 40%, 50%). As for the viscosity analyses, viscosity of the emulsions was lower when W was used as the protein source in the wall material. Lower viscosity values were obtained when carbohydrate wall materials based on L, S and HM were used. The best formulation was determined to be L+W (30%). This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) [project no: 2150948].

Key Words: microencapsulation, emulsion stability, milk fat
T97 Iodine-127 levels in bulk milk on Ontario dairy farms and its association with groundwater, milking management, and other risk factors. C. M. Rogerson,1 D. F. Kelton,1 V. R. Osborne,1 J. Levison,1 and S. M. Hamilton.1
1Department of Population Medicine, University of Guelph, Guelph, ON, Canada, 2Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, 3School of Engineering, University of Guelph, Guelph, ON, Canada, 4Earth Resources and Geoscience Mapping Section, Ontario Geological Survey, Sudbury, ON, Canada.

Several studies have investigated the associations between iodine content in milk and milking management practices and nutrition. Many reports have suggested that an increased milk iodine level is attributed to the use of iodine-based teat disinfectants and by the supplementation of iodine in rations. Little emphasis has been directed to investigating bulk milk iodine (BMI) levels in relation to the consumption of groundwater, which is known to contain varying levels of iodine naturally. The objectives of this study were (1) to determine the BMI content in milk sampled from 80 commercial dairy farms located in eastern (n = 58) and southwestern (n = 22) Ontario, and (2) to identify if the groundwater consumed by the lactating herd along with other factors are associated with higher BMI levels. The 80 participants completed a bilingual questionnaire that covered water consumption, nutrition, milking management practices, and well characteristics. The total iodine concentration (organic and inorganic) in milk and groundwater samples was established using inductively coupled plasma mass spectrometry. Independent variables of interest were screened and a linear regression model was fitted to assess multivariable associations between BMI levels and explanatory variables such as total iodine in groundwater samples, depth and age of well, water treatment, the use of an iodine-based disinfectants, and post-dip coverage goal. Results of the analysis suggest a strong correlation exists between the iodine content of groundwater in relation to BMI levels (P < 0.001). Post-milking practices including the use of an iodine-based teat disinfectant and overall coverage goal of the solution on teats were also significantly (P < 0.05) associated with increased BMI levels. These results suggest a significant association exists between BMI levels in relation to post-milking management practices and the iodine content in groundwater consumed by the lactating herd. Groundwater containing high levels of iodine that is used as a source of drinking water for dairy cattle should be treated to remove iodine and thereby prevent high BMI levels on farm.

Key Words: milk, iodine, dairy

T98 Mycoflora and occurrence of fumonisins in complete mixed rations from dairy farms in São Paulo, Brazil. J. E. P. Braga,1 A. Bosso,1 A. F. Rosa,1 R. Braghini,2 and C. R. Pozzi.1
1Instituto de Zootecnia, Nova Odessa, São Paulo, Brazil, 2Instituto de Ciências Biomédicas, São Paulo, São Paulo, Brazil.

Fumonisins are a group of mycotoxins mainly produced by Fusarium verticillioides and occur predominantly in maize and maize-based feeds. The contamination of feedstuffs with these mycotoxins poses a serious health concern to animals as well as human beings. The present study aimed to verify the mycoflora, water activity (aw) and presence of fumonisin B1 (FB1) and fumonisin B2 (FB2) in complete mixed rations samples (roughage + grain mixture, vol/vol) intended to lactating cows in 9 dairy farms in the state of São Paulo. The roughage was constituted of corn silage. The grain mixture was constituted by corn grains, soybean bran and cotton seed and mineral mixture. Samples of the diets were taken directly from the troughs batch of 15 cows, on 2 consecutive days at intervals of 24 h and every 15 d with a period of 45 sampling days per farm. It was extracted incremental samples in 25 spots to sum up a compound sample of 5 kg, which was homogenized and subdivided in 4 subsamples of 1.25 kg each. The serial dilution technique and pour plate were used to isolate the fungi. Samples were analyzed to verify the presence of FB1 and FB2 using an immunoaffinity column to clean up and HPLC with fluorescence detection. The mycoflora analysis of 288 samples revealed the occurrence of the genera Aspergillus (20.09%), Fusarium (14.16%) and Penicillium (11.48%). Fusarium verticillioides (0.97%) and Fusarium proliferatum (0.30%) were the most isolated species of feed samples. Water activity ranged from 0.91 to 0.93. With regard to the number of colony forming units (cfu), isolates of Fusarium species ranged from 1.30 × 105 to 1.37 × 106. FB1 (9.02%) was detected in 26 diet samples (n = 288) with concentrations ranging from 4.05 to 356.72 µg/kg with mean contamination of 11.0 ± 13.06 µg/kg. FB2 (28.12%) was detected in 88 diet samples (n = 288) with contaminations ranging from 7.90 µg/kg to 1,635.97 µg/kg with mean contamination of the 26.23 ± 46.08 µg/kg. Four farms presented the highest number of samples contaminated by FB2 (n = 74) when compared with FB1. The potential contamination of FB2 in corn samples requires further research.

Key Words: mycotoxin, dairy cattle, animal feed

T99 Reduction of Listeria monocytogenes in Queso Fresco by combination of phage endolysin PlyP100 and nisin. L. A. Ibarra-Sanchez,1 M. Van Tassell, and M. Miller, University of Illinois at Urbana-Champaign, Champaign, IL.

Fresh Hispanic-style cheeses (FHSC), such as Queso Fresco (QF), have been implicated in several outbreaks linked to Listeria monocytogenes, and effective biocontrol measures are needed to improve FHSC safety. The objectives of this study were to investigate the potential synergy between endolysin PlyP100 and nisin against L. monocytogenes in QF, and to examine pathogen resistance development after exposure to PlyP100 and nisin in QF. His-tagged PlyP100 was overexpressed in Escherichia coli and subsequently purified. PlyP100 and nisin were added to miniature QF at the following concentrations: 2.5 or 10 U/g PlyP100 with or without 250 µg/g nisin. Antilisterial activity of antimicrobial combinations were tested by inoculating cheese curds with approximately 4 Log cfu/g of L. monocytogenes cocktail, and survival of the pathogen was measured across 28 d of storage at 4°C. All experiments were repeated 3 times with samples prepared in duplicate. By the end of QF storage, 3 random L. monocytogenes isolates per cheese treatment, per independent experiment, were cultured and their sensitivity to PlyP100 and nisin was tested. PlyP100 reduced viable counts of L. monocytogenes in QF by up to approximately 1 Log cfu/g, and no regrowth was observed during 28 d storage. Nisin alone was ineffective to control the pathogen in QF, leading to subsequent regrowth. All treatments combining nisin and PlyP100 in QF achieved reduction of L. monocytogenes below the detection limit of plating. Additionally, in half of the QF samples with nisin + PlyP100, the pathogen was not recovered after enrichment. No difference in sensitivity to nisin or PlyP100 was observed in 36 random L. monocytogenes isolates from QF samples, regardless of whether antimicrobials were added to QF. In conclusion,
our results support the use of phage endolysin combined with nisin as a more efficient *Listeria* control measure in QF.

**Key Words:** Queso Fresco, *Listeria monocytogenes*, antimicrobial

**T100  Survival and growth of *Listeria monocytogenes* in a model cheese based on pH, moisture, and acid type.** S. K. Engstrom* and K. A. Glass, University of Wisconsin-Madison, Madison, WI.

High-moisture, low-acid cheeses, e.g., soft, Hispanic-style cheese, have been shown to support growth of *Listeria monocytogenes* during refrigerated storage. Previous studies have suggested that acetic acid has greater antilisterial activity than lactic acid, and that cheeses of lower pH values (e.g., 5.2) delay growth longer than cheeses of higher pH values (e.g., 5.8); however, no standard pH value for *Listeria* control has been identified. The objective of this research was to determine the effect of pH, acid type, and moisture on the growth of *L. monocytogenes* in a model cheese system stored at 4°C for up to 8 weeks. Cream, micellar casein, water, salt, lactose, and acid were combined in 16 formulations targeting 4 pH values (5.25, 5.50, 5.75, or 6.00), 2 moisture levels (50 or 56%) and using 2 acid types (lactic or acetic acid). Each formulation was inoculated with 3-log cfu/g *L. monocytogenes* (5-strain mixture). 25-g samples were vacuum-sealed and stored at 4°C for up to 8 weeks with triplicate samples enumerated on Modified Oxford agar weekly for *L. monocytogenes*. All formulations were tested in duplicate trials. Model cheeses formulated with lactic acid supported *L. monocytogenes* growth at pH ≥ 5.50 for both 50 and 56% moisture levels. Decreasing moisture from 56 to 50% in pH 5.50 model cheeses formulated with lactic acid delayed *L. monocytogenes* growth approximately 2 weeks, while the same decrease in moisture at pH values of 5.75 and 6.00 did not affect growth. Model cheeses formulated with lactic acid at pH 5.25 did not support *L. monocytogenes* growth at either moisture level. In contrast, acetic acid delayed growth compared with lactic acid at all pH values. For example, only pH 6.00 acetic acid treatments supported growth in 2 weeks at 4°C, whereas all cheeses with pH adjusted to ≥5.75 with lactic acid supported >2 log increase at the same sampling interval. These data confirm that acetic acid has greater inhibitory properties than lactic acid in high-moisture cheeses, and that modifying pH and/or moisture level will significantly influence *L. monocytogenes* growth.

**Key Words:** *Listeria monocytogenes*, cheese, model cheese
T101  Establishment and production of ryegrass and clover in two Colombian highland regions. J. Vargas, A. M. Sierra, Y. Avellaneda, O. L. Mayorga, and C. Ariza-Nieto*. CORPOICA, Bogota, Colombia.

In Colombia, specialized dairy systems are supported by forages use. However, edaphic and weather features are related to production and compositional quality of grasses. In this sense, it is important to recognize resilient fodder species to pastoral systems. The objective was to evaluate the establishment (covert proportion, %, Cp) and adaptation grade (0 to 3 scale, Ag) and production (dry matter yield (kg DM/ha−1, Dm) and net energy lactation (Mcal.kg DM−1, Nl)) in 5 perennial ryegrasses (3 diploid and 2 tetraploid) and 3 clovers (2 reds and one white) in 2 regions of Colombian highlands (Tuta and Mosquera at 2600 m above sea level). In each locality, it was established 8 m² plots of each species, which had 3 replicates. The establishment variables were evaluated twice a month for 5 mo. The production variables were evaluated during dry and rainy seasons. Ryegrasses and clovers vereties present similar establishment and of production responses (P > 0.05), Cp, Ag, Dm and Nl were 36.9 and 49.5, 1.5 and 1.9, 1699.1 and 1472.5, 1.4 and 1.62, respectively. The day of measure, but not the season, influenced the variables evaluated during production period (P < 0.01). In both ryegrasses and clovers, Ni was reduced by 0.34 and 0.22% as the regrowth day increased. There was a positive relationship (P < 0.01) between height, regrowth day, and dry matter yield (Table 1). In conclusion, evaluation day was more influential than specie and season on the establishment and production variables of ryegrasses and clovers. It is important to recognize grasses with a greater resilience to external factors to promote more sustainable dairy systems in Colombian highlands.

Table 1 (abstract T101). Relation between regrowth day, height, and dry matter yield of ryegrass and clover in two Colombian highland regions

<table>
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<th>Item</th>
<th>Mosquera Mean</th>
<th>Mosquera R²</th>
<th>Mosquera P-value</th>
<th>Tuta Mean</th>
<th>Tuta R²</th>
<th>Tuta P-value</th>
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<tr>
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</tr>
<tr>
<td>Height</td>
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<td>65.43</td>
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<tr>
<td>Regrowth day</td>
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<td>&lt;0.0001</td>
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<td>0.81</td>
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Key Words: grasses, legumes, pastoral system

T103  Effectiveness of a chemical additive on improving the aerobic stability of high-moisture corn submitted to aerobic spoilage at room and warm temperatures. E. Benjamim da Silva*1,2, R. M. Savage1, S. A. Polukis1, M. L. Smith1, A. M. Gray1, K. M. Pacer1, and L. Kung Jr.1, 1University of Delaware, Newark, DE, 2CAPES Foundation, Brasilia, DF, Brazil.

The objective of this experiment was to determine the effectiveness of Safesil (SF; 20% sodium benzoate, 10% potassium sorbate, and 5% sodium nitrite) from Salinity, Sweden, on improving the aerobic stability of high-moisture corn (HMC). HMC (32% moisture) was untreated (CTRL) or treated with SF (3 L/t). Twenty replicated silos (7.5 L) per treatment were packed at a density of 658 kg of DM/m³. Half of the silos were not air stressed, while the other half were submitted to a 12-h per week air stress. After 49 d, half of silos were subjected to aerobic spoilage at 22°C and the other half at 34°C. Samples were analyzed for microbial populations, fermentation profile, and aerobic stability (h before a 2°C increase above baseline after exposure to air at 22°C or 34°C). Data were analyzed by ANOVA as a factorial arrangement of treatments using JMP 12.0. There was an interaction (P < 0.01) between treatment and air stress on numbers of yeasts. CTRL subjected to air stress had higher yeast counts compared with no-stressed CTRL (7.01 vs. 4.44 log cfu/g fresh weight), whereas for SF, both stressed and no-stressed treatments, had lower numbers of yeasts (3.05 and 2.94 log cfu/g fresh weight), whereas for SF, both stressed and no-stressed treatments, had lower numbers of yeasts (3.05 vs. 2.94 log cfu/g, respectively). CTRL had a higher (P < 0.01) concentration of ethanol compared with SF (0.58 vs. 0.15%). The lower ethanol production in SF, was associated with a higher dry matter recovery (P < 0.05) compared with CTRL (99.75 vs. 98.25%). There was an interaction between treatment, air stress, and temperature on aerobic stability (P < 0.01). All SF silages, regardless of temperature during spoilage or air stress during storage, were stable for at least 250 h (time measurements were ended). At 22°C, CTRL no-stressed silage spoiled after 56 h, while CTRL air stressed spoiled at 11 h (P < 0.05). At 34°C, CTRL no-stressed silage spoiled at 26 h, while CTRL air stressed silage spoiled.
immediately ($P < 0.05$). These findings show that treatment of HMC with Safesil can overcome the effects of air stress during storage and exposure to warm temperatures at feed out.

**Key Words:** chemical additive, high-moisture corn, air stress

**T104 Effectiveness of a chemical additive on improving the aerobic stability of corn silage after short periods of ensiling.** E. Benjamim da Silva*1,2, R. M. Savage1, S. A. Polukis1, M. L. Smith1, A. M. Gray1, R. N. Mester1, and L. Kung Jr.1, *1University of Delaware, Newark, DE, 2CAPES Foundation, Brasilia, DF, Brazil.

The objective of this experiment was to determine the effectiveness of a chemical additive (SF; 20% sodium benzoate, 10% potassium sorbate, and 5% sodium nitrite) from Salinity (Gothenburg, Sweden) on improving aerobic stability of short-term ensiled corn silage. Whole plant corn was harvested at 39% DM, chopped, and untreated (CTRL), or treated with SF2 (2 L/t) or SF3 (3 L/t). Four replicated silos (7.5 L) were packed (224 kg of DM/m3) and opened after 1, 2, 4, and 46 d of ensiling. Untreated and SF2 ensiled for 46 d were analyzed after 48, 96, and 240 h of aerobic exposure. Silages were analyzed for microbial population, fermentation profile, and aerobic stability (h before a 2°C increase above baseline after exposure to air at 22°C). Data were analyzed by ANOVA as a factorial arrangement of treatments. After only 1 d of ensiling, SF2 was stable for 224 h and SF3 for 240 h, while CTRL was stable for 32 h. At d 2, 4 and 46 both SF2 and SF3 were stable for more than 240 h, while CTRL spoiled after 57, 81 and 117 h, respectively. Numbers of yeasts were similar among all treatments from 1 to 4 d. Only at d 46, there were fewer yeasts ($P < 0.05$) in treated silages (1.14 log cfu/g fresh weight) compared with CTRL (3.22). Concentrations of ethanol were the same among treatments at all openings. On silages ensiled for 46 d, at 48 and 96 h after aerobic exposure, no differences in pH were observed among treatments. However, at 240 h of aerobic exposure, CTRL pH was higher ($P < 0.05$) than SF2 (5.40 vs. 3.66). Numbers of yeasts were lower ($P < 0.05$) at all aerobic sampling time points and mold counts were lower ($P < 0.05$) after 240 h for SF2 compared with CTRL. These findings suggest that the additive is not immediately fungicidal, but its fungistatic effects apparently alter the metabolism of yeasts sufficiently enough to cause substantial improvements in aerobic stability. As the improvement on aerobic stability was observed as soon as after 1 d of ensiling, this additive might be useful for producers who cannot wait several weeks for silage to cure and that must feed out silage immediately or very soon after harvest.

**Key Words:** sensory additive, leucocyte, grazing dairy cow

**T105 Sensory additive effects on leucocyte and metabolic profile of grazing dairy cows.** L. M. Gómez1, P. Aguirre1, F. Bargo*2,3, G. Teda2, and I. Ipharraguerre1,2, *1Sollà, Medellin, Colombia, 2Lucta SA, Barcelona, Spain, 3Universidad Buenos Aires, Buenos Aires, Argentina, 4University of Kiel, Kiel, Germany.

There is a lack of research on potential effects of supplementation on metabolic profile in grazing dairy cows. Therefore, 45 Holstein dairy cows (147 DIM, 544 kg BW) were assigned to a 3 × 3 Latin square design replicated 15 times to evaluate the effect of sensory additive compound by natural plant extracts with a sweet flavor (ProEfficient, PE) on leucocyte and metabolic profile of grazing dairy cows supplemented with concentrate. Cows were blocked by parity in 15 blocks and within blocks randomly assigned to 3 treatments: 0 kg/d concentrate (0C); 5 kg/d control concentrate (CC); and 5 kg/d CC with 30 g/d of PE (PEC). Cows grazed a kikuyu (*Pennisetum clandestinum*) pasture for 84 d in 28-d periods at a pasture allowance of 35 kg DM/cow. Pasture averaged 24.2% CP, 55.5% NDF, and 60.3% in vitro DM digestibility (IVDMD). Concentrates (44.3% corn, 11.2% wheat bran, 10.0% corn gluten feed, 8.6% rice meal, 8.4% soybean meal, 8.0% sunflower meal, 9.5% mineral premix) were fed twice daily at milking and averaged 18.4% CP, 17.8% NDF, and 85.5% IVDMD. Blood samples were collected at 0900 h (3 h after supplementation) from the tail vein on d 3 of each period. One BD Vacutainer blood-collection tube and one plasma BD Vacutainer K3,EDTA were analyzed for hematological parameters, white blood cells (WBC), differential WBC count, fibrinogen concentration, and parameters of blood biochemistry at the Animal Diagnosis Laboratory of Universidad de Antioquia. Data were analyzed using a mixed model that included the fixed effects of treatment, block, period, their 2-way interactions, and the random effect of cow within block. Supplementation with PEC ($P = 0.07$) improved the total count of leucocytes compared with 0C (8.10 vs. 7.62 cells × 103 μL, SEM 0.37). Concentrate supplementation increased ($P = 0.03$) granulocytes count (3.31 vs. 2.85 cells × 103 μL, SEM 0.17). Lymphocytes percentage was lower ($P = 0.10$) with PEC than with 0C (57.8 vs. 61.3%, SEM 1.55). Total plasmatic proteins increased ($P < 0.05$) with PEC vs 0C (8.14 vs 7.98 g/L, SEM 0.08). The cells and components of immune system (leucocytes and total plasma proteins) tended to improve by adding PE to the concentrate supplemented to grazing dairy cows.

**Key Words:** sensory additive, leucocyte, grazing dairy cow

**T106 Effect of a homolactic inoculant alone and in combination with a heterolactic inoculant on the fermentation and aerobic stability of high-moisture corn.** M. L. Smith*1, R. M. Savage1, E. Benjamim da Silva1, S. A. Polukis1, S. J. Dietz2, K. M. Pacer3, T. P. Karnezos2, and L. Kung Jr.1, *1University of Delaware, Newark, DE, 2PMI Nutritional Additives, Shoreview, MN.

The objective of this study was to evaluate the effect of a homolactic inoculant alone and in combination with a heterolactic inoculant on the fermentation and aerobic stability of high-moisture corn. Four replicate piles of high-moisture corn (68% DM) were either untreated (CON) or treated with 165,000 cfu of homolactic acid producing bacteria/g of fresh material (HI; Purina FI Enhanced) or 600,000 cfu of homo- and heterolactic acid producing bacteria/g of fresh material (HI+LB; Purina SI Buchneri) (Land O’Lakes, Inc., Arden Hills, MN) and ensiled in 7.5 L silos for 30 and 90 d. The data were analyzed as a 3 × 2 factorial arrangement with the main effects of treatment (T) and d of ensiling (D), and their interaction (T × D), using the Fit Model procedure of JMP (SAS Institute Inc., Cary, NC) and differences were reported as significant when $P \leq 0.05$ using Tukey’s test. The aerobic stability (hr; $T \times D$; $P = 0.02$) of HI+LB was greater after 30 (263) and 90 (240) d of ensiling when compared with CON (40 and 54) and HI (97 and 43). After 30 and 90 d of ensiling, yeast counts (log cfu/g; $T \times D$; $P < 0.01$) were lowest for HI+LB (3.14 and 1.33) when compared with CON (3.51 and 4.55), with ethanol concentration (% DM; $T \times D$; $P < 0.01$) of HI+LB (0.52) being lower than CON (0.80) after 90 d of ensiling. Lactic acid concentrations after 30 and 90 d varied across treatments, of HI+LB (0.52) being lower than CON (0.80) after 90 d of ensiling. propionic acid (% DM; $P < 0.01$) was greatest for HI+LB (0.23) and lower for CON (0.18) and HI (0.19). High-moisture corn inoculated with HI+LB was more aerobically stable and had fewer yeasts as early as 30 d after
ensiling, with the concentration of ethanol being lower at 90 d of ensiling when compared with CON.

**Key Words:** aerobic stability, high-moisture corn

### T107 Effect of a homolactic inoculant alone and in combination with a heterolactic inoculant on the fermentation and aerobic stability of snaplage. M. L. Smith*1, R. M. Savage1, E. Benjamimm da Silva1, S. A. Polukis1, S. J. Dietz1, M. B. Palillo1, T. P. Karnezos2, and L. Kung Jr.1, 1University of Delaware, Newark, DE, 2PMI Nutritional Additives, Shoreview, MN.

The objective of this study was to evaluate the effect of a homolactic inoculant alone and in combination with a heterolactic inoculant on the fermentation and aerobic stability of snaplage. Five replicate piles of snaplage (70% DM) were either untreated (CON) or treated with 165,000 cfu of lactic acid producing bacteria/g of fresh material (HI; Purina FI Enhanced) or 600,000 cfu of lactic acid producing bacteria/g of fresh material (HI+LB; Purina SI Buchneri) (Land O’Lakes, Inc., Arden Hills, MN) and ensiled in 7.5 L silos for 30 and 90 d. The data were analyzed as a 3 × 2 factorial arrangement with the main effects of treatment (T) and d of ensiling (D), and their interaction (T × D), using the Fit Model procedure of JMP (SAS Institute Inc., Cary, NC) and differences were reported as significant when \( P \leq 0.05 \) using Tukey’s test. The aerobic stability (hr; \( T \times D; P < 0.01 \)) of CON (44), HI (41), and HI+LB (54) were similar at 30 d; but at 90 d of ensiling, HI+LB (213) was increased when compared with CON (92) and HI (104). Yeast counts (log cfu/g; \( P = 0.03 \)) and ethanol concentration (% DM; \( P = 0.02 \)) were lower for HI+LB (4.38 and 0.29) when compared with CON (5.19 and 0.37), with HI (4.83 and 0.31) being similar to both CON and HI+LB. After 30 d of ensiling, lactic acid concentrations (% DM) were greatest for HI+LB (0.12 \( P < 0.01 \)) of CON (0.09 and 0.00) and HI (0.07 and 0.06) when compared with CON (0.09 and 0.00) and HI (0.07 and 0.00). Snaplage inoculated with HI+LB had fewer yeasts and lower concentration of ethanol at 30 and 90 d of ensiling when compared with CON, with markedly increased aerobic stability at 90 d of ensiling when compared with CON and HI.

**Key Words:** aerobic stability, silage, snaplage

### Table 1 (abstract T109). Undigested NDF on organic matter basis (uNDFom) of dry forages analyzed by wet chemistry (% of dry matter)

<table>
<thead>
<tr>
<th>Forages1</th>
<th>uNDFom30 Mean SE</th>
<th>uNDFom120 Mean SE</th>
<th>uNDFom240 Mean SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa Premium</td>
<td>25.2 0.3</td>
<td>24.0 0.5</td>
<td>23.1 0.7</td>
</tr>
<tr>
<td>Alfalfa #1</td>
<td>26.3 0.5</td>
<td>25.4 0.3</td>
<td>24.6 0.4</td>
</tr>
<tr>
<td>Timothy 1st Premium</td>
<td>29.5 0.2</td>
<td>23.5 2.0</td>
<td>21.3 1.2</td>
</tr>
<tr>
<td>Timothy 1st #1</td>
<td>27.7 0.5</td>
<td>20.9 1.2</td>
<td>19.2 1.1</td>
</tr>
<tr>
<td>Timothy 1st #2</td>
<td>33.7 0.8</td>
<td>24.9 1.0</td>
<td>22.7 1.2</td>
</tr>
<tr>
<td>Sudan P/S pressed</td>
<td>21.4 0.4</td>
<td>13.8 0.9</td>
<td>11.5 1.0</td>
</tr>
<tr>
<td>Sudan P/S no pressed</td>
<td>22.4 0.6</td>
<td>14.2 1.1</td>
<td>12.3 1.1</td>
</tr>
<tr>
<td>Sudan F/S pressed</td>
<td>29.7 0.7</td>
<td>17.4 0.4</td>
<td>15.5 0.9</td>
</tr>
<tr>
<td>Sudan F/S no pressed</td>
<td>29.8 0.7</td>
<td>18.6 0.4</td>
<td>16.7 0.4</td>
</tr>
<tr>
<td>Klein #1 pressed</td>
<td>27.0 0.9</td>
<td>21.9 0.2</td>
<td>20.5 0.5</td>
</tr>
<tr>
<td>Klein #1 no pressed</td>
<td>37.7 1.4</td>
<td>19.1 0.3</td>
<td>17.9 0.4</td>
</tr>
<tr>
<td>Ryegrass straw</td>
<td>44.8 1.9</td>
<td>31.4 0.5</td>
<td>29.4 0.3</td>
</tr>
<tr>
<td>Fescue straw</td>
<td>43.1 1.1</td>
<td>31.4 0.5</td>
<td>30.6 1.0</td>
</tr>
</tbody>
</table>

1P/S = Premium/Standard; F/S = Fine/Standard.

### T108 Accuracy and precision of forage analysis by commercial laboratories. J. Severe* and A. J. Young, Utah State University, Logan, UT.

Nutritional analysis of feedstuffs is the foundation of progressive feed management. Feed analysis provides producers with nutritional information needed to optimize animal health and production. Peer review and popular literature indicate widespread perceived differences between feed laboratories. Objectives of this study were to determine variation in feed analysis between US feed laboratories and forage types. Twelve laboratories were selected to analyze 3 hay types: mixed grass (MG), pre-bloom alfalfa (BLA), and pre-bud alfalfa (BDA) in a blind test. Samples were sent 3 times, a month apart, in duplicate, and analyzed for DM, CP, NDF, and ADF. Mean, standard deviation, and range of RFV for MG, BDA, and BLA were: 118, 13.6, 87–161; 141, 13.9, 101–176; and 237, 31.7, 158–290; respectively. CP results were: 10.8, 1.3, 8–15; 24, 1.9, 21–29; and 25, 2.5, 23–29; respectively. ADF results were 32, 3.2, 26–42; 33, 2.1, 27–40; and 22, 2.5, 18–29; respectively. NDF results were 51, 4.3, 40–60; 42, 3.5, 35–54; and 29, 3.8, 22–40; respectively. Out of 216 samples submitted, 7 (3%) returned results that had obvious clerical errors and were corrected before statistical analyses. Significant inter and intra-laboratory differences were found for CP, ADF, and NDF; especially with BDA forage type. About 71% of labs had RFV values with ranges greater than 30 points for BDA; USDA hay quality groupings span 20 points. Before laboratory submissions, DM was determined for all samples by drying for 72 h at 55°C (PDM). For 216 submissions 49% produced negative differences when PDM was subtracted from laboratory-derived DM (LDM), indicating that samples increased moisture content after mailing. Western states laboratories (WSL) averaged positive differences, while eastern states laboratories (ESL) were primarily negative. Differences in humidity between the feed source and laboratory can have effects on forage analysis and laboratories should take steps to control changes. In summary, there can be large variations between and within laboratories that may affect the accuracy of forage analysis and can vary based on forage type.

**Key Words:** laboratory analysis, forage analysis


...
Dry forages fed in Japan are not commonly analyzed for undigested neutral detergent fiber on an organic matter basis (uNDFom). This study was conducted to evaluate (1) uNDFom composition of dry forages fed in Japan, (2) accuracy of near-infrared (NIR) analysis for uNDFom of dry forages, and (3) uNDFom differences on prediction of dry matter intake (DMI) and milk production. Forages (2 alfalfa hays, 3 timothy hays, 4 sudan grasses, 2 klein grasses, 1 ryegrass straw and 1 fescue straw) with different grades or processing were obtained. The forages were ground to pass a 1-mm screen and sent to 3 laboratories in the US (Lab A, B and C) to conduct wet chemistry and NIR analyses for uNDFom at 30, 120, and 240 h. Wet chemistry results of all laboratories were averaged for each analyte to create reference values. Mean difference between the reference values and NIR results, and standard deviation of the difference were plotted to calculate Euclidean distance from the center point (ED) for measures of NIR similarity to wet chemistry. The NIR accuracy described as ED ranged from 2.30 to 3.14 at Lab A and from 3.66 to 4.35 at Labs B and C. Wet chemistry results were used to model rations by the Cornell Net Carbohydrate and Protein System v 6.55. Predicted DMI and milk production varied as a function of forage uNDFom content. It is possible to evaluate nutritional values of dry forages, which could affect DMI and milk production based on NIR or wet chemistry analysis for uNDFom composition.

Key Words: NIR, undigested NDF, wet chemistry

T110 Carbon footprint of dairy production systems in Québec: Barley versus corn silage. J. Guyader*, S. Little, R. Kröbel, C. Benchaar, and K. A. Beauchemin, Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.

In Canada, corn silage (CS) is increasingly being fed to dairy cows at the expense of barley silage (BS), as its lower fiber and higher starch content improves animal performance and reduces enteric methane (CH₄) emissions. A life cycle analysis was conducted to compare the carbon (C) footprint (total greenhouse gas [GHG] as CO₂ equivalent/L of fat- and protein-corrected milk) of milk production from Canadian confinement dairy systems using CS or BS. A typical farm in Québec (Canada) was simulated to represent the 6-year lifespan of a dairy cow, from calving to culling. Diets fed to cows consisted of 54% CS or BS, 6% grass hay and 40% concentrates (dry matter basis). The impact of digestibility (measured as total digestible nutrient [TDN] content) of each of these silages on GHG emissions was also investigated. Based on an experimental work, milk production was assumed to average 34.7 and 31.9 kg/day for cows fed CS and BS of medium TDN content respectively. Milk production was assumed to be positively correlated with the TDN content of silages and the number of cows was adjusted to obtain similar milk production between farms. We assumed that forages as well as barley and corn grains were cultivated on-farm whereas all other feed ingredients were purchased. Greenhouse gas emissions were quantified with the Holos model. Methane emissions included enteric fermentation and manure storage, assuming that percentage of energy intake lost as CH₄ was constant regardless of silage TDN content. Carbon dioxide emissions included energy for crop production and processing and transportation of purchased feed. Nitrous oxide emissions accounted for nitrogen (N) degradation from manure, N leaching and volatilization. For medium TDN content silages, total GHG emissions were 13% lower for farms using CS compared with BS, even if the lower enteric CH₄ emission with CS was partially offset by increased CO₂ emissions from the additional purchased feed protein sources (+9%). For both forage sources, increasing silage TDN content reduced the C footprint of the dairy farm. Finally, when the dairy farm used highly digestible BS, the C footprint was lower than when using the least digestible CS.

Key Words: barley silage, corn silage, life cycle analysis

T111 Effect of seed variety and cutting date on nutritive values and in vitro digestibility of teff grass. B. Saylor*, B. Bradford, and D. Min, Kansas State University, Manhattan, KS.

Declining groundwater supplies are among the most pressing issues facing the dairy industry today. The water needed for forage production represents the great majority of total water use on most dairy farms, posing a major challenge in the pursuit of improved drought resilience. The objective of this experiment was to investigate the effect of variety and cutting date on the nutritive values and digestibility of teff grass (Eragrostis tef), a warm-season annual grass native to Ethiopia that is well adapted to drought conditions. Eighty pots were blocked by location in a greenhouse and randomly assigned to 4 teff varieties (Tiffany, Moxie, Corvallis, and Dessie) and to 5 cutting dates (40, 45, 50, 55, or 60 d after planting [DAP]). Harvested samples were dried, weighed, and analyzed for CP, NDF, and 24 h in vitro NDF digestibility (IVNDFD). It was found that seed variety had no effect (P > 0.10) on dry matter (DM) yield, CP, NDF, or IVNDFD. Yield of DM increased linearly (P < 0.001) from 4.1 to 26.4 ± 0.45 g/pot as harvest date increased from 40 to 60 DAP. Similarly, NDF concentration increased linearly (P < 0.001) from 51.7 to 63.5 ± 0.81% of DM with increasing harvest date. Concentration of CP decreased linearly (P < 0.001) from 28.7 to 11.2 ± 0.49% of DM and IVNDFD decreased linearly (P < 0.001) from 60.8 to 41.2 ± 1.0% as harvest date increased from 40 to 60 DAP. To assess carryover effects of cutting date on nutritive values, 2 additional cuttings were taken from each pot. It was found that increasing the first-cutting harvest date from 40 to 60 DAP decreased (P < 0.001) CP concentration in both the second and third cuttings. Additionally, increasing DAP reduced (P < 0.001) DM yield in the subsequent cuttings. Across all seed varieties and harvest dates, concentration of CP decreased (P < 0.001) and concentration of NDF increased (P < 0.001) linearly with each additional cutting. Results indicate that, under greenhouse conditions, the first cutting of teff grass should be taken between 40 and 50 DAP to optimize forage quality and digestibility in that cutting and any additional cuttings.

Key Words: drought, teff grass, dairy cattle

T112 Effect of homofermentative bacteria and cellulase addition to sugarcane at ensiling on silage chemical composition. L. R. de Q. Carvalho, V. L. Bany, A. A. Pinheiro, M. Dias, P. A. Helrigel, and E. A. Collao-Saenz*, Universidade Federal de Goiás-UFG, Jataí, Goiás, Brazil.

Sugarcane is a high-energy yielding crop, and its silage can be used as an alternative feed for dairy cows in tropical regions. The objective of this study was to evaluate the effect of a bacterial additive (BAC), and cellulase enzyme (ENZ) on chemical composition and fermentation of sugarcane silage. Inclusion or no inclusion of a BAC (Lactobacillus plantarum and Pediococcus pentosaceus, strains CH5796 and 2354, respectively; 2.5 × 10¹⁰ cfu/g) and 3 doses of ENZ, 0, 3 and 6% wt/wt (g cellulase/100g cellulose) added on sugarcane silage were evaluated after 30 d of fermentation in a completely randomized design with a factorial arrangement. Sugarcane was harvested at about 24.7% DM and 15.9 °Brix and ensiled in 18 4-L capacity PVC mini-silos with...
The objective of this study was to determine the cell wall (CW) composition along the corn stalk. Three phytomers of corn plants from 8 corn hybrids were used (2 replicates per hybrid). A center phytomer (C) corresponded to the phytomer of ear insertion. Upper (U) and lower (L) phytomers corresponded to 3 phytomers above and below, respectively, relative to phytomer C. Each phytomer was cut into 4 sections: top internode (T), middle internode (M), bottom internode (B), and node (N). For each replicate of each hybrid, sections within phytomers from internode (T) to node (N) were composited, and the CW concentration and composition were determined. Data were analyzed as for a randomized complete block design with repeated measures (subject = phytomer). The CW concentration did not change between phytomers (< 0.69) but increased from B to T within the phytomer (30.3 to 35.1% DM, respectively; P < 0.34) but decreased from B to T within the phytomer (36.2 to 34.9% CW, respectively; P < 0.01). Section N had the lowest concentration of GLU (31.8% CW). Concentrations of UA, ARA, and XYL decrease and the concentration of GLU increases in more mature sections of the phytomer of the corn plant, showing changes in nutritional quality.

Key Words: corn, uronic acids, neutral sugars
Table 1 (abstract T114).

<table>
<thead>
<tr>
<th>Legume</th>
<th>Grass</th>
<th>5-yr average estimated milk (Mg/ha/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White clover</td>
<td>Timothy</td>
<td>8.52</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>8.12</td>
<td></td>
</tr>
<tr>
<td>Tall fescue</td>
<td>8.79</td>
<td></td>
</tr>
<tr>
<td>Orchardgrass</td>
<td>8.12</td>
<td></td>
</tr>
<tr>
<td>Meadow fescue</td>
<td>7.91</td>
<td></td>
</tr>
<tr>
<td>Meadow bromegrass</td>
<td>9.28</td>
<td></td>
</tr>
<tr>
<td>Birdsfoot trefoil</td>
<td>Timothy</td>
<td>10.30</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>10.05</td>
<td></td>
</tr>
<tr>
<td>Tall fescue</td>
<td>9.76</td>
<td></td>
</tr>
<tr>
<td>Orchardgrass</td>
<td>10.35</td>
<td></td>
</tr>
<tr>
<td>Meadow fescue</td>
<td>9.22</td>
<td></td>
</tr>
<tr>
<td>Meadow bromegrass</td>
<td>11.23</td>
<td></td>
</tr>
<tr>
<td>Grazing-type alfalfa</td>
<td>Timothy</td>
<td>10.56</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>9.51</td>
<td></td>
</tr>
<tr>
<td>Tall fescue</td>
<td>9.01</td>
<td></td>
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<tr>
<td>Orchardgrass</td>
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<tr>
<td>Meadow fescue</td>
<td>8.65</td>
<td></td>
</tr>
<tr>
<td>Meadow bromegrass</td>
<td>10.39</td>
<td></td>
</tr>
</tbody>
</table>

SEM 0.245

T115 The effects of Lactobacillus buchneri and various air stresses on the fermentation and aerobic stability of corn silage.

Silages can spoil when exposed to air during storage and feedout. The objective of this study was to determine the effects of treating whole plant corn with Lactobacillus buchneri 40788 on the fermentation and aerobic stability of silage stored with or without air stress during storage. Whole-plant corn was harvested at 34% DM and was untreated or treated with L. buchneri 40788 (LB), final application rate of 400,000 cfu/g fresh forage and Pediococcus pentosaceus, 100,000 cfu/g; Lallemand Animal Nutrition, Milwaukee, WI). Five replicated silos (7.5 L, density of 224 kg of DM/m³) for each treatment were not air-stressed (NS), air-stressed early (ES, 3 h/wk for wk 1–9), or air-stressed late (LS, 3 h/wk for wk 10–19). Air-stressed silos had 3 1.60-cm holes plugged with stoppers, 2 located on the bottom of the bucket and one on the lid of the bucket that were opened during periods of air stress. After 126 d of ensiling at 22°C, silos were opened and analyzed for microbial populations, fermentation profile, and aerobic stability. Numbers of yeasts were lowest (P < 0.05) in all treatments with LB regardless of air stress. Untreated LS silage had the greatest number of yeasts among all treatments. LB silages had greater (P < 0.01) concentrations of acetic acid then control silages as expected (2.28 vs. 1.22%), and silages subjected to ES had greater (P < 0.05) acetic acid concentrations compared with LS silages (2.13 vs. 1.52%). There was a tendency (P < 0.09) for air stress during storage to reduce aerobic stability (227 h for NS, 165 h for ES, and 140 h for LS). Regardless of air stress, LB silages were more stable (P < 0.01, 276 h) vs. untreated silages (76 h). These findings suggest that treatment with LB can markedly improve the aerobic stability of corn silages even when they are air-stressed during storage, and that air stress occurring near the time of feed out is more detrimental on aerobic stability during feedout than air stress occurring during early storage.

Key Words: aerobic stability, Lactobacillus buchneri, air stress

T116 The impact of storage strategy and time on the quality and dry matter loss of wet distillers grains. A. Echeverría¹, M. De León¹, R. Gimenez¹, M. Ault², and O. Queiroz³, ¹National Institute of Agricultural Technology, Manfredi, Córdoba, Argentina, ²Dpto. Tecnico Bovinos, TEKNA SA, Rio Cuarto, Córdoba, Argentina, ³Animal Health and Nutrition Chr-Hansen, Córdoba, Córdoba, Argentina.

The objectives of this trial were to evaluate the quality and DM loss of wet distillers grain (WDGS) mixed or not with different fibers sources during short and long storage times. To replicate a conventional Ag-bag silo, 24 bags of 90 cm long and 38 cm diameter were made using the same 150-μm UV treated polyethylene plastic. Eight laboratory bags were randomly assigned to one of the 3 treatments: (1) control (CON; WDGS 100%); (2) WDGS plus alfalfa hay (WDH; WDGS 87%+ alfalfa hay 17%); (3) WDGS plus corn silage (WDC; WDGS 70%+ corn silage 30%). The experiment had a completely randomized design with 3 treatments, opening dates (30 and 180 d) and 4 replicates per treatment. Data were analyzed by ANOVA using the GLM procedure of SAS and the model included the treatment, time and treatment by time interaction. The DM content was similar between treatments (average 35.41%; SEM = 0.72). Protein (28.0 vs. 26.17 and 19.67%; SEM = 0.56), fat (12.93 vs. 11.88 and 10.22%; SEM = 0.17) and NDF (47.16 vs. 42.28 and 38.88%; SEM = 0.85) content were higher to WDGS in comparison with WDC and WDC respectively, ADF (22.60 vs. 18.41 vs. 17.46%; SEM = 0.78) and ash (6.49 vs. 4.97 and 4.31%; SEM = 0.08) content were higher for the WDH in comparison with WDGS and WDC. The lowest pH (4.08 vs. 4.34 and 4.30; SEM = 0.03) and deoxynivalenol concentration (1.52 vs. 2.11 and 2.08 µg/kg; SEM = 0.11) were obtained with the treatment WDC. Zearalenone (165.27, 180.61 and 115.50 µg/kg SEM = 33.36) and aflatoxin (1.03, 1.53 and 1.24 µg/kg, SEM = 0.248) concentrations were similar between treatments. Extended periods of storage (180 d) increased DM loss in all treatments, but in less extent in treatment WGC when compared with WDGS or WDH (10.86 vs. 13.85 and 19.35; SEM = 0.72). Based on DM loss, mycotoxin and pH data, treatment WDC is the most suitable to be used for extended period of storage. The use of by-products combined or not with fiber sources should always account for significant DM loss, as observed in this trial.

Key Words: by-products, dry matter loss, storage


The objective of the present work is to study the effect of combining 3 tropical fruit (passion fruit, pineapple and banana) residue with saboya grass before silanging on the chemical composition and the ruminal in vitro degradation obtained. The forage and residue were chopped and homogenized. Four combinations were prepared: saboya grass as the only silanging material and saboya grass mixed with a 15% of passion fruit, pineapple or banana residue as fresh base. The silang was done...
in experimental micro-silos (6 replicas per treatment). The chemical composition was analyzed into a random complete design. Also, the rumen degradation kinetics in a random block design. After 60 d, the micro-silos were opened and some samples were taken to determine the chemical composition and the in vitro rumen degradation of the dry material (MS), the neutral detergent fiber (FND) and the detergent acid fiber (FAD) at 0, 3, 6, 12, 24, 48, and 72 h. chemical composition was analyzed in a complete random design. A complete random block design was used in this study with 3 treatments and 6 replications. Fruit residue had relatively small effect over the silaging chemical composition. Effective degradability of the MS did not show differences between the pineapple and passion fruit silaging (P > 0.05) was bigger than other silaging (P < 0.05). Pineapple silaging and in a smaller way the one which had passion fruit residue, shown higher effective degradability of the FND and FAD compared with the silaging made of saboya grass or saboya grass with banana residue (P < 0.05). These results suggest that making silaging using a saboya grass combined with pineapple and passion fruit residue might be an efficient way to dispose of them, reducing environmental risk and improving the cradle’s food.

**Key Words:** saboya grass, fruit residue, silaging

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**T118 Effect of Lactobacillus buchneri 30319 alone or in combination with Lactobacillus plantarum 40027 (MTD/1) on the aerobic stability of high-moisture corn.** S. A. Polukis, M. L. Smith, E. B. da Silva, R. M. Savage, R. N. Mester*, M. B. Palillo, and L. Kung Jr., University of Delaware, Newark, DE.

The objective of this study was to evaluate the effect of L. buchneri 30319 (LB), alone or with L. plantarum 40027 (MTD/1) (LP, Volac Intl. Ltd., Hertfordshire, UK), with and without air stress during storage, on the aerobic stability of high-moisture corn (HMC). HMC (68% DM) was (a) untreated (C), or treated with (b) LB - 400,000 log cfu/g of fresh weight), or c) LB and LP - 100,000 cfu/g (LBLP). Five individually replicated silos (7.5 L) for each treatment were packed (density of 653 kg DM/m³) and ensiled for 30 and 90 d between 21 and 23°C. Additional silos for untreated (C-AS) and treated silage with LB (LB-AS) were prepared and air-stressed before the 90-d opening only. Air-stressed silos had 3 1.60-cm holes plugged with stoppers, 2 located on the bottom of the bucket and one on the lid of the bucket. Stoppers were opened for 2 h every 2 wk through 42 d and then 2 h/wk through 90 d of storage. Each day was analyzed separately using the Fit Model procedure in JMP (SAS Institute Inc., Cary, NC) as a completely randomized block. Significance was declared at P < 0.05. LB and LBLP had fewer yeasts (<2.00 log cfu/g) than C at 30 (5.46 log cfu/g) and 90 d (4.83 log cfu/g) of ensiling. At 90 d C-AS had the highest numbers of yeasts (6.23 log cfu/g) among treatments. Yeasts were <2.00 log cfu/g in LB-AS at 90 d. At 30 d there was a difference in molds between C (3.79 log cfu/g) and LB and LBLP (both <2.00 log cfu/g) but molds were not different among treatments at 90 d. At 30 d, aerobic stability was improved equally by LB (>250 h) and LBLP (>250 h) when compared with C (32 h). At 90 d, aerobic stability was also improved for LB (242 h) and LBLP (>250 h) compared with C (52 h). Air stressed resulted in a decrease in stability (25 h) compared with C (52 h) but stability was still >250 h for LB-AS. Treatment with LB improved the aerobic stability of HMC when added alone or in combination with LP. Air stress during storage decreased the aerobic stability of HMC but it did not affect the stability of silage treated with LB.

**Key Words:** aerobic stability, high-moisture corn, air stress

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**T119 Tall fescue as an alternative to timothy silage fed with or without alfalfa to dairy cows.** A. M. Richard*, I. Charbonneau1, R. Gervais1, G. F. Tremblay2, and G. Belanger3, 1Département des sciences animales, Université Laval, Québec; QC, Canada, 2Agriculture and Agri-Food Canada, Québec, QC, Canada.

Tall fescue has a good summer regrowth but is not always considered as an alternative to timothy because of its possible lack of palatability in dairy rations. We evaluated the effect of (1) replacing timothy with tall fescue, offered as sole forage or in association with alfalfa; and (2) feeding tall fescue as silage (35% DM) or haylage (55% DM). Treatments consisted of modifying the forage portion of the diet (70:30 forage:concentrate) as follows: (1) 100% timothy silage; (2) 100% tall fescue silage; (3) 60:40 timothy:alfalfa silages; (4) 60:40 tall fescue:alfalfa silages; (5) 100% tall fescue haylage. Fifteen Holstein cows were randomly assigned to treatments in a triple 5 × 5 Latin square design. Treatment periods lasted 21 d with the last 3 d used for data and sample collection. Preplanned contrasts tested were timothy vs. tall fescue, sole grass species vs. grass:alfalfa mixtures, interaction between grass species and grass:alfalfa mixture, and tall fescue silage vs. tall fescue haylage. Grass species did not affect DMI (23.5 kg/d; P = 0.36), milk yield (27.5 kg/d; P = 0.62), and milk fat content (4.21%; P = 0.55). Feeding grass:alfalfa mixtures resulted in higher DMI (24.3 vs. 22.6 kg/d; P < 0.01) and milk yield (28.3 vs. 26.7 kg/d; P < 0.01), but in lower milk fat content (4.15% vs. 4.27%; P < 0.01) than feeding sole grass species. Milk protein content was not affected by grass species (3.48%; P = 0.52) when offered in association with alfalfa, but was higher with timothy than tall fescue when offered as sole grass species (3.53 vs. 3.40%; P < 0.01). Dry matter intake was higher (22.6 vs. 20.8 kg/d; P < 0.01), milk protein content tended to be lower (3.40% vs. 3.44%; P = 0.06), but milk yield (27.0 kg/d; P = 0.58) and milk fat content (4.25%; P = 0.53) were similar when tall fescue silage was fed as compared with tall fescue haylage. Results show that tall fescue is a good alternative to timothy for lactating dairy cows, and confirm the beneficial effect of feeding grass species in association with alfalfa. Using tall fescue haylage instead of silage reduced DMI, but did not affect milk yield.

**Key Words:** forage, dry matter intake, milk yield

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**T120 The use of the Cornell Net Carbohydrate and Protein System in corn silage hybrid testing programs.** A. B. Lawton*, J. R. Lawrence¹, M. E. Smith², W. S. Burhans³, M. E. Van Amburgh¹, and T. R. Overton¹, ¹Department of Animal Science and PRO-DAIRY, Cornell University, Ithaca, NY, ²Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY.

Corn silage (CS) testing programs aid in evaluating hybrids for subsequent use. Ranking metrics such as Rumen Fermentable Carbohydrate–Fill Index (RFCFI; calculated as [(digested NDF at 30h+starch)/undegested NDF (uNDF) at 30h]), and Milk/Ton, as calculated by the Cornell Net Carbohydrate and Protein System were analyzed in a complete random design. A completely random block design was used in this study with 3 treatments and 6 replications. Fruit residue had relatively small effect over the silaging chemical composition. Effective degradability of the MS did not show differences between the pineapple and passion fruit silaging (P > 0.05) was bigger than other silaging (P < 0.05). Pineapple silaging and in a smaller way the one which had passion fruit residue, shown higher effective degradability of the FND and FAD compared with the silaging made of saboya grass or saboya grass with banana residue (P < 0.05). These results suggest that making silaging using a saboya grass combined with pineapple and passion fruit residue might be an efficient way to dispose of them, reducing environmental risk and improving the cradle’s food.

**Key Words:** forage, dry matter intake, milk yield
63.2 kg). This approach accounts for DMI differences based on uNDF240 supply and rumen fill. The reported ME AMY was more limiting than MP AMY and accounts for nutrient and digestibility characteristics. Comparisons of ME AMY with RFCFI and MILK2006 were made for both locations (Table 1). A stronger correlation existed between ME AMY and RFCFI, than MILK2006, and was greater for the NNY location, which had greater yields and lower digestibility values than the CNY location. Utilizing CNCPS may enhance the information available from CS testing programs.

**Key Words:** CNCPS, corn silage, hybrid trial

### Table 1 (abstract T120). Correlation between CNCPS v.6.5.5 predicted ME allowable milk yield on a DMI equivalent or uNDF240 intake corrected basis

<table>
<thead>
<tr>
<th>CNCPS measurement</th>
<th>Ranking metric</th>
<th>Central NY</th>
<th>Northern NY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted ME AMY, DMI equivalent</td>
<td>RFCFI</td>
<td>0.69</td>
<td>0.93</td>
</tr>
<tr>
<td>Predicted ME AMY, DMI equivalent</td>
<td>MILK2006</td>
<td>0.18</td>
<td>0.51</td>
</tr>
<tr>
<td>Predicted ME AMY, uNDF240 adjusted DMI</td>
<td>RFCFI</td>
<td>0.63</td>
<td>0.89</td>
</tr>
<tr>
<td>Predicted ME AMY, uNDF240 adjusted DMI</td>
<td>MILK2006</td>
<td>0.49</td>
<td>0.42</td>
</tr>
</tbody>
</table>

1AMY = allowable milk yield.
2RFCFI = Rumen Fermentable Carbohydrate–Fill Index.
3uNDF240 = undigested NDF at 240 h.

The objective of this trial was to compare the effect of silage covering systems on fermentation, nutritional quality, and estimated organic matter loss of corn silage after 156 days of storage in a drive-over pile. S. Li1, E. Uriarte2, K. Wang3, D. Bu4,5, K. Rich6, C. Banchero6, K. Bolsen*, 1University of Manitoba, Winnipeg, MB, Canada, 2Ibero-American University, Puebla, Mexico, 3State Key Laboratory of Animal Nutrition, Beijing, China, 4CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, Beijing, China, 5World Agroforestry Centre, East and Central Asia, Beijing, China, 6Silostop, London, UK, 7Kansas State University, Manhattan, KS.

The objective of this trial was to compare the effect of silage covering systems on fermentation, nutritional quality, and estimated OM loss in the outer layer of whole-plant corn silage stored in a large drive-over pile on a dairy farm in the People’s Republic of China. On 22 and 23 September 2015, whole-plant corn was packed into about 50 linear meters in the center of a 22,000-ton capacity pile. The apex of the forage was about 4 meters, and the whole-plant had an average DM content of 32 percent. The silage covering systems were (1) conventional white-on-black polyethylene (PE) plastic (PE plastic) and (2) oxygen barrier (OB) underlay film (www.silostop.com) plus conventional white-on-black PE (OB film). The conventional PE plastic protected the OB underlay film from UV light and physical damage. The surface of packed forage in the center of the pile was divided into eight, 5-meter by 5-meter areas; four areas were covered with PE plastic; four areas, with OB film. Covering systems were arranged alternately, and there was a 1-meter border around each area. The covering materials were removed before feed-out after 156 days of storage, and samples collected at 0 to 45 cm from the surface at three locations within each area. Samples of the crop at harvest and silage were analyzed for fermentation components and chemical composition. Loss of OM was estimated from the ash content in the crop at harvest and in the silage. Silage pH and lactic acid content were the only fermentation traits affected (P < 0.05) by covering system. Silage protected with OB film had higher nutritional quality than silage protected with PE plastic, as evidenced by a lower (P < 0.05) ADF and NDF content and higher (P < 0.05) NDF digestibility. Ash content was lower (P < 0.05) in silage covered with OB film than in silage covered with PE plastic. Estimated loss of OM in the upper 45-cm layer of silage was 14.2% under OB film versus 20.2% under PE plastic. Whole-pile estimated net economic benefit to the OB underlay film compared to PE plastic was US$0.30 to 0.50 per ton of crop ensiled.

**Key Words:** silage, covering, film
The objective of this study was to evaluate 3 milk replacer (MR) feeding programs on calf performance up to 4 mo of age. Neonatal Holstein male calves (n = 48; 34 ± 1.5 kg BW) were randomly assigned to either a moderate rate of MR (MOD; 0.66 kg/d for 39 d then 0.33 kg/d for 3 d), an ad libitum rate of MR (ADLIB; offered twice daily between 0630 h and 0830 h and between 1430 and 1630 h for 35 d, 0.66 kg/d for 4 d, and 0.33 kg/d for 3 d), or a step-up rate of MR (STEPUP; increased from 0.32 to 0.62 kg/d in first 12 d, 0.66 kg/d for 27 d, and 0.33 kg/d for 3 d). The MR (25% CP, 18% fat) was fed 2× daily up to d 39 and 1× daily thereafter. Texturized starter (40% starch, 21% CP) and water were offered ad libitum. Calves were housed in individual pens through d 56. Calf BW was measured initially and weekly thereafter. Hip widths (HW) were measured initially and every 2 wk thereafter. Feces were scored and feed intake recorded daily. From 56 to 112 d, calves were moved to group pens (4 calves/pen). The same starter blended with 5% chopped grass hay was offered ad libitum. BW and HW were measured on d 56, 84, and 112. Data, within period, were analyzed as a completely randomized design with repeated measures when appropriate. Experimental units were calf in first period and pen in second. Total MR intake per calf averaged 27, 51, and 25 kg for MOD, ADLIB, and STEPUP programs, respectively, with a range of 42 to 63 kg for ADLIB. In the first 56 d, starter intake and feed efficiency were less for ADLIB vs. MOD (P < 0.05), while fecal scores and abnormal fecal score days were greater for calves fed ADLIB vs. MOD (P < 0.05). Calves fed STEPUP had lesser ADG than calves fed MOD (P < 0.05). In the second 56 d, initial BW was greater for ADLIB vs. MOD (P < 0.05), though final BW was not different between MOD and ADLIB or STEPUP (P > 0.05). Calves previously fed MOD had greater ADG, feed efficiency, and HW change than calves fed ADLIB (P < 0.05). Though BW were greater at weaning when MR was fed ad libitum, calves were less efficient and growth advantages were lost by 4 mo of age.

Key Words: calf, feeding rate, growth

This study looked into the effects of milk replacer (MR) feeding rate and Neo-Terramycin (NT) feeding in MR on calf performance and digestion. Neonatal Holstein male calves (n = 48; 34 ± 1.5 kg) were randomly assigned to 3 treatments, a moderate rate of MR (MOD), a moderate rate containing NT (Neopectamycin-Oxytetacycline, 1300 g/t; NTMOD), and a high rate of MR (HIGH). The MR rate for MOD and NTMOD was 0.66 kg/d for 39 d, then 0.33 kg/d for 3 d, and for HIGH was 0.85 kg/d for 5 d, 0.17 kg/d for 30 d, and 0.53 kg/d for 7 d (DM basis). The MR with NT was fed for 14 d, non-med for 1 d, NT for 14 d again, non-med for 1 d, then NT for the last 12 d. All MR had 25% CP and 18% fat (DM). Textured starter (40% starch, 20% CP; DM) and water were fed ad libitum through d 56. Calves were individually housed and bedded with straw. Medical treatments were recorded daily. Calves were weighed initially and weekly thereafter. Body condition score (BCS) and hip widths were measured initially and every 2 weeks thereafter. During d 47 to 51 of the trial, feces were collected from 5 calves per treatment to estimate digestibility using acid insoluble ash as marker. Data were analyzed as a completely randomized design with repeated measures when appropriate. Pre-planned contrast statements of MOD vs. NTMOD and MOD vs. HIGH were used to separate the means. Pre-weaning starter intake was less (P < 0.05), ADG tended to be greater (P = 0.06) and BCS change was greater (P < 0.05) for calves fed HI vs. MOD. Pre-weaning medical treatment days were less (P < 0.05) for calves fed NTMOD vs. MOD. Post-weaning ADG was less (P < 0.05) for calves fed NTMOD vs MOD. Digestibility of DM, OM, ADF, and NDF were greater (P < 0.05) for calves fed MOD vs. NTMOD and HIGH. Digestibility of NDF was 28, 17, and 18% for MOD, NTMOD, and HIGH respectively. Fat digestibility (P < 0.05) was greater for calves fed MOD vs. HIGH. In this study, the reduction of starter intake by feeding more MR and the prolonged feeding of NT in MR reduced digestion immediately post-weaning.

Key Words: antibiotics, medication, growth

Holstein bull calves (n = 48, 2–3 d of age) were fed milk replacer powder (MR) at 0.66 kg DM/d for 46 d and 0.33 kg/d for 5 d (MRM) or 0.85 kg/d for 5 d, 1.07 kg/d for 37 d, and 0.53 kg/d for 7 d (MRH), reconstituted to 14% DM. Calf starters (CS), offered from d 1, were a pelleted containing 10% starch or a textured feed with whole corn and oats containing 40% starch (HS). Water was available at all times. From 0 to 8 wk, calves were housed in individual pens. From 9 to 16 wk, calves were grouped (4 calves/pen) within treatment and fed 95% of the same CS plus 5% chopped grass hay. During the last 5 d of wk 5, chrome oxide was added to MR at each feeding of 5 calves per treatment to estimate digestibility. During wk 8, fecal grab samples were taken from the same calves once daily for 5 d. During wk 11, 13, and 16, fecal samples were carefully collected from the floor of all group pens once daily for 5 d. All post-weaned fecal samples were analyzed for acid-insoluble ash to estimate digestion. Data were analyzed as a 2 × 2 factorial arrangement of MR and CS in a repeated measures mixed model ANOVA. Digestion of most nutrients increased with age and CS intake. Feeding MRH during wk 0–8 reduced (P < 0.01) digestion of NDF (33.0 vs. 50.4%), ADG (22.7 vs. 41.5%) and starch (75.0 vs. 84.4%). Feeding HS during wk 0–8 increased (P < 0.05) digestion of DM (80.8 vs. 72.6%), OM (81.5 vs. 73.3%), starch (84.8 vs. 74.6%), and CP (76.6 vs. 71.6%), and reduced (P < 0.05) digestion of NDF (36.4 vs. 47.0%) and ADF (26.5 vs. 37.7%). From wk 11–16, feeding MRH reduced (P < 0.01) digestion of DM (77.9 vs. 79.9%), OM (78.8 vs. 80.7%), CP (80.4 vs. 83.2%), fat (85.8 vs. 87.4%), NDF (36.6 vs. 60.0%) and ADF (25.2 vs. 57.9%). From wk 11–16, feeding HS increased (P < 0.01) digestion of DM (82.1 vs. 75.7%), OM (83.0 vs. 76.4%), CP (84.0 vs. 79.6%), and reduced (P < 0.05) digestion of starch (96.2 vs. 97.5%), NDF (48.3 vs. 66.6%), and ADF (47.6 vs. 62.4%). Feeding large amounts of MR inhibited OM and fiber digestion to 4 mo of age.
whereas high CS increased nutrient digestion. Changes to MR feeding programs should consider post-weaning digestion.

**Key Words**: Calf, digestion, starch

**T124** Estimates of metabolizable energy of dry feed in calves fed two types of starters and two levels of milk replacer. J. D. Quigley*, T. M. Hill, F. X. Suarez-Mena, T. S. Dennis, L. L. Deikun, and R. L. Schlotterbeck, Provi nni North America, Brookville, OH.

Holstein bull calves (n = 48, 2–3 d of age) were fed milk replacer (MR) at 0.66 kg/d for 46 d and 0.33 kg/d for 3 d (MRM) or 0.85 kg/d for 5 d, 1.07 kg/d for 37 d, and 0.53 kg/d for 7 d (MRH). Calf starters (CS), offered from d 1, were pellet (LSP; 10% starch) or pellet plus whole corn and oats (HST; 40% starch). Calves were housed in individual pens from 0 to 8 wk. From 9 to 16 wk, calves were grouped within treatment (n = 3/pen) and fed 95% of the same CS plus 5% chopped grass hay. During the last 5 d of wk 5, chromic oxide was added to MR at each feeding of 5 calves per treatment to estimate digestion. Fecal grab samples were taken daily from the same calves for 5 d during wk 8. During wk 11, 13, and 16, fecal samples were collected daily from the floor of all group pens for 5 d. Post-weaned fecal samples were analyzed for acid-insoluble ash. Apparent digestibility of CP, fat, NDF, ADF and starch were used to estimate ration ME (MEe) using equations from 2001 Dairy NRC, corrected for apparent digestibility. MEe of CS during wk 5 were corrected for MR intake using lactose, CP and fat digestibilities of 95, 93 and 93%, respectively. MEe during wk 11, 13, and 16 included 95% CS and 5% hay. Ration ME was also predicted using 2001 NRC calculations (MEn). Data were analyzed as a repeated measures mixed model ANOVA with calf (wk 1–8) and pen (wk 9–16) as experimental unit. Including all wk, MEe were higher in HST than LSP (P < 0.02) and when MRM was fed (P < 0.06). Excluding wk 5, there was no effect (P > 0.10) of CS form or MR feeding on MEe.

**Key Words**: calf nutrition, growth, health

**T125** Performance of calves fed milk replacer or whole milk and traditional starter or an alternative concentrate feeding program. E. L. M. Silva*1, S. J. Bertics3, E. B. Alves2, D. M. Donnelly3, J. R. R. Dórea3, C. M. M. Bittar1, and D. K. Combs3, 1University of Sao Paulo, Piracicaba, SP, Brazil, 2Federal University of Lavras, Lavras, MG, Brazil, 3University of Wisconsin, Madison, WI.

Traditional calf starters are formulated to balance nutrient needs of calves fed restricted amounts (4L/d) of reconstituted milk replacer (MR) or whole milk (WM). We compared growth and intake of calves fed higher rates of MR or WM and traditional calf starter (CS) or an alternative (AC) feeding program that more closely matches NRC (2001) guidelines. The AC program consisted of feeding cracked corn ad libitum from 1 to 21d of age; then during wk 4 a 2:1 mixture of cracked corn; calf grower (20% CP); then at wk 5 a 9:4:6 mixture of grower and soybean meal was fed along with alfalfa hay ad libitum. Holstein heifer calves (n = 75) were assigned randomly to 1 of 5 treatment groups: T1: A negative control in which calves were offered 4 L/d of MR(24% CP, 22% Fat) from d2 to 6 wk of age, at wk 7 they were fed 2 L/d until they were weaned at wk 8. T2: Calves were offered 8 L/d of MR until 5 weeks old. At wk 6 and 7 they were fed 4 L/d and 2 L/d respectively. Calves on T1 and T2 were fed ad libitum CS. T3: Calves received MR in the same manner as calves assigned to T2 and the AC feed program. T4 and T5: Calves were fed in the same manner as calves assigned to T2 and T3 respectively, except that they were fed pasteurized WM instead of MR. Growth, feed intake and health scores were measured. Data were analyzed as repeated measures over time with the MIXED procedure (SAS Inst. Inc.). During the first 8 weeks of the study ADG were higher in calves fed WM (T4 and T5) than calves fed MR (T1, T2 and T3; P < 0.05) (Table 1). Growth rate (ADG) did not differ between calves offered CS or AC. Calves fed a restricted amount of MR (T1) had higher feed intake than others groups (P < 0.05). Calves on T1, T2 and T3 had slightly higher morbidity (higher health scores) than calves fed WM (T4 and T5; P < 0.05). Feeding calves WM increased growth and decreased morbidity. Replacing calf starter with the alternative concentrate program did not affect performance.

**Key Words**: calf nutrition, growth, health

**Table 1 (abstract T125).** Performance of calves through eight weeks of age

<table>
<thead>
<tr>
<th>Item</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>SEM</th>
<th>T1 vs T2,3,4,5</th>
<th>T1,2,3 vs T4,5</th>
<th>T2 vs T3</th>
<th>T4 vs T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG (g/d)</td>
<td>701.3</td>
<td>713.4</td>
<td>634.6</td>
<td>803.3</td>
<td>764.8</td>
<td>32.6</td>
<td>0.10</td>
<td>0.00</td>
<td>0.10</td>
<td>0.41</td>
</tr>
<tr>
<td>Feed intake (g/yr)</td>
<td>849.9</td>
<td>687.2</td>
<td>589.8</td>
<td>770.2</td>
<td>639.2</td>
<td>37.4</td>
<td>&lt;0.001</td>
<td>0.90</td>
<td>0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>Health score</td>
<td>0.45</td>
<td>0.49</td>
<td>0.50</td>
<td>0.36</td>
<td>0.37</td>
<td>0.06</td>
<td>0.76</td>
<td>0.03</td>
<td>0.90</td>
<td>0.85</td>
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</table>

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randomly assigned to treatments to obtain a 2x2 factorial arrangement (2 RFI levels and 2 diet energy levels). Diets were offered in a 120 d trial. Statistical analyses were performed using a MIXED procedure in SAS 9.4 with pen as experimental unit. Dry matter intake was not affected by diet (7.38 vs. 7.83 kg DM for HiE and LoE, respectively; P = 0.14), or by RFI (7.40 vs. 7.81 kg DM for HiRFI and LoRFI, respectively; P = 0.17) or their interaction (P = 0.66). Daily gain (ADG) was affected by diet with heifers fed HiE having greater ADG than heifers fed LoE (1.14 vs. 0.97 kg/d; P < 0.01). Also, RFI affected ADG with LoRFI heifers having greater ADG than HiRFI heifers (1.09 vs. 1.02 kg/d; P = 0.03), with the interaction of RFI and diet not significant (P > 0.10). Feed efficiency was improved for heifers fed the HiE diet (6.44 vs. 8.02 kg DM/kg gain for HiE and LoE, respectively; P < 0.01), but was not affected by RFI (P = 0.48) or the interaction of RFI and diet (P = 0.62). Overall, feed efficiency of pre-breeding heifers was not dependent on genomic RFI. Heifers with LoRFI had greater ADG but this was likely due to a numerical increase in intake. Feed efficiency of heifers was reduced when heifers were fed the LoE diet, but resulted in more optimal ADG than feeding a higher energy diet ad-libitum.

**Key Words:** dairy heifer, diet energy, residual feed intake

**T127 Effect of limit feeding and genomic residual feed intake on dairy heifer growth and feed efficiency**, M. S. Akins1*, K. T. Williams1, H. Su2, W. K. Coble3t, N. M. Esser4, P. C. Hoffman1,5, and K. A. Weigel1, 1Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, 2China Agricultural University, Beijing, China, 3USDA Dairy Forage Research Center, Marshfield, WI, 4Marshall Agricultural Research Station, University of Wisconsin-Marshall, WI, 5Vita Plus Corporation, Madison, WI.

The objective of this study was to evaluate growth and feed efficiency of dairy heifers differing in predicted genomic residual feed intake as a lactating cow (RFI) and either fed ad-libitum or limit-fed. Post-bred Holstein heifers (128, ages 14–20 mo) were blocked by BW (heavy, 516 kg; medium-heavy, 485 kg; medium-light, 457 kg; and light, 420 kg) with 32 heifers per block. Heifers within block were sorted by genomic RFI to obtain 2 pens of high and 2 pens of low RFI within each weight block (8 heifers per pen). Diet treatments were i) a control diet (CON) comprised of corn silage and alfalfa silage (59.2% TDN, 12.7% CP, and 47.8% NDF, DM basis), and ii) a diet limit-fed (LIMIT) to 90% of CON intakes (62.4% TDN, 13.4% CP, and 42.7% NDF, DM basis). The LIMIT diet contained the same forages as CON but included corn and soybean meal to obtain similar nutrient intakes as CON. Treatments were randomly allocated to obtain a 2x2 factorial arrangement and implemented within a 120-d trial. Data were evaluated using the MIXED procedure in SAS 9.4 with pen as experimental unit. Feed intake was greater (P = 0.01) for heifers fed CON diets (11.0 vs. 9.98 kg DM/d). Feed intake was unaffected (P > 0.34) by RFI with no interaction between diet and RFI. Protein intake was greater (P = 0.04) for heifers fed CON compared with heifers fed LIMIT (1.41 vs. 1.33 kg CP/d). Intake of dietary ME tended to be greater for CON fed heifers (P = 0.07), but intakes of NEm and NEg were similar (P > 0.28) between diets. The ADG of heifers was affected by diet (P < 0.01) with CON fed heifers having greater ADG than LIMIT (0.88 vs. 0.80 kg/d) potentially due to greater digesta fill for CON. The ADG of heifers was also affected (P < 0.01) by RFI with Low RFI heifers gaining more than High RFI heifers (0.86 vs. 0.83 kg/d). Feed efficiency of heifers was unaffected by diet (P = 0.70), but was improved (P = 0.01) in Low RFI heifers compared with High RFI heifers (12.1 vs. 12.8 kg DM/kg gain). Body condition gain was unaffected by RFI or diet (P > 0.36). Overall, heifers with Low genomic RFI had improved feed and growth efficiency whether fed ad-libitum or limit-fed diets.

**Key Words:** dairy heifer, residual feed intake, limit feeding

**T128 Evaluation of a nutrition model for calves raised under tropical conditions using individual animal data**, V. L. Souza1, C. M. M. Bittar1, J. K. Drackley2, R. Almeida3, and D. P. D. Lanna1, 1Esalq/USP, Piracicaba, SP, Brazil, 2University of Illinois, Urbana, IL, 3Universidade Federal do Paraná, Curitiba, PR, Brazil.

Model evaluation using data from calves fed several types of milk replacers and calf starters under different environmental conditions is important to identify whether the model can be useful for dairy nutritionists. Following our previous studies, 501 sets of individual calf data from 16 studies carried out at the University of São Paulo (ESALQ) in Brazil were used to evaluate the updates published by the Agriculture Modeling and Training Systems (AMTS) calf model (AMTS.Cattle.Pro). These studies provided all inputs required by the model. Descriptive statistics were generated using SAS. Analyses for model adequacy were performed with R and the Model Evaluation System. The average daily gain (ADG) observed at weaning was used to evaluate the model (7 or 8 weeks of age). The mean square error of prediction (MSPE), mean bias, concordance correlation coefficient (CCC), and analysis of linear regression were calculated. Calves (36.3 ± 6.3 kg BW at birth; 60.7 ± 14.2 kg BW at weaning) were fed different amounts of milk replacer (4.5 ± 0.9 L/d, 12.5% DM, average 21 ± 1.9% CP and 17 ± 2.0% fat in dry solids) or whole milk (5.3 ± 0.9 L/d), and were weaned at 57 ± 3 d of age (ADG 0.745 ± 0.383 kg/d). Calf starters contained 21.3 ± 1% CP. The ADG predicted by dietary metabolizable protein (MP) showed lower values of mean bias and MSPE and higher CCC compared with the ADG predicted by dietary metabolizable energy (ME, Table 1). The model showed to be more accurate than precise (Cb > r value). The low precision of the model may be related to variation in starter intake (0.820 ± 0.549 kg/d) of young calves before weaning. The updates published by the AMTS model can be used in the evaluation of early nutrition programs for young calves to make predictions with good accuracy but a low precision.

**Table 1 (abstract T128).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>ME allowable gain</th>
<th>MP allowable gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model-predicted ADG (kg/d)</td>
<td>0.729</td>
<td>0.748</td>
</tr>
<tr>
<td>Mean bias (Y – X)</td>
<td>0.016</td>
<td>-0.003</td>
</tr>
<tr>
<td>MSPE (kg × kg)</td>
<td>0.138</td>
<td>0.099</td>
</tr>
<tr>
<td>MSPE decomposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean bias (%)</td>
<td>0.189</td>
<td>0.007</td>
</tr>
<tr>
<td>Systematic bias (%)</td>
<td>4.623</td>
<td>1.181</td>
</tr>
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</tr>
<tr>
<td>P-value (a = 0)</td>
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</tr>
<tr>
<td>P-value (b = 1)</td>
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**Key Words:** dairy calf, growth model

**T129 Monthly body weight change in wild type and slick haired post-weaned Puerto Rican Holstein heifers**, J. M.
In Puerto Rico, the slick hair genotype/phenotype has been shown to increase heat tolerance and milk yield in mature dairy cows. However, it is unknown if this superior thermoregulatory capacity may result in growth differences during the post-weaning period. Therefore, this study aimed to evaluate the change in body weight (BW) over time of 25 Puerto Rican Holstein heifers with regular (WT; n = 14) or slick (SL; n = 11) haired coats. Hair coat types were first chosen visually and then genonomically confirmed. The heifers studied were born from October 21, 2013 to February 14, 2015, weaned at 60 d of age, and weighed monthly from 4 to 34 mo of age. Data were analyzed by the GLIMMIX, GLM, and REG procedures of SAS. Hair coat type and age interacted to affect BW (P = 0.0005). However, no differences were observed in BW between the WT and the SL heifers (mean values over the 30 mo of 279.33 ± 5.78 and 275.06 ± 6.91 kg, respectively; P = 0.3182); and as age progressed from 4 to 34 mo, BW increased in both, the WT and the SL heifers (93.52 ± 11.42 to 446.35 ± 28.27 kg and 85.69 ± 9.97 to 381.37 ± 35.81 kg, respectively; P < 0.0001). The coat type by age interaction may be explained by the fact that in the WT heifers the growth curve was best described by the linear regression BW = 12.28 age + 54.41 (R² = 0.88; P < 0.0001); while in their SL counterparts, it was best explained by the quadratic regression BW = −0.32 age² + 22.75 age + 6.24 (R² = 0.74; P < 0.0001). These different growth curves suggest that, under our conditions, the SL heifers may reach earlier and smaller mature body weights than their WT counterparts. Therefore, future studies should evaluate any possible differences in mature body weight or age at maturity between both hair coat types.

Key Words: slick-haired Holstein heifer, heifer growth, postweaning period


In specialized dairy systems, there are problems due to the low development of calf rumen function, causing a delay in the beginning of productive life of the replacement animals. The aim of this study was to evaluate the supplementation of functional additives on the development of rumen. A total of 49 one-day-old Holstein calves were randomly assigned to 6 experimental groups: (1) Control (C), (2) oregano essential oil (EOO), (3) native yeast (NY), (4) Combination1 (EOO+Y1), (5) Combination2 (EOO+Y2), (6) Combination3 (EOO+Y3). Whole milk (2.8%CP, 3.2%fat) was offered twice daily until d 90. Calf starter (18%CP, 2.43 ME Mcal/kg DM) was used for all treatments and was available free choice. Milk intake (4.27 ± 4.37L/d, ADFI (463 ± 529g/d), and ADG (568 ± 675g/d) were similar in all groups (P > 0.05). At the end of the trial (112 d), 3 calves per treatment were selected and slaughtered to extract rumen samples and take measurements of ruminal papillae development. The surface area of the papillae, as a function of their length and width, was determined in 9 regions of the rumen: caudal portion of caudal ventral blind sac (A), left side caudal dorsal sac (LB), left side cranial dorsal sac (LC), left side cranial ventral sac (LD), left side ventral portion of caudal ventral blind sac (LE), right side caudal dorsal sac (RB), right side cranial dorsal sac (RC), right side cranial ventral sac (RD), right side ventral portion of caudal ventral blind sac (RE). Data were analyzed as a completely randomized design with a factorial arrangement of treatments (6 × 9) using PROC GLIMMIX. The rumen papillae with greater surface area were observed in the cranial-ventral portions (RD 2.46, LD 1.84 mm²), while the less surface area were found in LC, RC, A (1.14, 1.22, 1.17 mm², respectively). Calves fed with NY treatment had the greatest papillae surface area in all the regions (P < 0.05). The interaction region × treatment was affected due to the supplementation of the additive (P < 0.001), however, no difference were observed in A, LB, RB, RE regions (P > 0.05), whereas in RD calves supplemented with OEO showed the greatest surface papillae area (3.25 mm²). In conclusion, supplementation of native yeasts as a functional additive improves calf rumen development.

Key Words: ruminal papillae, oregano essential oil, native yeast
bacteria count of milk varies within and across dairy farms. Both batch and high temperature short time (HTST) pasteurizers were used, as well as UV (UV) treatment of milk. Milk samples at each farm were collected for 7 consecutive days to determine total solids (TS), protein, butterfat, somatic cell count, and antibiotic presence, as well as bacteria count. On d 1 through 6, a post-pasteurized milk sample was collected at morning or evening calf feeding. On d 7, 3 samples for bacteria count were taken at the morning calf feeding: pre-pasteurized, immediately post-pasteurized, and post-pasteurized before feeding the last calf. Samples were stored between 1.67°C and 5.56°C, and then sent to Eurofins DQCI (Mounds View, MN) for all analyses. Data were analyzed by PROC FREQ and PROC MEANS of SAS; PROC UNIVARIATE of SAS was used for within and across farm variation. Mean TS, protein, and fat of post-pasteurized waste milk across all farms were 12.8%, 3.41%, and 3.92%, respectively. Minimum TS, protein, and fat of post-pasteurized waste milk were 7.67%, 2.13%, and 1.55%, respectively. Maximum TS, protein, and fat of post-pasteurized waste milk were 18.1%, 4.96%, and 9.47%, respectively. The coefficient of variation for TS, protein, and fat were 6.58%, 7.90%, and 17.32% within a farm, respectively; and 8.54%, 11.8%, and 19.2% across farms, respectively. Bacteria counts post-pasteurization were categorized as failed (>100,000 cfu), poor (20,001 – 100,000 cfu), or good (≤20,000 cfu). The percentage of farms in each category were as follows: failed- 27.44%, poor- 14.14%, good- 58.42%. In terms of last calf fed, 36.11% of farms failed, 18.23% were poor, and 45.66% were good. Antibiotics were detected in 56.8% of samples collected. Pasteurized waste milk is a highly variable source of nutrition for dairy calves and on-farm pasteurization does not consistently deliver waste milk with a low bacteria count.

**Key Words:** calf, pasteurized waste milk, pasteurizer
T133  Transcriptional changes in the early lactation mammary gland involved immune signaling pathways but were not affected by NSAID treatment. C. M. Ylioja¹, A. J. Carpenter¹, J. J. M. Kim¹, J. Doelman², and J. P. Cant³.¹Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ²Nutreco Nederland BV, Boxmeer, the Netherlands.

Previous studies have shown that non-steroidal anti-inflammatory drug (NSAID) treatment in early lactation can have a positive impact on whole-lactation milk production in older cows. Our objective was to evaluate transcriptional changes in the mammary gland that could explain increased production responses. Sodium salicylate (SS; 125 g/d) or water (CON) were administered via oral drench to multiparous cows (n = 8/treatment) once daily for 3 d beginning approximately 24 h after parturition, and mammary tissue was collected on d 1, 4, and 45 postpartum. Day 1 tissue was collected immediately preceding the initial drench, and d 4 tissue was collected the day following the final drench. Total RNA extracted from tissue was deep sequenced and analyzed for differential gene expression using DESeq2. Only 16 of 18,286 genes were differentially expressed (false discovery rate < 0.1) on d 45 due to NSAID treatment. Given the lack of milk yield and a low mammary transcriptome response to SS, additional analyses focused on time-dependent effects. Of the > 8500 genes that were differentially expressed (DE) over time, those meeting cutoff values of 1.5-fold change and adjusted p-value of < 0.05 were used for functional analysis across time points in Ingenuity Pathway Analysis software. Analysis of transcriptional differences over time showed downregulation of pathways related to immune cell recruitment and differentiation, including cytokine signaling, TLR activation, inflammasome signaling, and interferon signaling, as well as cell growth and differentiation between wk 1 and 6 of lactation. STAT3 and PPAR signaling were upregulated on d 45 compared with the earlier time points. Additionally, DE genes in our data set showed extensive overlap with pathways related to cholesterol synthesis and retinoid X receptor signaling. Despite the low overall transcriptional effects of SS, transcriptome analysis emphasizes the extensive involvement of immune-related signaling pathways in the switch from lactogenesis to galactopoiesis.

Key Words: NSAID, transcriptome, immune function

T134  Peroxisome proliferator-activated receptor gamma (PPARγ) agonist does not overcome the effect of trans-10,cis-12 conjugated linoleic acid (CLA) but stimulate lipogenic gene expression in mammary explants cultured in vitro. W. B. Junior, P. C. Carraro, E. D. Silva, and D. E. Oliveira*, Santa Catarina State University, Lages, SC, Brazil.

The PPARγ is a ligand-dependent transcription factor coordinating lipogenic genes in the mammary gland and can be modulated by conjugated linoleic acid (CLA) and/or chemical agonists. This study used the PPARγ agonist Triazolinediona (TZD) to evaluate the effect on lipogenic gene expression. Mammary explants were cultured in vitro for 24 h with the following treatments: (1) Control: 400 µL of mammary epithelial growth medium; (2) TZD: Control + 40 µL of TZD (10 µmol/L); (3) CLA: Control + 30 µmol CLA (315 µmol/L) and (4) TZDCLA: Control + TZD (10 µmol/L) + 30 µmol CLA (315 µmol/L). The CLA used was a mixture 50:50 of cis-9,trans-11 and trans-10,cis-12. The RNA was extracted, complementary DNA (cDNA) synthesized and qRT-PCR carried out, measuring the gene expression of acetyl-CoA-carboxylase α (ACCα transcript from promoter III), fatty acid synthase (FASN), peroxisome proliferator-activated receptor gamma (PPARγ), sterol regulatory element binding protein-1 (SREBP1), sterol regulatory element-binding protein cleavage-activating protein (SCAP), stearoyl CoA desaturase (SCD), insulin-induced gene 1 (INSIG1), and insulin-induced gene (INSIG2). The data were analyzed using the PROC MIXED using treatment and sample as fixed effects. Analysis of the data set showed extensive overlap with pathways related to cholesterol metabolism and sample as fixed effects and the geometric mean of ribosomal protein 18 (S18) and β-actin as a covariate. Compared with Control, TZD treatment increased the gene expression of SREBP1 (P = 0.0001), INS1G1 (P = 0.0001), INS1G2 (P = 0.005), FASN (P = 0.0001), ACCα (P = 0.0001), SCD1 (P = 0.0001) e PPARγ (P = 0.0001) in 10.1-, 7.9-, 8.5-, 78.3-, 87.5-, 62.7-, and 6.2-fold, respectively. The CLA compared with Control, decreased the expression of FASN (P = 0.04), SCD (P = 0.01) e ACCα (P = 0.05), in 2.8-, 2.1- and 0.4-fold, respectively. Comparing TZDCLA and CLA treatments, TZDCLA decreased the expression of FASN (P = 0.01) and INS1G2 (P = 0.0005) in 2.7 and 15.5 fold, respectively. Overall, our results showed a positive and consistent effect of TZD increasing the gene expression of PPARγ and its targeted genes and CLA reducing the expression of genes involved in milk fat depression.

Key Words: milk fat depression, milk fat synthesis, nuclear receptor

T135  Effects of feed restriction on synthetic capacity of the bovine mammary gland. D. J. Seymour*, J. J. M. Kim¹, J. Doelman², and J. P. Cant³.¹Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, ²Nutreco Nederland BV, Boxmeer, the Netherlands.

Mechanisms that regulate the milk synthetic response to nutrient supply in lactating dairy cows remain largely unexplained. The objective of this study was to evaluate short- and long-term changes in expression of mammary genes related to secretory cell turnover and milk-synthesizing activity per cell in lactating Holstein dairy cattle subjected to restricted feeding. Pairs of cows (234 ± 27 DIM, n = 7) were blocked by date and average daily milk yield and fed either 100 or 60% of ad libitum intake for 14 d. The feed restriction treatment commenced with 16 h of no access to feed. Milk production dropped 4.8 kg/d by d 1 of restriction and remained at that level until d 14 (P < 0.01). On d 1, plasma glucose and BHBA concentrations did not differ between treatment groups (P > 0.67), but FA concentrations were 2 times higher (P < 0.01) in restricted cows. There were no differences in these metabolite concentrations between treatment groups on d 13 (P > 0.18). Mammary mRNA expression of milk protein genes and genes related to protein synthesis and secretion were not affected after 16 h of feed withdrawal (P > 0.10), but expression and protein abundance of cyclin D1 were downregulated 56 and 42% (P ≤ 0.04), respectively. After 14 d, cyclin D1 expression in mammary tissue was no longer low (P > 0.32) but expression of the pro-apoptotic DNA damage-inducible transcript 3 (aka CHOP) was elevated 69% (P = 0.04). There were no differences between treatments in mammary parenchymal DNA mass or proportions of proliferating and apoptotic cells on d 14 (P > 0.37). However, parenchymal tissue and protein mass were 24 and 29% lower, respectively, in restricted versus unrestricted cows (P = 0.03) and the glands produced 45% less milk daily per gram of parenchymal DNA. Results suggest that both mammary cell number and activity per cell are acutely regulated within 16 h of a change in total

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dietary nutrient supply, and that chronic changes in milk yields can be sustained without chronic changes in cell proliferation or apoptosis rates.

Key Words: lactation, feed restriction, cell turnover

T136  Comparison of metabolites and hormones involved in the control of energy partitioning during the lactation of dairy ewes and goats. M. F. Lunesu1, A. Prandi2, A. Comin3, G. C. Bomboi1, P. Sechi1, P. Nicolussi1, M. Decandia4, and A. Cannas*1, 1University of Sassari, Sassari, Italy, 2University of Udine, Udine, Italy, 3Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy, 4Dipartimento di Ricerca nelle Produzioni Animali, Agris, Olmedo, Italy.

This research studied the evolution of metabolites and hormones involved in the control of energy partitioning during early and mid-lactation of dairy ewes and goats and assessed in mid-lactation interactions with the type of carbohydrates used in the diet. Twenty Sarda ewes and 20 Saanen goats were compared from 15 ± 5 in milk (DIM; mean ± st.dev.) to 134 ± 5 DIM in the same feeding conditions. Since parturition, each species was fed a high starch diet (20.4% starch, 35.5% NDF, DM basis), whereas from 92 ± 11 DIM each species was allocated to 2 dietary treatments: high starch (HS; 20.0% starch, 36.7% NDF, DM basis) and low starch-high digestible fiber (LS: 7.8% starch, 48.8% NDF, DM basis) diets. The LS diet was obtained by substituting cereal grains with soyhulls. Blood samples were collected monthly and analyzed for plasma glucose, NEFA, growth hormone (GH), IGF-1 and leptin. Data were studied by using the PROC MIXED procedure of SAS for repeated measurements. From early to mid-lactation, glucose concentration was higher in ewes than in goats (54.6 vs. 48.4 mg/dl ± 1.2 (mean±SEM); P < 0.0001). NEFA concentration was lower in ewes than in goats (0.25 vs. 0.31 mmol/L ± 0.03; P = 0.036). IGF-1 concentration did not differ (108.8 vs. 94.2 ng/mL ± 11.64; P > 0.1). Goats had higher plasma GH (4.47 vs. 2.28 ng/mL ± 0.57; P < 0.001), with a marked peak in early lactation not observed in ewes, higher leptin concentration (26.3 vs. 11.4 ng/ml ± 2.1; P < 0.0002), and lower plasma insulin content (0.11 vs. 0.26 μg/L ± 0.02; P < 0.0001) than the ewes. In mid-lactation, metabolites and hormones were not affected by the diets in both species. In conclusion, this experiment found that (1) the ewes had a hormonal profile more directed to the partitioning of dietary energy in favor of body reserve accumulation, rather to milk production, than the goats; (2) in mid lactation the hormonal status was not affected by the prevalent type of carbohydrate (starch or digestible fiber) of the diets; iii) blood leptin was much higher in goats than in ewes, despite the latter accumulated much more body reserves than the former.

Key Words: energy partitioning, lactating ewe, lactating goat

T137  Effects of extracellular Zn and G protein-coupled receptor 39 silencing on immortalized bovine mammary epithelial (MAC-T) cells. J. E. Shaffer, L. K. Mamedova*, and B. J. Bradford, Kansas State University, Manhattan, KS.

Both form and concentration of supplemental Zn has been shown to impact milk production and mammary health in dairy cattle. However, the physiological mechanisms by which these effects are produced remain to be fully elucidated. One potential route is by direct effect on mammary epithelial cells (MEC). Zinc is known to act as a ligand for GPR39, a G protein-coupled receptor expressed in a variety of cell lines and tissues, where it promotes cell survival and proliferation by a Gq pathway characterized by intracellular Ca++ release followed by phosphorylation of kinases including ERK and AKT. The objective of this study was to characterize the presence and activity of GPR39 in an immortalized bovine MEC line (MAC-T). Using RT-qPCR, GPR39 was found to be expressed in a variety of bovine tissues as well as in MAC-T cells. Two siRNA constructs (siGPR39a and siGPR39b) were designed and utilized in vitro for the knockdown of GPR39 expression in MAC-T cells. Cells were cultured on 12-well plates, transfected with siGPR39a, siGPR39b, or a universal negative control (siCON) 24 h before subsequent treatments. Cells were then treated for 10 min with either a Zn-free physiological saline solution (0 Zn), or 100 μM Zn (100 Zn), and after another 10 min, cells were harvested for RNA and protein. Transcript abundance was determined by RT-qPCR and protein phosphorylation by Western blot. There was a tendency for 100 Zn to increase GPR39 mRNA abundance compared with 0 Zn in siCON cells (P = 0.096). In 100 Zn cells, transcript abundance of GPR39 was reduced 63% by siGPR39a (P = 0.02) and 57% by siGPR39b (P = 0.04). No effects of GPR39 knockdown, Zn treatment, or their interaction were observed on phosphorylation of AKT or ERK. 2 common intermediates of Gq signaling. In summary, extracellular Zn was not observed to activate Gq signaling in MAC-T cells regardless of GPR39 expression.

Key Words: zinc, MAC-T, lactation physiology

T138  The bovine milk microbiome and somatic cell count. S. L. Brooker*1, K. M. Yahvah1, B. A. Casperson2, J. E. Williams1, B. Shafii1, W. Price1, J. Tinker1, and M. A. McGuire1, 1University of Idaho, Moscow, ID, 2Purdue University, West Lafayette, IN, 3Boise State University, Boise, ID.

Efforts to determine causative agents in mammary inflammation in dairy cows are critical to animal welfare and economic viability. Two key questions to address are 1) what factors are important in maintaining a healthy milk microbiota and 2) what factors lead to the manifestation of bacterial infection or inflammation. Quarter milk samples from 103 mostly Holstein cows were obtained from 2 different dairies in Idaho. Characterization of the microbial community was performed by culture independent Illumina sequencing of amplicons from the V1-V3 hypervariable region of the 16S rRNA gene to determine relative abundance of bacteria present. Almost 45% of the reads were unclassified at the genus level, showing one of the limitations of this study. From the cows, 350 quarters had low somatic cell count (SCC) (<200,000 cells/mL), 26 had mid SCC (200,000–400,000 cells/mL), 3 quarters omitted due to missing SCC. Milk microbial communities were characterized with major membership by genera such as Staphylococcus (5–20%), Corynebacterium (5–10%), and Clotiodium XI (5%). Higher SCC quarters tended to have elevated amounts of Staphylococcus and Streptococcus. Using the nonnegative matrix factorization (NMF) methodology, the community structure was best described by Caryophanon, Coxiella, Gemella, and Luteipluratus, though the contribution differed greatly between quarters, SCC, and dairy. Overall, diversity (Shannon Diversity) was similar across quarters within an individual (16.22 – 17.68) whereas the diversity markedly decreased as SCC increased (18.04, 12.16, and 11.50 for low SCC, mid SCC, and high SCC, respectively). This pattern was also reflected by dairy. The dairy with a larger number of mid and high SCC quarters and had an overall lower average diversity (12.43) relative to the other dairy (21.87). In summary, quarters, though independent glands, appear to be biological replicates of the system under healthy conditions. More work is needed to determine the various aspects of microbial community structure that may confer health or disease states for mastitis in dairy cows. This work was supported by the Idaho State Board of Education.

Key Words: milk, microbiome, diversity
Effects of supplementary folic acid and vitamin B12 feed-restriction on immune cell functions and blood cell population in dairy cows. N. Vanacker*, C. Girard, M. Duplessis, and P. Lacasse, Agriculture and Agri-Food Canada, Sherbrooke Research and Development Center, Sherbrooke, QC, Canada.

Cows undergoing negative energy balance often experience a state of immunosuppression and are at greater risk of infectious diseases. The present study aimed at evaluating the impact of a folic acid and vitamin B12 supplement and feed restriction on some immune parameters. Sixteen cows at 45 ± 3 DIM were assigned to 8 blocks of 2 cows each according to their milk production during the previous week, 45 ± 6 kg/d, then within each block, they randomly received weekly intramuscular injections of either saline (C) or 260 mg of folic acid and 10 mg of vitamin B12 (V) for 5 wk. On wk 5, the cows were fed 75% of their ad libitum intake, 24 (±2.5) kg of DM/d, during 4 d. Blood sample samples were taken before the beginning of the experiment, just prior feed restriction and after 3 d of feed restriction to evaluate blood cell population, phagocytosis capacity and oxidative burst of polymorphonuclear leukocyte (PMN). The vitamin supplement did not affect any of the tested variables. Feed restriction reduced (P < 0.05) the percentage of PMN positive for phagocytosis. Accordingly, the percentage of PMN that were positive for oxidative burst after being stimulated with PMA was reduced by feed restriction (P < 0.05). Feed restriction did not affect blood cell population. In conclusion, feed restriction affected the functions of PMN, suggesting that the greater risk of infectious diseases in cows experiencing a negative energy balance is related to impaired immune cell functions.

Key Words: energy balance, phagocytosis, oxidative burst

Differential effects of lipopolysaccharide on expression of major milk protein genes in mouse mammary epithelial cells. Q. Tian*, A. Spitzer, and F.-Q. Zhao, Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT.

Mastitis is an endemic disease in the dairy industry and causes large economic losses to dairy farmers due to reduced milk yield and quality. Mastitis is inflammation of one or more mammary glands caused by bacterial infection. Lipopolysaccharide (LPS) is a major outer membrane component of gram-negative bacteria and a major endotoxin that elicits strong mammary inflammation. The major objective of this study was to investigate the effects of LPS on milk protein gene expression in mammary epithelial cells (MEC), using HC11, a mouse MEC line, as a model. HC11 cells were cultured with 0–500 µg of LPS for 3–24 h to assess cell viability through MTT assay and relative gene expression via real-time reverse transcription PCR. LPS reduced HC11 cell viability in a dose- and time-dependent manner as doses of ≥250 µg/mL reduced cell viability significantly at 4 h, but the threshold decreased to ≥100 µg/mL at 24 h (P < 0.05). However, as little as 0.1 µg/mL of LPS dramatically induced mRNA expression of inflammation markers IL-6, IL-1β and TNFα at 3 h and 24 h of treatment (P < 0.05). When HC11 cells were cultured in medium containing lactogenic hormones prolactin and glucocorticoids, treatment of the cells with 0.1–25 µg/mL of LPS for 3 h and 24 h reduced β-casein gene (CSN2) expression by 40–80% (P < 0.05), but surprisingly increased αS1-casein (CSN1S1) expression by 20–226 fold (P < 0.05). Expression of α-lactalbumin gene (LALBA) was also increased at low concentrations of LPS (0.5 and 1 µg/mL) at 3 h, but decreased by 37–72% at all concentrations tested at 24 h (P < 0.05). In summary, our data demonstrated novel differential effects of LPS on expression of 3 major milk protein genes in MECs, suggesting potential functional differences among these proteins during mastitis. Especially, the dramatic rise of αS1-casein expression by LPS raised a possible immune function of this protein in the mammary gland.

Key Words: gene expression, mastitis, milk protein
T140  Surface properties of fat globules and proteomic analysis of MFGM during temperature processing of milk. J. Ortega-Anaya* and R. Jiménez-Flores, The Ohio State University, Columbus, OH.

Processing milk at different temperatures has an impact on the physicochemical properties of the milk fat globule membrane (MFGM) which affect the surface properties of fat globules due to changes in the distribution and concentration of intrinsic proteins as well as other interacting as a consequence of the temperature. The aim of this study was to analyze the change in surface properties, such as zeta-potential and size distribution during different heat treatments and correlate it to shifts in proteins identified by 2D electrophoresis and a proteomic approach by tandem mass spectrometry. A pilot-scale study was performed using 5 conditions: no heating (raw), cooling at 4°C, batch (63°C for 30 min), high temperature-short time (HTST, 72°C, 16 s) and ultra-high temperature pasteurization (UHT, 121°C, 3 min). We identified 115 proteins classified in 12 families based on their function. The thermal treatments changed not only the native distribution of MFGM identified but also increased the adsorption and aggregation, especially of soluble protein complexes. The change in surface properties and charge distribution was a direct consequence of thermal denaturation that favors intermolecular disulfide bonds of proteins at the MFGM surface since the formation of SS interaction was proven to correlate with heat treatment of milk. Among the pasteurization treatments, UHT resulted in the highest variation on surface charge and loss of proteins such as butyrophilin compared with HTST processing which had less protein denaturation. This promises to deliver MFGM isolates that maintain nutritional and functional activities when they are included in food products.

Table 1 (abstract T140). Protein distribution on the MFGM and relative abundance after different heat treatments

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Key Words: MFGM, protein, surface properties

T141  The effect of emulsifying salts in binary combinations on the structure of casein micelles at varying pH, temperature, time and concentration. M. Culler, T. Thomas, M. Zaffuto*, A. Peleschak, and F. Harte, Pennsylvania State University, University Park, PA.

The Code of Federal Regulations (CFR) lists 13 emulsifying salts (ES) for pasteurized process cheese. Commercial manufacturers typically use a blend of ES to achieve a desired textural profile. Although it is known that ES act by changing the mineral equilibrium causing micellar dissociation, there are unexplained differences between specific ES. The aim of this study is to measure the effects of combinations of disodium phosphate (DSP), tetrasodium diphosphate (TSPP), sodium hexametaphosphate (SHMP), dipotassium phosphate (DPP) and trisodium citrate (TSC) on casein micelle stability in response to changes in temperature, pH, time, and emulsifying salt concentration (ES). Binary ES stock solutions were made in mM concentrations of 0:200, 50:150, 100:100, 150:50, and 200:50 in a 1-in-20 dilution of protein free serum (PFS) (3 kDa MWCO) in water. ES solutions were mixed with more diluted PFS to achieve total [ES] up to 198 mM. Skim milk was added to make up 2% of the volume, and turbidity was measured at 400 nm immediately (t = 0), after 30 s and after 30 min. ES solution, milk and diluted PFS solutions were pH-adjusted before measurement to either 5, 5.8, or 6.8. Duplicate measurements were conducted at room temperature and 50°C. Data were modeled using an exponential decay equation to calculate a threshold [ES] (Ct) that caused a rapid decrease in turbidity. When ES were combined, the resulting turbidity curves displayed characteristics of both individual ES curves. Increasing the interaction time universally decreased the Ct, while increasing temperature increased average system turbidity. As the ratio of the ES was varied, the resultant curve most closely resembled the curve of the highest [ES]. For example, when DSP and TSPP were mixed at room temperature, pH 6.8, and t = 0, the Ct was 8.8 mM for 150mM TSPP and 50mM DSP, however when the [ES] were reversed, the Ct increased to 59 mM. After displaying a decrease in turbidity when [TSPP] was increased to 50 mM (from 0.668 to 0.144 AU), turbidity increased (to 0.2785 AU) as [TSPP] increased to 198 mM, suggesting temporary aggregate formation not strongly observed when [SHMP] was increased.

Key Words: casein, emulsifying salt

T296  Period2 gene silencing increases the synthesis of casein protein in bovine mammary epithelial cells. L. Y. Hu1*, Y. J. Jing1, M. Z. Wang1, Q. Y. Xu1, J. L. Ouyang1, and J. L. Loor2* 1College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China, 2Mammalian NutriPhysioGenomics, Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL.

Studies have confirmed that Period2 (PER2), a core component of circadian oscillator, plays a key role in regulating biological circadian rhythm fluctuations including protein metabolism through transcriptional effects. This study employed bovine mammary epithelial cells (BMEC) as a model to investigate the effect of the suppression of PER2 expression on synthesis of casein protein. BMEC cells were established by enzymatic digestion of mammary tissue from mid-lactation cows and cultured in specific growth medium (DFEM/F12 as basis). Second passage BMEC were divided into a treatment and a control group both of which were transiently-transfected with siRNA or a negative control oligonucleotide. After 72 h incubation to ensure the suppression of PER2 expression, the mRNA abundance and protein abundance of CSN1S1, CSN1S2, CSN2, CSN3, and PER2 were measured by qRT-PCR and ELISA.
analysis of data were performed using an independent sample t-test module (SPSS16.0), with differences considered significant at $P < 0.05$. The results showed that PER2 silencing enhanced the synthesis of CSN1S1, CSN1S2, CSN2, and CSN3 in part through upregulating the mRNA abundance of CSN1S1 and CSN1S2. In conclusion, our results provide the basis for further studies aiming at understanding how PER2 regulates mammary gland function and milk protein synthesis.

**Key Words:** PER2 gene, mammary epithelial cell, casein

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**T298 Effects of milk-flavoring constituents on the fluorometric assay of bovine alkaline phosphatase.** E. M. Brock and Z. Ustunol,* Michigan State University, East Lansing, MI.

Determining the activity of residual alkaline phosphatase (ALP, EC 3.1.3.1) in milk validates adequate pasteurization and confirms that cross-contamination of raw milk has not occurred, thus ensuring product safety. However, flavored milks have been shown to yield false positive or false negative ALP activities, which inaccurately represents the microbiological safety of the milk sample. The aim of this study was to investigate the effects of 3 common milk-flavoring constituents on ALP activity using the fluorometric assay. Preliminary work determined no significant differences ($P > 0.05$) in ALP activity as a function of dual-stage homogenization (2,000 psi) or differing pasteurization methods including high temperature, short time (72°C for 15 s) and low temperature, long time (63°C for 30 min); therefore, homogenization and varying pasteurization conditions were not included as experimental treatments in the present study design. Raw milk was standardized to 3.25% milkfat. The batch of milk was divided into 4 treatment groups. Sucrose, pure vanilla extract, or cocoa solids were added at 8, 0.1, and 1.5%, respectively. A control (no ingredients) was included as the fourth group. All 4 treatments were pasteurized at high temperature, short time conditions. Experimentation was independently replicated 3 times, and each analysis was done in duplicate. ALP activity did not significantly differ among treatment groups ($P > 0.05$) and was adequately inactivated ($<350$ mU/L). However, the treatment containing cocoa solids had the highest ALP activity at 106.70 mU/L in comparison to the control at 19.32 mU/L, suggesting the primary polyphenols of the ingredient are inducing a conformational change of the enzyme or interacting with the quantified fluorescence produced. With flavored milk sales rising in recent years, ensuring product safety and understanding potential interactions is imperative in the dairy industry.

**Key Words:** alkaline phosphatase, flavored milk, fluorometric assay
Physiology and Endocrinology II

T142  Changes in duodenal protein expression in dairy calves at birth and 48 hours of age. 
S. L. Gelsinger*1 and A. J. Heinrichs2,
1The University of Wisconsin-Madison, Madison, WI, 2The Pennsylvania State University, University Park, PA.

The capacity for macromolecular absorption in the gastrointestinal tract of the newborn calf erodes with time after birth and ceases between 24 and 48 h of age. Little is known about mechanisms governing this process. Eight bull calves, whose births were observed, were removed from their dam before standing. Calves were slaughtered at birth or 48 h of age (n = 4/age). Calves slaughtered at 48 h received 4 L of colostrum within 4 h of birth and milk replacer at 24 and 36 h. Cross sections were collected from the duodenum midway between the pyloric sphincter and the liver. Sections were rinsed in deionized water, snap-frozen in liquid N and stored at −80°C. Samples were pooled to create 2 pools per age (0, 48) with 2 calves represented in each pool. Protein expression from 48-h pools was compared with 0-h pools using 2-2-D fluorescent gels. Proteins with approximately 2-fold greater expression from calves in one age group were further analyzed using mass spectrometry to identify unique proteins. Alcohol dehydrogenase and fructose-bisphosphate aldolase B expressions were 1.8-fold higher in calves at birth. Fifteen proteins had increased expression 48 h after birth; several identified proteins were involved in multiple physiological pathways. Functional groupings based on gene ontology were: immunity (n = 5), angiogenesis (n = 4), lipid metabolism (n = 3), protein synthesis (n = 3), nutrient delivery (n = 2), cell differentiation (n = 2), cell growth (n = 2), nervous system maturation (n = 1), and vitamin A metabolism (n = 1). Greatest increases in expression were for peroxiredoxin-6 (immunity), serum albumin (nutrient transport), and pre-mRNA processing factor 19 (protein synthesis). These proteins increased by 11.1-6.9 and 4.5-fold 48-h after birth, respectively. Results indicate that feeding colostrum to calves may initiate blood vessel, nervous, and other tissue development and may also be important for vitamin A metabolism. Subsequent studies aim to differentiate between effects of calf age and colostrum feeding.

Key Words: calf, intestinal development, protein expression

T143  Reproductive management strategies for first service in replacement dairy heifers. 
M. Masello*1, M. M. Perez1, G. E. Granados1, M. L. Stangaferro1, B. Ceglowski2, M. J. Thomas2, and J. O. Giordano1, 1Cornell University, Ithaca, NY, 2Dairy Health & Management Services, Lovellie, NY.

Reproductive programs that rely primarily on timed artificial insemination (TAI) may reduce days to pregnancy in dairy heifers. Thus, the objective was to compare the reproductive performance of heifers managed for 1st service with programs that relied primarily on insemination to estrus (AI) or TAI. Holstein heifers from 2 farms were randomized to receive 1st service at 12 mo of age [end of the voluntary waiting period (VWP)] with sexed semen after (1) PG+AIE (n = 279): AIE after PG injections every 14 d (up to 3) starting at the end of the VWP, (2) PG+TAI (n = 294): AIE after 2 PG injections 14 d apart (end of VWP at 2nd PGF). Heifers in PG+AIE and PG+TAI not AIE 9 d after the last PGF received the 5d-Cosynch protocol (5dCP = CIDR+GnrH-5d-CIDR-out+PGF-3d-GnrH+TAI), 3) TAI (n = 277): ovulation synchronization with the 5dCP protocol. Heifers not AIE by 72 h after PGF (end of VWP) received GnrH+TAI. After the 1st AI, heifers in all groups received AIE and TAI after 5dCP (initiated 3 ± 3 d post-AI) with conventional semen. Hazard of AI and pregnancy (days to event) for up to 45 d after

VWP were analyzed with Cox’s regression and binomial outcomes by logistic regression in SAS. Results are presented in Table. More heifers were AIE in the PG+AIE and PG+TAI than the TAI group. However, the hazard of AI was greater in the TAI than the other groups. Pregnancies per AI were similar for all AI combined, AIE, or TAI services. Time to pregnancy was reduced for the TAI than the PG+AIE group but it was similar for the TAI and PG+TAI and the PG+AIE and PG+TAI groups. We conclude that a program for first AI that relied primarily on TAI reduced time to AI when compared with programs that resulted in most AI (>80%) and reduced time to pregnancy when compared with a program with almost 100% AIE. Supported by NYFVI AOR15–020.

Table 1 (abstract T143).

<table>
<thead>
<tr>
<th>Item</th>
<th>PG+AIE</th>
<th>PG+TAI</th>
<th>TAI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazard of AIE, HR</td>
<td>1.16</td>
<td>1.85</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>All AI P/AI 1st AI, %</td>
<td>42.3</td>
<td>46.9</td>
<td>43.0</td>
<td>0.54</td>
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<tr>
<td>P/AI AIE 1st AI, % (no.)</td>
<td>42.4</td>
<td>50.4</td>
<td>50.0</td>
<td>0.17</td>
</tr>
<tr>
<td>P/AI TAI 1st AI, % (no.)</td>
<td>—2</td>
<td>30.0</td>
<td>41.3</td>
<td>0.17</td>
</tr>
<tr>
<td>Hazard of pregnancy 1</td>
<td>1.02</td>
<td>1.23</td>
<td></td>
<td>0.07</td>
</tr>
</tbody>
</table>

1HR TAI vs PG+TAI 1.58 (1.33–1.87).
2PG+AIE group: TAI P/AI not included because n = 3.
3HR TAI vs PG+TAI 1.20 (0.99–1.46).

Key Words: estrus, timed AI, heifer

T144  Response of patatin-like phospholipase domain-containing protein 3 abundance to fatty acid treatment in bovine primary hepatocytes. 
H. T. Holdordt*, R. S. Pralle, M. T. Lavarias, Z. Zhang, T. L. Chandler, and H. M. White, University of Wisconsin-Madison, Madison, WI.

Patatin-like phospholipase domain-containing protein 3 (PNPLA3) is a membrane bound lipase that has been associated with the accumulation, and potentially with the subsequent depletion, of liver lipids in dairy transition cows. Hepatic PNPLA3 abundance is increased postcalving during remobilization of liver lipids, and is correlated with nonesterified fatty acids (NEFA) concentrations. Fatty acids are known regulators of hepatic gene and protein abundance. Therefore, the objective of this study was to determine if hepatic PNPLA3 abundance is responsive to incremental changes in concentration of fatty acids in primary hepatocytes. Primary hepatocytes isolated from 4 Holstein calves were maintained as monolayer cultures for 24 h before treatment. Treatments consisted of a NEFA free control, palmitic acid (C16:0), oleic acid (C18:1n-6), α-linolenic acid (C18:3n-3), and a fatty acid cocktail with a profile of fatty acids reflective of NEFA at parturition. The fatty acid cocktail consisted of 3% C14:0, 27% C16:0, 23% C18:0, 31% C18:1n6, 8% C18:2 n-6, and 8% C18:3n-3. Abundance of PNPLA3 was determined by Western blot analysis and normalized to total lane protein. For analysis, PNPLA3 was expressed relative to a fatty acid free control, and transformed as log(relative abundance +1) because data were not normally distributed. Data were analyzed for main effects of treatment and concentration, and linear and quadratic contrasts of concentration using PROC MIXED in SAS 9.4. Addition of any fatty acids decreased (P < 0.05) PNPLA3 compared with fatty acid free
control. Increasing fatty acid concentrations linearly decreased \( P < 0.05 \) PNPLA3 abundance. Fatty acid treatment had an effect \( P < 0.05 \) on PNPLA3 abundance with the fatty acid cocktail tending to result in greater PNPLA3 abundance than palmitic acid. These data indicate that PNPLA3 abundance may be regulated by fatty acids. This could suggest that changing fatty acid concentrations during the transition period could play a role in regulating PNPLA3 abundance during this time.

**Key Words:** adiponutrin, transition cow, fatty liver syndrome

**T145 Influence of adipocyte size and adipose depot on the expression of adipokines in dairy cows at the end of pregnancy.** J. De Koster*, M. Van Poucke, M. Hostens, K. Hermans, W. Van den Broeck, L. Peelman, and G. Opsomer, 1Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, 2Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Gent, Belgium, 3Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Gent, Belgium, 4Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Gent, Belgium.

The aim of this study was to determine the mRNA expression of adiponectin (ADIPOQ), leptin (LEP), interleukin 6 (IL6) and tumor necrosis factor (TNF) in adipose depots from cows with a variable body condition score (BCS 2.75–5) at the end of the dry period. Ten pregnant dairy cows were euthanized 10 to 13 d before the expected parturition date. Immediately after euthanasia, samples of subcutaneous, omental, mesenteric, perirenal and intrapelvic adipose depot were taken and snap frozen in liquid nitrogen or fixed in 4% formaldehyde. Expression of the different genes was determined by RT-qPCR according to the MIQE guidelines. Expression is represented as relative mRNA abundance after normalization with 3 reference genes (MARVELD1, EIF3K, LRP10) determined using geNorm. Size of the adipocytes was determined by measuring the area of 100 adipocytes on histological sections after HE staining. Statistical analyses were performed using SAS version 9.4. A generalized linear mixed model was built with expression of the individual genes as dependent variable, depot, adipocyte area and the interaction between depot and adipocyte area included as independent variables and cow was included as random factor. Pairwise comparisons between depots were checked using the Tukey post-hoc test. Adipocyte size (10,243 ± 1,347\( \mu m^2 \)) was positively related to the expression of LEPT (0.38 ± 0.05; \( \beta = 102.87 \)), IL6 (0.23 ± 0.04; \( \beta = 78.44 \)), TNF (1.03 ± 0.13; \( \beta = 66.50 \)) and ADIPOQ (0.43 ± 0.04; \( \beta = -22.79 \); not significant) and the size of the adipocytes. The expression of the different adipokines was clearly different between the different adipose depots. In general, the expression of pro-inflammatory adipokines (LEPT and IL6) was greater in the internal compared with the subcutaneous adipose depots. In conclusion, the results of the present study indicate a pro-inflammatory state of adipose depots with dysregulation of inflammatory processes and contribute to metabolic and infectious disorders in overweight dairy cows at the end of pregnancy, which might induce a dysregulation of inflammatory processes and contribute to metabolic and infectious disorders in overweight dairy cows at the end of pregnancy. Thus, adipose tissue, adipokine, transition period

**Key Words:** adiponectin, transition cow

**T147 Effects of fatty acid supplementation on oxidative status of red blood cells in dairy cows fed a ration with low n-3 fatty acid content.** D. Revski, D. Dipasquale, U. Bernabucci, S. Haubold, C. Kröger-Koch, A. Tuchscherer, H. J. Schuberth, H. Hammon, and M. Mielenz*, 1Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, 2Department of Agriculture and Forestry Sciences, University of Tuscia, Viterbo, Italy, 3BASF SE, Limburgerhof, Germany, 4Immunology Unit, University of Veterinary Medicine, Hannover, Germany.

Reactive oxygen species (ROS) are formed under physiological and pathophysiological conditions. High-yielding dairy cows exhibit a high metabolic activity during lactation that increases the risk of oxidative stress (OST). Fatty acids (FA) can increase ROS but specific essential FA and conjugated linoleic acid (CLA) are also discussed to prevent OST. Common diets in dairy farming often deliver lower amounts of n-3 FA compared with pasture based systems. The objective was to evaluate the effects of a diet low in n-3 FA content, followed by FA supplementation on the oxidative status (OS) in red blood cells (RBC). Four rumin fistulated Holstein cows (126 ± 4 d in milk) fitted with abomasal infusion tubes were arranged in a 4 x 4 Latin square model. Cows were fed a TMR based on corn silage and supplemented twice per day with 3 successively rising lipid dosages. Each dosage was given for 2 wk, resulting in a 6-wk treatment period, followed by a 3-wk wash out phase. Supplements were coconut oil delivering medium-chain FA (38, 77 and 153 g/d, respectively), linseed-safflower oil mix, delivering n-3 FA (EFA; 42, 82 and 163 g/d, respectively), LUTALIN (9,11 and 281
Fatty acid composition of red blood cell membranes of dairy cows fed a diet with low n-3 fatty acid content and effects of fatty acid supplementation. D. Revskij¹, S. Haubold¹, C. Kröger-Koch¹, H. Kienemberger¹, M. Rychlík¹, A. Tuchscherer¹, A. Tröschér⁴, H. J. Schuberth², H. M. Hammon¹, and M. Mielenz*¹, ¹Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, ²Bavarian Biomolecular Mass Spectrometry Center, Technical University of Munich, Freising, Germany, ³Analytical Food Chemistry, Technical University of Munich, Freising, Germany, ⁴BASF SE, Limburgerhof, Germany, ⁵Immunology Unit, University of Veterinary Medicine, Hannover, Germany.

Incorporation of dietary n-3 fatty acids (FA) in plasma membranes influences membrane fluidity and function. Common diets for dairy cows often deliver lower amounts of n-3 FA compared with pasture-based systems. Objective was to evaluate effects of a diet low in n-3 FA (Trial 1) and with additional FA supplementation (Trial 2) on the FA composition of red blood cell (RBC) membranes. Cows were fed a corn silage based TMR for 24 weeks (n = 5) in Trial 1 and 12 weeks in Trial 2 (n = 4). In Trial 1 RBC were collected in week −1, 0, 1, 2, 8, 16, 24. In Trial 2 cows, fitted with abomasal infusion tubes, were arranged in a 4 × 4 Latin square model after the 12 weeks and supplemented with 3 successively rising lipid dosages for 2 weeks, respectively, followed by a 3 week wash out phase. Supplements were coconut oil, linseed-safflower oil mix, the latter delivering n-3 FA (EFA), LUTALIN (c9,t11 + t10,c12 CLA; Ludwigshafen, Germany) or both, EFA + CLA. Membranes were isolated at the end of each treatment and wash out period. Data were analyzed with the MIXED procedure of SAS using a repeated measures ANOVA model with treatment as fixed effect and week in milk as covariate. LSMMEANS were compared by Tukey test. Linolenic acid as well as c9,t11 and t10,c12 CLA increased dose dependently (P < 0.05) in milk fat and plasma of the respective treatment groups. The analyzed indicators of OST in RBC were not affected by the different supplements. These data indicate that a diet low in n-3 FA may has no effects on the OS in RBC of mid-lactating dairy cows.

Key Words: dairy cow, oxidative stress, essential fatty acids

T149 Sorbic acid is rapidly absorbed but does not affect plasma leptin and adiponectin concentrations in milk-fed calves. M. Mielenz*¹, S. Görs³, A. Tuchscherer¹, H. Sauerwein², and J. J. G. C. van den Borne³, ¹Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, ²Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Bonn, Germany, ³Wageningen University, Animal Nutrition Group, Wageningen, the Netherlands.

Adipose tissue contributes to whole body energy partitioning and the adipokines leptin and adiponectin have been associated with lipid and glucose metabolism. Propionate as a ligand for the free fatty acid binding receptors FFAR1 and FFAR2 increases leptin secretion in mice by activation of FFAR2 and increases leptin mRNA abundance in subcutaneous adipose tissue of sheep. Potassium sorbate is commonly used as a preservative in milk replacer. Interestingly, sorbic acid is a specific ligand for FFAR2 in cattle, but effects on adipokine release are unknown. Aim of the current study was therefore to analyze effects of dietary sorbic acid supplementation on plasma concentrations of sorbic acid, leptin and adiponectin in milk-fed calves. Eight predominantly milk-fed male Holstein Friesian calves (145 ± 2 kg of BW) were equipped with semipermanent catheters in the jugular vein. Four calves were fed with a milk replacer containing 2% sorbic acid at 0630 and 1530 h for 2 consecutive days. Blood samples were taken at −10, 15, 30, 60, 120, 180, 240, 300, and 360 min relative to the last feeding. Sorbic acid uptake was analyzed in pooled samples after protein precipitation with acetonitrile by HPLC on a Rezex ROA-organic acid column (Phenomenex, Aschaffenburg, Germany) at 60°C and a flow of 0.4 mL/min with 0.5 mM sulfuric acid as eluent and UV detection at 262 nm. Plasma concentrations of leptin and adiponectin were measured by ELISA. Data were analyzed with the MIXED procedure of SAS using a repeated measurement ANOVA model with treatment as fixed effect. There were no feed refusals of milk replacer containing 2% sorbic acid. One (control) calf could not be sampled due to malfunction of the catheter. An immediate increase of sorbic acid was observed in plasma with a peak at 15 min after the onset of feeding. Plasma leptin and adiponectin concentrations were not affected by supplementing sorbic acid. In conclusion, uptake of sorbic acid from milk replacer occurs very rapidly in calves but sorbic acid supplementation (2%) did not significantly affect plasma leptin and adiponectin in milk-fed calves.

Key Words: sorbic acid, milk replacer, adipokine

T150 Markers of mineral metabolism in non-lactating, non-pregnant Holstein cows fed DCAD rations with low, medium, or high concentrations of calcium and challenged with hypocalcemia. A. P. Prichard*, C. E. Wimmler¹, L. A. Amundson¹, A. Cheng¹, M. Klister¹, T. Munoz¹, S. R. Weaver¹, A. D. Rowson², S. S. Bascom², D. E. Nuzback², K. P. Zanzalari², and L. L. Hernandez¹, ¹University of Wisconsin-Madison, Madison, WI, ²Phibro Animal Health Corporation, Teaneck, NJ.

Feeding a negative dietary cation-anion difference (-DCAD) ration has proven to be an effective method of preventing hypocalcemia. Urine pH is a common method of determining level of diet acidification. Furthermore, urine analyses of Ca, Mg, and P are indicators of mineral status in the cow, and urine deoxypyridinoline (DPD) is a common measurement for bone turnover. Multiparous, non-lactating, non-pregnant Holstein cows were fed -DCAD rations with low, medium, or high concentrations of Ca for 21 d and subjected to a controlled induction of hypocalcemia with 5% ethylene glycol tetraacetic acid (EGTA). Low Ca (LC) cows (n = 5) were fed 0.45% Ca, medium Ca (MC) cows (n = 6) were fed 1.13% Ca, and high Ca (HC) cows (n = 6) were fed 2.02% Ca. Aver-
age DCAD was −15.1 mEq/100g DM. Urine and blood samples were collected and urine pH measured daily during the feeding period. Cows were reduced to 60% of preinfusion blood ionized Ca (iCa) during the EGTA challenge, and evaluated until they reached 90% of preinfusion concentrations. A urine sample was collected before the start of EGTA challenge and 30 min later. Urine pH was less than 6.0 in all cows, but was lowest in LC cows during the feeding period (P = 0.02). Blood pH, hematocrit, and serum total Ca, Na, K, total protein and serotonin did not differ among treatments during the feeding period. Blood iCa and glucose were significantly elevated in HC compared with the LC and MC cows during the feeding period (P < 0.05). All urine analyses were corrected to creatinine concentration. Urine Ca, P, Mg and DPD did not differ among treatments at any time. During the EGTA challenge, urine pH decreased over time (P = 0.005), but was not different among treatments. These data suggest that urine mineral concentrations remained relatively constant regardless of dietary calcium, and were unaffected by EGTA challenge. Additionally, a HC diet increased blood iCa and glucose concentrations during the feeding period compared with MC and LC cows, suggesting feeding HC rations with −DCAD may improve metabolic markers of health in the dairy cow.

Key Words: urine, calcium, DCAD

T151 Identification of metabolic differences in dairy cows consuming corn stover and rice straw through liver metabolomics and transcriptomics. H. Sun1,2, H. Liu1, D. Wang1, L. L. Guan*,2, and J. Liu1,1Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China, 2Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada.

Both corn stover and rice straw as extensively available crop byproducts are rich in neutral detergent fiber and low in crude protein, which lead to lowered performance when they are fed to dairy cows. However, the metabolic mechanism behind is not clearly defined. This study was conducted to investigate the metabolic profiling and gene functions of liver using gas chromatography–time of flight/mass spectrometry based metabolomics and RNA-seq based transcriptomics when cows fed with rice straw (RS, n = 6) and corn stover (CS, n = 6), respectively. A total of 267 metabolites were measured in the liver. The principal component analysis (PCA), partial least squares discriminant analysis, and orthogonal partial least squares discriminant analysis showed that the metabolite profiles were separated based on diet. Among them, 24 significantly different metabolites (VIP > 1 and P < 0.05) were identified in the liver between CS and RS groups with 12 metabolites significantly higher and 12 metabolites significantly lower in the CS group. Pathway analysis based on significantly different metabolites revealed 5 key pathways (Impact value >0.5): linoleic acid metabolism, Gly, Ser and Thr metabolism, Phe, Tyr and Try biosynthesis, Ala, Asp and Glu metabolism, and Val, Leu, and Ile biosynthesis. In addition, 22 significantly different expressed genes (P < 0.0005, FDR <0.2) were identified in the liver between 2 groups. The molecular functions based on differential expressed genes indicated that the function of identical protein binding was upregulated in liver of the CS-fed cows (FDR = 0.0016) compared with those fed RS. Integrated analysis of metabolomics and transcriptomics revealed that Val, Leu, and Ile biosynthesis, and linoleic acid metabolism were the 2 top functional pathways that altered between CS and RS diets. Our results provide fundamental understanding on liver metabolic function and the mechanism behind which may provide information for future low quality forage utilization.

Key Words: liver, metabolomics, transcriptomic

T152 Expression of IgG receptor and tight junction protein in neonatal calf intestine. S. L. Gelsinger*,1, L. L. Hernandez1, and A. J. Heinrichs2,1The University of Wisconsin-Madison, Madison, WI, 2The Pennsylvania State University, University Park, PA.

Capacity for immunoglobulin G (IgG) absorption in the gastrointestinal tract of newborn calves erodes with time after birth and ceases between 24 and 48 h of age. Loose tight junctions in intestinal epithelium and endocytosis via the neonatal Fc receptor (FcRn) have been suggested as possible mechanisms of IgG absorption. The objective of this experiment was to compare gene expression of FcRn and tight junction protein, ZO1, in intestinal sections of calves up to 48 h of age. Bull calves (n = 16) were removed from their dam and slaughtered at 0, 12, 24, or 48 h of age (n = 4/age). All calves except those slaughtered at birth received 4 L of colostrum within 4 h of birth. Calves slaughtered at 48 h also received milk replacer at 24 and 36 h. Cross sections were collected from the duodenum, jejunum, ileum, and colon, rinsed in deionized water, and snap-frozen in liquid N. Total RNA was used to create cDNA and real-time polymerase chain reaction was performed. Fold changes in expression were normalized, first to geometric mean values for 2 housekeeping genes, and then to average values from the same intestinal section of calves slaughtered at birth. Data were ranked and mixed model analysis included intestinal section and calf age as fixed effects, and calf as a random repeated effect. Expression of FcRn and ZO1 differed by intestinal section and FcRn expression tended to increase with time after birth. Mean (±SEM) fold changes in ZO1 expression were 1.05, 0.95, 1.14, and 1.02 (±0.04) in duodenum, jejunum, ileum, and colon, respectively. No differences were observed with calf age. Mean fold changes in FcRn expression were 2.14, 2.53, 2.35, and 1.99 (±0.45) in duodenum, jejunum, ileum, and colon, and 1.27, 1.63, 2.70, and 3.41 (±0.63) at 0, 12, 24, and 48 h, respectively. Intestinal expression of FcRn was lowest at birth, whereas capacity for IgG absorption is greatest at birth; therefore, it is unlikely that FcRn is a major route for IgG absorption in neonatal calves. Tight junction formation as indicated by ZO1 expression was highly variable and not related to time after birth. Subsequent studies of protein expression are needed to confirm these results.

Key Words: calf, IgG absorption, neonatal Fc receptor (FcRn)

T153 Effect of one versus two prostaglandin F2α injections on progesterone concentrations and luteolysis in dairy cows subjected to a 5-d CIDR-Cosynch. J. Spencer*,1, K. Carnahan1, W. Price2, B. Shafii2, and A. Ahmadzadeh1,1Animal and Vet. Sci., University of Idaho, Moscow, ID, 2Statistical Program, University of Idaho, Moscow, ID.

The objective of this study was to examine the effects of one or 2 doses of prostaglandin F2α (PG) on progesterone (P4) concentration profiles, and luteal function in dairy cows subjected to a 5-d CIDR-Cosynch protocol. On d 0, 43 cows received GnRH (100µg; i.m.), and a CIDR was inserted. On d 5, the CIDR was removed and cows were assigned randomly to receive one dose of PG (25mg) (1PG; n = 15), or 2 doses (25mg/dose) of PG 12 h apart (2PG; n = 19). On d 5 and after CIDR removal, serial blood samples were collected every 12 h from d 5 to 8 to measure P4 concentrations. On d 8, all cows received a second GnRH injection and were inseminated. Ovaries were examined by ultrasonography on d 0, 5 and 8, and ovarian structures were recorded. Nine cows were determined acyclic and/or did not have luteal tissue at CIDR removal, thus were removed from this study. A repeated measures, generalized linear mixed model ANOVA was used to determine differences in P4 concentrations. The model included treatment, the repeated factor time, and time by treatment interaction as fixed effects, and cow
within treatment was the random effect. The $P_4$ data were assumed to follow a lognormal distribution. On d 5, all cows had a CL or developed luteal tissue, and $P_4$ concentrations were similar between groups ($P > 0.1$) before treatments. As expected, $P_4$ concentrations decreased over time in both treatments ($P < 0.01$). However, there was a significant effect of treatment by time interaction on $P_4$ concentrations ($P < 0.01$). By 24 h after treatments, $P_4$ concentrations were lower ($P < 0.01$), and remained lower ($P < 0.01$) throughout the remainder of the experiment in 2PG compared with 1PG. By 72 h post-treatment, the average $P_4$ concentrations were 1.03 ng/mL and 0.05 ng/mL for 1PG and 2PG, respectively. These results indicate differences in $P_4$ response between 1PG and 2PG in cows subjected to a 5-d CIDR-Cosynch. Compared with 1PG, 2PG injections (12 h apart) was more effective in inducing luteolysis, as $P_4$ concentrations were significantly less by the time of AI.

Key Words: prostaglandin F$_{2\alpha}$, progesterone, luteolysis

T154 Delaying administration of prostaglandin F$_{2\alpha}$ by 24 hours during a Double-Ovsynch protocol decreased fertility of lactating Holstein cows to timed artificial insemination. A. M. Niles*, A. E. Jones, P. D. Carvalho, and P. M. Fricke, Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.

To determine timing and frequency of PGF$_{2\alpha}$ treatment on luteal regression, lactating Holstein cows (n = 562) were submitted to a Double-Ovsynch protocol as follows: 2 25 mg PGF$_{2\alpha}$ (Lutalyse Sterile Solution, 5 mg/mL) treatments on d 7 and 8 (Control; 3 d, G2; 16 h, TAI) during July and August, 2017. Cows were randomly assigned to treatments with PGF$_{2\alpha}$ (dinoprostone tromethamine; Zoetis) to induce luteal regression during the Breeding-Ovsynch protocol as follows: 2 25 mg PGF$_{2\alpha}$ (Lutalyse Sterile Solution, 5 mg/mL) treatments on d 7 and 8 (Control; n = 182); one 25 mg PGF$_{2\alpha}$ (Lutalyse Hycon, 12.5 mg/mL) treatment on d 7 (D7; n = 195); or one 25 mg PGF$_{2\alpha}$ (Lutalyse Hycon) treatment on d 8 (D8; n = 185). Blood samples collected from all cows on d 7 and at G2 of the Breeding-Ovsynch protocol were assayed for progesterone (P4) by RIA. Data were analyzed by logistic regression using the GLIMMIX procedure of SAS. Overall, primiparous cows had more ($P < 0.01$) P/AI than multiparous cows 39 (46% vs. 23%) and 74 (42% vs. 19%) d after TAI and there was no treatment by parity interaction. D8 cows had fewer ($P < 0.01$) P/AI than D7 cows 39 (31% vs. 40%), and 74 (32% vs. 34%), respectively) d after TAI. Overall, cows (n = 45) with low P4 ($<0.1$ ng/mL) at PGF$_{2\alpha}$ had fewer ($P < 0.01$) P/AI 39 d after TAI than cows (n = 504) with high P4 ($\geq0.5$ ng/mL) at PGF$_{2\alpha}$ (13% vs. 36%). Among cows with high P4 at PGF$_{2\alpha}$, cows with low P4 ($<0.5$ ng/mL) at G2 had more ($P < 0.01$) P/AI 39 d after TAI than cows with high P4 ($\geq0.5$ ng/mL) at G2 (38% vs. 17%, respectively), and more (P < 0.01) D8 cows had high P4 ($\geq0.5$ ng/mL) at G2 (30%) than Control and D7 cows (9% and 10%, respectively). We conclude that delaying PGF$_{2\alpha}$ treatment by 24 h during the Breeding-Ovsynch portion of the Double-Ovsynch protocol resulted in decreased fertility due to delayed luteal regression before TAI. Supported by USDA NIFA Hatch project 1006519

Key Words: Double-Ovsynch, PGF$_{2\alpha}$, luteal regression

T155 A higher plane of nutrition in pre-weaning Holstein heifer calves alters transcriptome profiles in mammary parenchyma and fat pad. S. Zhan*1,2, A. J. Geiger3, J. C. McCann1, M. Vailati-Riboni1, R. M. Akers1, and J. J. Loor1, 1University of Illinois, Urbana-Champaign, Urbana, IL, 2Virginia Polytechnic Institute and State University, Blacksburg, VA.

Objectives were to study how pre-weaning nutrition alters the mammary transcriptome of Holstein calves using RNA-sequencing. The control (CON) group was fed 0.44 kg/d of milk replacer (MR, 20.9% CP, 19.8% fat), while the higher-plane of nutrition group (HIG, 28.9% CP, 26.2% fat) was fed 1.08 kg/d of MR. Starter feed was introduced at the end of wk 4 and MR was reduced in both treatments to 50% at wk 8 to induce weaning. Calves (n = 6 per diet) were sacrificed upon weaning (wk 8). Parenchyma (PAR) and mammary fat pad (MFP) tissue were removed and snap-frozen in liquid-N until RNA extraction. A total of 167 differentially expressed genes (DEG) (adjusted p-value <0.05, fold change >1.5) in the MFP and 487 DEG in the PAR were detected in response to HIG. Of those, 108 were upregulated and 59 were downregulated in the MFP and 319 were upregulated and 168 were downregulated in the PAR. The Dynamic Impact Approach with the Kyoto Encyclopedia of Genes and Genomes pathway database was used for bioinformatics analysis of DEG. In the MFP, the most-impacted pathways by HIG included ‘Glycan Biosynthesis and Metabolism’, ‘Metabolism of Co-factors and Vitamins’, ‘Lipid Metabolism’, and ‘Carbohydrate Metabolism’. In PAR, most-impacted pathways included ‘Lipid Metabolism’, ‘Amino Acid Metabolism’, ‘Metabolism of Co-factors and Vitamins’, ‘Energy Metabolism’, and ‘Glycan Biosynthesis and Metabolism’. Upstream transcription regulator analysis of DEG affected by HIG (fold change > [1.5] versus CON) using Ingenuity Pathway Analysis identified 4 transcription regulators (TR) (in MFP (FOS, EGR1, ZFP36, and EGR2) and 16 in PAR (DLX4, PAX1, EGR2, FOXM1, E2F7, E2F2, E2F3, CCNE1, BRCA1, KLF4, TP73, KLF15, FOSB, FOSS, PER2, MYOCD, and FOS)). Several downstream upregulated and downregulated target genes were associated with these TR, underscoring the role of molecular networks on tissue-weaning nutrition regulates mammary development. The relevance of these molecular alterations to future milk production performance remain to be determined.

Key Words: accelerated nutrition, bioinformatics, milk replacer

T156 Impact of higher plane of nutrition and post-weaning exogenous estrogen on transcriptome profiles in mammary parenchyma and fat pad of Holstein heifer calves. S. Zhan*1,2, A. J. Geiger3, J. C. McCann1, M. Vailati-Riboni1, R. M. Akers1, and J. J. Loor1, 1University of Illinois, Urbana-Champaign, Urbana, IL, 2Sichuan Agricultural University, Chengdu, Sichuan, China, 3Virginia Polytechnic Institute and State University, Blacksburg, VA.

Objectives were to study how pre-weaning nutrition and estradiol alter the mammary transcriptome of Holstein calves using RNA-sequencing. Twenty-four Holstein calves were assigned to 2 experimental milk replacers (MR, n = 12/group): control (CON, 20.9% CP, 19.8% fat) MR fed at 0.44 kg/day, or enhanced MR (HIG, 28.9% CP, 26.2% fat) fed at 1.08 kg/day. Starter feed was introduced at the end of wk 4 and MR was reduced in both treatments to 50% at wk 8 to induce weaning. MR was fed for 8 weeks. At the end of wk 8, calves were given an estradiol (E2) implant or placebo. Treatment groups were (n = 6/group): CON plus placebo (R), CON plus E2 (R-E2), HIG plus placebo (EH), and HIG plus E2 (EH-E2). Two weeks post-E2 all calves were sacrificed and mammary parenchyma (PAR) and fat pad (MFP) removed and snap-frozen in liquid-N until RNA extraction. Twenty-two (10 upregulated, 12 downregulated) and 351 (69 upregulated, 282 downregulated) differentially expressed genes (DEG, adjusted p-value <0.05, fold change (FC) > [1.5]) were detected in the MFP in response to HIG and
E2, respectively. A total of 369 (210 upregulated, 159 downregulated) and 427 (103 upregulated, 324 downregulated) DEG were identified in the PAR in response to HIG and E2, respectively. Bioinformatics analysis revealed that in the MFP, the most-impacted metabolic pathways by HIG included ‘Metabolism of Cofactors and Vitamins’, ‘Amino Acid Metabolism’, and ‘Carbohydrate Metabolism’. Among the most-impacted pathways by E2 were ‘Lipid Metabolism’, ‘Amino Acid Metabolism’, and ‘Glycan Biosynthesis and Metabolism’, with most being downregulated. Upstream transcription regulator analysis of DEG affected by HIG and E2 using Ingenuity Pathway Analysis identified 17 and 4 transcription regulators (TR) in MFP, while 12 and 427 (103 upregulated, 324 downregulated) DEG were identified E2, respectively. A total of 369 (210 upregulated, 159 downregulated) DEG were identified in the PAR in response to HIG and E2, respectively. Several downstream upregulated and downregulated target genes are linked with these TR, underscoring the role of molecular networks on tissue development in response to HIG and E2. These results provide an integrated understanding of the molecular basis of how pre-weaning nutrition and post-weaning exogenous estrogen regulate mammary development.

Key Words: accelerated nutrition, estrogen, bioinformatics

T157 Perinatal effects of feeding rumen-protected methionine donors to dams on hepatic gene expression in Holstein calves. C. Besperhok Jacometo*1, P. Montagner2, Z. Zhou3, F. Lopes4, D. Bogotá, DC, Colombia, 2Universidade Federal de Pelotas, Pelotas, RS, Brasil, 3University of Illinois, Urbana, IL, 4Adisseo SA, São Paulo, SP, Brazil, 5Adisseo NA, Alpharetta, GA.

The aim of this study was to assess the effect of feeding a methionine (MET) or choline (CHO) source to dams on neonatal calf liver expression of genes related to methyl-donor pathways and energy metabolism. The experiment was conducted as a randomized complete block design with 2 × 2 factorial arrangement of MET (Smartamine M, Adisseo NA) and CHO (ReaShure, Balchem Inc.) level (with or without). Eighty Holstein calves born to cows receiving during the last ~4 wk of pregnancy MET (0.08% DM; n = 20), CHO (60 g/d; n = 20), MIX (MET+CHO; n = 20) or control (CON; n = 20) were evaluated. Immediately after birth calves were separated from the dam, fed first colostrum, housed individually and fed a common milk replacer twice daily. Liver biopsies were harvested (n = 8/group) at 4 d of age for qPCR analysis. Data were analyzed using the MIXED procedure of SAS, with MET and CHO as a fixed effect, and also a methyl donor contrast effect was tested. Both methyl donors upregulated (P < 0.05) the expression of CBS, BHMT and MTR, while they downregulated (P < 0.001) SAHH expression, all of which are related to the one-carbon and methionine pathways and the transsulfuration pathway. MAT2A expression was downregulated (P = 0.05) in liver of MET calves and upregulated (P = 0.02) in CHO calves. Expression of PEMT, MAT1A and CHDH was not affected (P > 0.05) by maternal treatment. Glutathione metabolism enzyme (GCCL and GSR) expression also was not affected by maternal treatment (P > 0.05). Regarding taurine metabolism, maternal supplementation with CHO upregulated (P = 0.05) CSAD hepatic expression, but CDO expression was not affected (P > 0.05). Expression of genes related to carbohydrate metabolism and hepatokines (PC, PCK1, SLC2A2 and FGF21) was not affected by maternal diet (P > 0.05). However, the glucocorticoid receptor was upregulated (P = 0.06) by maternal MET (P = 0.08) or CHO (P = 0.09) but not by their combination (MIX, P = 0.25). Overall, the data suggest that maternal feeding with methyl donors during the last ~4 wk of gestation elicited changes in neonatal calf hepatic gene expression and the response is different according to the methyl donor source.

Key Words: amino acids, fetal programming, nutrigenomics

T158 Short-term feeding of a rumen-protected carbohydrate increases plasma insulin concentrations in early postpartum dairy cows. M. C. Lucy*1, A. R. Castillo2, J. P. Russi3, G. Diaz-Pérez1, S. G. Moore1, L. M. Mayo1, and R. Doyle1, 1University of Missouri, Columbia, MO, 2University of California, Cooperative Extension, Merced, CA, 3RUSITEC, Piedritas, Buenos Aires, Argentina.

Low blood glucose concentrations early postpartum are associated with low blood insulin concentrations, postpartum metabolic disorders, and infertility. The hypothesis was that short-term feeding of a rumen protected carbohydrate (RPC; 56% soybean meal, 40% soluble carbohydrates, 3.2% urea, and 0.8% minerals) would increase blood insulin concentrations by increasing glucose supply from the gastrointestinal tract. Lactating dairy cows (4 Holstein and 1 Guernsey; 17 ± 2 DIM; 30.9 ± 4.6 kg milk and 13.7 ± 2.6 kg DMI per day) were jugular catheterized, barn housed, and milked 2×. During the first 24 h (d 1), cows were fed a nutritionally balanced TMR (corn silage, haylage, wet brewer grains, dry corn, alfalfa hay, rumen protected and unprotected soybean meal, soyhulls, and premix). After 24 h (d 2, 3, and 4), cows were fed the TMR with the RPC added at 10% of diet DM. On d 5, cows were switched to control TMR. Blood was sampled every 2 h for d 1 to 5 through a jugular catheter. Plasma was isolated and analyzed for insulin, glucose, β-hydroxybutyrate (BHB) and nonesterified fatty acids (NEFA). Data were analyzed for the effects of day, time, and day by time with cow as a random effect (Proc GLM of SAS). There was an effect of day (P < 0.001) on plasma insulin concentrations (0.23, 0.24, 0.31, 0.40, and 0.36 ng/mL; SEM = 0.027; d 1 to 5, respectively). The increase in blood insulin was associated with a decrease in plasma glucose (54.4, 55.7, 51.0, 50.9, and 51.3 mg/dL; SEM = 0.7; d 1 to 5) and an increase (P < 0.001) in plasma BHB (1.64, 1.78, 2.01, 2.19, and 1.97 mmol/L; SEM = 0.06; d 1 to 5; P < 0.001). There was no effect of day on plasma NEFA but there was an effect of time (P < 0.001). Milk produced and DMI were similar (P > 0.10) for d 1 to 5. In conclusion, short-term feeding of the RPC increased blood insulin concentrations. The increase in blood insulin was associated with a decrease in blood glucose and an increase in BHB. Feeding RPC to early postpartum dairy cows effectively alleviated depressed insulin and shifted associated metabolite concentrations.

Key Words: insulin, glucose, bypass carbohydrate

T159 Relationship between liver functionality index and fertility in dairy cows. E. Trevisi*1, F. Piccioni-Cappelli, M. Mezzetti, A. Ferrari, and A. Minuti, Istituto di Zootecnica, Facoltà di Scienze Agrarie, Alimentari ed Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy.

During the transition period many cows experienced severe negative energy balance, reduced immunocompetence, inflammatory status, oxidative stress and hypocalcemia which have an impact on the immediate and the later physiological conditions. Although some indexes of the above disorders are related to poor fertility, more accurate prognostic biomarkers are needed. With this aims 52 periparturient cows have been accurately monitored for fertility traits from dry period to first insemination (AI), obtained with the estrus synchronization, and until diagnosis of pregnancy or culling. Diagnosis of pregnancy was performed with ultrasonography at 28 d post-AI and was confirmed 14 d later. Health status, BCS, milk yield, somatic cell counts and a wide inflammometabolic profile (including interleukins (IL) 1β and 6) have been individually measured. Moreover, liver functionality index (LFI), which combines the post calving variations of albumin, cholesterol and bilirubin has been calculated. All data (BCS and blood biomark-
ers) were analyzed with the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), with each animal as the experimental unit. Cows were divided into classes of pregnancy status: pregnant at 1st AI (EP, 15), pregnant within 250 DIM (MP, 24) or later (LP, 5), and not pregnant (INF, 8). During the transition period, LP (but not INF) vs EP showed (often with statistical significance): the most severe reduction of BCS; the highest concentrations of IL1β (in dry period only); the highest concentrations of haptoglobin, ceruloplasmin, globulin, bilirubin, NEFA, BHB, urea and GOT after calving; the lowest levels of paraoxonase and cholesterol. The LFI was inversely related to days open interval and tended to be higher in EP (0.7 ± 2.1 points) vs other groups (−0.6 ± 2.6 points), with marked individual variation. Significant correlations (P < 0.05) with days open and some biomarkers at 7 DIM (i.e., bilirubin, globulin, paraoxonase, Ca-P ratio) and at the day of the AI (IL1β, Ca:P ratio, globulin) have been observed. These data suggest that parameters related to the acute phase response within 28 DIM allow to identify cows at risk of reproductive disorders.

**Key Words:** fertility, transition period, inflammation profile


This study aimed to evaluate the effect of calcium salts of medium-chain fatty acids (MCFA) on milk production performance and plasma hormone concentrations in lactating dairy cows. Fifteen multiparous Holstein cows (initial days in milk: 183 ± 15.2, parity: 2.9 ± 0.2, initial BW: 674 ± 15.9 kg) were managed in freestall barns, provided experimental diets twice daily, and milked before each feeding. Cows were fed a total mixed ration (TMR) containing 3.3% ether extract (EE), 37.0% NDF and 1.72 MCal/kg NE2 on a dry matter (DM) basis. Cows were randomly assigned to dietary treatments using a replicated 3 × 3 Latin square design with 21-d periods. Three treatments were arranged as control, 0.5% and 1.0% MCFA. Calcium salts of medium-chain fatty acids containing 80% caprylic acid and 20% capric acid was added to the TMR at 0.5 and 1.0% of DM intake (DMI), respectively. Milk samples were collected on the last 3 d and blood and rumen samples were collected on the final day of each treatment. Repeated measure ANOVA tests were performed to determine effects of treatment using the PROC MIXED procedure of SAS. Ruminal fermentation, DMI, BW, BCS, milk yield and composition, or plasma metabolite (glucose, nonesterified fatty acids, triglyceride and total-cholesterol) concentrations were not affected by MCFA. Entodinium spp. counts in the rumen were lower (P = 0.05) with 1.0% MCFA (4.83 ± 0.08; LSM ± SE) compared with those in the control (5.05). Relative plasma ghrelin levels were higher (P = 0.04) with 1.0% MCFA (1.48 ± 0.17) than with the control (1.00). Relative plasma IGF-1 levels were higher (P = 0.03) with 1.0% MCFA (1.03 ± 0.03) than with 0.5% MCFA (0.91). In addition, relative plasma insulin levels were higher (P = 0.02) with 0.5% MCFA (1.87 ± 0.24) than the control (1.00). The insulin: glucagon ratio tended to be lower (P = 0.14) with 1.0% MCFA (1.64 ± 0.55) than with the control (2.39) or with 0.5% MCFA (2.77). In conclusion, MCFA has the potential to shift nutrient metabolism toward a catabolic state via alterations of plasma hormone dynamics in lactating dairy cows.

**Key Words:** medium-chain fatty acids, ghrelin, metabolic hormone

**T161 The effect of body condition score and lipolysis intensity on the biosynthesis of oxylipids in periparturient dairy cows.** G. A. Contreras, C. Strieder Barboza, J. de Souza, J. Gandy, A. L. Lock, and L. M. Sordillo, Department of Large Animal Clinical Sciences, East Lansing, MI; Department of Animal Science, East Lansing, MI.

Periparturient dairy cows with high body condition score (BCS) exhibit enhanced adipose tissue (AT) lipolysis that promotes uncontrolled inflammatory responses. Among the fatty acids (FA) released during lipolysis, polyunsaturated (PUFA) modulate inflammation through their oxidized byproducts (oxylipids). Linoleic acid (LA) and arachidonic acid (ARA) derived oxylipids, hydroxy-ocatadecadienoic acids (HODE), and hydroxy-eicosatetraenoic acids (HETE) act as pro-inflammatory mediators, while oxylipids from eicosapentanoic acid (EPA) are anti-inflammatory. Currently, there is minimal information available on the effect of lipolysis and BCS on oxylipid biosynthesis in dairy cows. We hypothesized that periparturient PUFA and oxylipid profiles are dependent on BCS and lipolysis intensity. Holstein cows with high (HB; BCS ≥ 3.75, n = 5) or moderate (MB; BCS ≤ 3.5, n = 4) BCS were selected at dry-off. Blood and subcutaneous AT samples were collected at −27 ± 7 (D1) and −10 ± 5 d (D2) prepartum and at 8 ± 3 d postpartum (PP). Targeted lipidomic analysis was performed on samples using HPLCMS/MS. The statistical model included the random effect of block and the fixed effect of treatment, time, and their interaction (analyzed in SAS). Plasma FA concentrations increased as parturition approached peaking at PP. Cows with HB had higher plasma FA compared with MB, reflecting a BCS effect on lipolysis intensity (P < 0.01). Plasma ArA and EPA were decreased at D2, compared with the other time points for all cows (P < 0.01). Cows with HB had lower plasma content of ArA and EPA compared with MB at all time points (P < 0.05). Concentrations of ArA and EPA in AT, as well as LA content in AT and plasma, remained unchanged during the experiment and were not influenced by BCS. In AT, 9-HODE, 5-HETE, and 11-HETE were increased at PP compared with D1 and D2 (P < 0.05). Concentrations of 9-HODE, 13-HODE, 5-HETE, and 15-HETE in AT were decreased in HB compared with MB (P < 0.05). Our results demonstrate that prepartum adiposity may limit the availability of plasma PUFA, such as EPA, which serve as substrates for anti-inflammatory oxylipids. Furthermore, periparturient lipolysis enhances HODE and HETE biosynthesis in AT and their release into circulation.

**Key Words:** adipose tissue remodeling, lipolysis, oxylipids

**T162 pH from mammary gland secretions is acidic at the time of parturition in mares.** I. F. Canisso, F. S. Lima, R. E. Ellerbrock, and G. Amorim, University of Illinois, Urbana-Champaign, IL.

Assessment of mammary gland secretion (MGS) pH is an inexpensive method to determine impending parturition in mares. However, previous studies have shown that some mares may fail to show changes in MGS before foaling. Following collection of MGS, it is unknown the ideal conditions for sample storage and time for assessment of pH. These questions are relevant in practice for optimal use of pH of MGS. Our objectives were (1) to determine MGS pH and electrolyte concentrations prepartum and at parturition, (3) to characterize milk pH in the first week postpartum, and (3) to evaluate pre-foaling MGS...
T164 Interaction of pre-calving DCAD diet and serotonin infusions on hypocalcemia in Holstein multiparous cows. C. J. Slater*, E. L. Endres, P. M. Crump, and L. L. Hernandez, University of Wisconsin-Madison, Madison, WI.

Hypocalcemia affects 50% of dairy cows. Our lab has previously demonstrated that infusions of the serotonin precursor 5-hydroxy-L-tryptophan (5-HTP) increases circulating calcium levels in the transition cow. It is unknown whether feeding a negative DCAD diet alters the relationship between 5-HTP and hypocalcemia. The main objective of this study was to determine whether feeding a negative DCAD diet before calving in conjunction with a 5-HTP treatment could further diminish the magnitude of hypocalcemia at the time of calving. We utilized a randomized complete block design with a 2 × 2 factorial arrangement. 32 multiparous Holstein cows were fed either a positive (+130 mEq/kg) or negative (−130 mEq/kg) DCAD diet 21 d before calving and were treated daily with saline or 5-HTP (1 mg/kg) IV starting 7 d before estimated calving date. Cows were blocked by parity and were randomly assigned to one of 4 treatment groups: positive DCAD plus saline (+DCAD/CON), positive DCAD diet plus 5-HTP (+DCAD/5-HTP), negative DCAD plus saline (−DCAD/CON), and negative DCAD plus 5-HTP (−DCAD/5-HTP), resulting in an n = 8 per group. Total calcium (tCa), ionized calcium (iCa), and feed intake (DMI) were recorded. iCa was significantly elevated pre-calving (P < 0.02) in the -DCAD/5-HTP group compared with the other treatment groups as well as on d 0 and 1 after calving (P < 0.001). While differences in iCa were not significant across the pre or post-calving periods, iCa was numerically higher on d 0 and significantly higher on d 1 in the -DCAD/5-HTP (P < 0.05) cows compared with all other groups. While there was a DCAD by treatment interaction on DMI (P = 0.03) pre-calving, post-calving DMI differences were not significant. These findings demonstrate that feeding a -DCAD diet in conjunction with 5-HTP pre-calving can increase post-calving circulating Ca concentrations and therefore diminish the magnitude of hypocalcemia at the time of calving.

Key Words: DCAD, serotonin, hypocalcemia

T165 Use of milk progesterone (P4) data to predict non-pregnancy in dairy cows subjected to timed AI. B. O. Omontese*, A. R. Santos, L. G. Silva, V. R. Merenda, and R. S. Bisinotto, Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN.

Objectives were to evaluate the use of qualitative on-farm milk P4 measurements to predict non-pregnancy in lactating dairy cows. Jersey cows (n = 752) from 2 herds were subjected to timed AI (d −8 GnRH, d −3 and −2 P4; d 0 GnRH and AI). Milk was sampled on d −3, 0, 7, and 28 relative to AI. Samples were exposed to a lateral flow test (LFT) strip and classified into 3 groups: G1 = test line not visible or lighter than reference; G2 = test line similar to reference; G3 = test line darker than reference. Based on previous work, these groups indicate milk P4 concentrations of 17.1, 5.8 and 0.7 ng/mL respectively. Pregnancy was diagnosed on d 34 and 62 after AI. Data were analyzed by multivariable logistic regression and orthogonal contrasts were built (C1: G1+G2 vs. G3; C2: G1 vs. G2). Portions of cows in G1, G2, and G3 at each sampling point are depicted in the table below. Pregnancy per AI (P/AI) in G3 cows on d −3 was smaller (P < 0.01) compared with G2 and
G1. Cows in G1 and G2 at AI had smaller \( (P = 0.01) \) P/Al than those in G3. Cows in G3 on d 7 had smaller \( (P < 0.01) \) P/Al compared with cows in G2 and G1. No difference was observed between G1 and G2 cows on d \(-3\), 0, and 7. Cows in G3 on d 28 had the smallest \( (P < 0.01) \) P/Al followed by herdmates in G2 then G1. Pregnancy loss (PL) tended to be greater \( (P = 0.07) \) for cows in G2 at AI compared with G3. Milk P4 group on d 7 tended \( (P = 0.09) \) to influence PL, which was lowest in G2, followed by G1 then G3. Cows in G3 on d 28 had greater \( (P < 0.01) \) PL compared with G2 and G1. On-farm milk P4 data can be used to predict non-pregnancy and potentially allow for early resynchronization.

**Key Words:** progesterone, reproduction, synchronization

### Table 1 (abstract T165). Pregnancy per AI [Adj. % (no.)] and pregnancy loss [Adj. % (no.)]

<table>
<thead>
<tr>
<th>Item</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>Group</th>
<th>C1</th>
<th>C2</th>
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<tr>
<td>% total</td>
<td>51.8</td>
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<td>26.4</td>
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<tr>
<td>P/Al d 62</td>
<td>37.2 (387)</td>
<td>38.3 (159)</td>
<td>17.4 (196)</td>
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<td></td>
</tr>
<tr>
<td>PL</td>
<td>8.0 (161)</td>
<td>7.3 (67)</td>
<td>14.1 (41)</td>
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<tr>
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<tr>
<td>% total</td>
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<tr>
<td>P/Al d 62</td>
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### T166  Effect of eCG administration on day 7 postpartum on resumption of ovarian cyclicity and uterine involution in dairy cows. E. Rojas Cañadas*1,2, P. Lonergan2, and S. T. Butler1, 1Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland, 2School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland.

The objective was to assess the effect of eCG administration on d 7 post-partum (pp) on ovarian cyclicity, uterine health and uterine involution in lactating dairy cows. Healthy cows \( n = 34 \), 21 primiparous (PR) and 13 multiparous (MP) \) were enrolled in the study. Cows were stratified by parity and BCS, and randomly assigned to receive either 500IU eCG \( (n = 16) \) or 2mL 0.9 saline \( (control = 18) \) on d 7 pp by IM injection. Ovaries were examined by transrectal ultrasound (US) from d 10 pp until ovulation or regression of the first follicle wave; the diameter of the dominant follicle was recorded at each exam. US exams were conducted on d 21, 28, 35 and 42 pp to measure the diameter of the cervix and the uterine horns. Vaginal discharge score was recorded on a 1 to 5 scale on d 14, 21, 28, 35 and 42 pp. Endometrial cytology samples were collected on d 42 pp, and the percentage of polymorphonuclear leukocytes determined. Milk samples were collected 3 times per week from d 14 pp until d 60 pp for progesterone determination. All data were analyzed using mixed models in SAS. The model included treatment and lactation as fixed effects and cow as a random effect. Treatment did not affect ovulation of the first follicle wave \( (10/16) \) vs. \( (11/18) \), days to commencement of luteal activity (CLA) \( (22.6 \pm 22.0 \text{d}) \), duration of the first luteal phase \( (11.9 \text{ vs. } 11.4 \text{d}) \) or incidence of subclinical endometritis \( (3/16 \text{ vs. } 1/18) \) in eCG vs Control cows, respectively. An interaction between treatment and parity was detected for CLA \( (P = 0.03) \), whereby CLA was shorter for MP eCG cows \( (21.0 \text{ d}) \) compared with MP Control cows \( (28.4 \text{ d}) \), but was not different in PR cows \( (21.5 \text{ vs. } 21.0 \text{ d}) \). There were no effects of treatment on uterine involution or clinical endometritis. Overall, eCG treatment on d 7 pp had little effect on the reproductive tract measurements collected.

**Key Words:** eCG, postpartum, cyclicity

### T167  Effect of short wavelength light from white LED on melatonin and appetite-related hormones in calves. M. Mon*1, A. Shinoda2; T. Watanabe2, S. Kushibiki3, T. Obitsu1, and T. Sugino1, 1The Research Center for Animal Science, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan, 2Showa Denko K. K., Tokyo, Japan, 3National Institute of Livestock and Grassland Science, Tsukuba, Japan.

Short wavelength light (blue light) promotes feelings hunger in human, but the effect in calves is unclear. This study aimed to investigate the effect of blue light from white LED on melatonin and appetite-related hormone secretion. Six Holstein heifer calves with a mean body weight of 86.3 kg at 8-wk of age were used and housed in an insulated daylight shed at 20°C with a 16:8 h light-dark cycle (LDPP). Calves were fed a calf starter and kleingrass hay ad libitum. Feed intake was recorded daily and calves were weighed weekly. Animals were assigned to 2 treatments for 3 weeks each in a 2 × 2 crossover design as follows: exposed to white LED including blue light \( (12 \text{ µmol/cm}^2/\text{s}, 880 \text{ lx}) \) during the light period and no light \( (0 \text{ lx}) \) during the dark period \( (WHITE) \), or exposed to 594 nm (yellow) monochromatic LED light \( (12 \text{ µmol/cm}^2/\text{s}, 930 \text{ lx}) \) for 2 h before the dark period \( (YELLOW) \). Blood samples were collected from a jugular vein on the final day of each treatment. Data were analyzed by ANOVA to determine effects of treatment, time as repeated measures, and their interaction. Dry matter intake (DMI) of calf starter did not differ between the groups, but DMI of kleingrass hay ad libitum. Feed intake was recorded daily and calves were weighed weekly. Animals were assigned to 2 treatments for 3 weeks each in a 2 × 2 crossover design as follows: exposed to white LED including blue light \( (12 \text{ µmol/cm}^2/\text{s}, 880 \text{ lx}) \) during the light period and no light \( (0 \text{ lx}) \) during the dark period \( (WHITE) \), or exposed to 594 nm (yellow) monochromatic LED light \( (12 \text{ µmol/cm}^2/\text{s}, 930 \text{ lx}) \) for 2 h before the dark period \( (YELLOW) \). Blood samples were collected from a jugular vein on the final day of each treatment. Data were analyzed by ANOVA to determine effects of treatment, time as repeated measures, and their interaction. Dry matter intake (DMI) of calf starter did not differ between the groups, but DMI of kleingrass hay was higher \( (P < 0.05) \) in the YELLOW group \( (236 ± 63.8 \text{ g/day}; \text{LSM} ± \text{SEM}) \) than in the WHITE group \( (152 ± 63.8 \text{ g/day}) \). Body weight gain did not differ between the groups. Plasma melatonin concentrations
gradually increased from 2 h before the dark period in the YELLOW group (9.11 ± 2.18 pg/mL), but did not increase in the WHITE group (1.78 ± 2.18 pg/mL). Higher plasma melatonin levels were maintained (P < 0.01) during the dark period (21.3 ± 1.81 pg/mL) compared with those during the light period (1.23 ± 1.81 pg/mL) and were not different between the groups. Plasma ghrelin concentrations were higher (P < 0.01) during the dark period than during the light period, and were higher (P < 0.01) for the WHITE group (0.08 ± 0.02 ng/mL) than for the YELLOW group (0.06 ± 0.02 ng/mL). Plasma GLP-1 concentrations were not affected by treatment. Results suggest that exposure to white LED for 2 h before the dark period under LDPP suppresses melatonin secretion and DMI in calves.

**Key Words:** white LED, melatonin, ghrelin

**T168** Delaying PRID Delta removal by 24 h during a 5-day PRID-synch protocol decreased expression of estrus before timed AI without affecting fertility in Holstein heifers. V. G. Santos*,1, P. D. Carvalho1, C. Maia2, B. Carneiro2, A. Valenza3, and P. M. Fricke1,
1Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, 2Diessen Serviços Veterinários Lda, Evora, Portugal, 3CEVA Santé Animale, Libourne, France.

Our objective was to evaluate the effect of delaying PRID removal during a 5-d PRID-synch protocol on expression of estrus before timed artificial insemination (TAI) and pregnancies per artificial insemination (P/AI) in Holstein heifers. Our hypothesis was that delaying PRID removal would decrease the percentage of heifers in estrus before scheduled TAI while not affecting P/AI thus reducing the need for estrus detection during the protocol. Nulliparous Holstein heifers at ~14 mo of age were randomly assigned to receive their first AI after synchronization with: 1) a 5-d PRID-synch protocol with PRID removal on Day 5 (PRID5; D0, GnRH + PRID; D5, PGF -PRID; D6, PGF; D8, GnRH + TAI; n = 230), or 2) a 5-d PRID-synch protocol with PRID removal on Day 6 (PRID6; D0, GnRH + PRID; D5, PGF; D6, PGF -PRID; D8, GnRH + TAI; n = 232). Heifers were inseminated if observed in estrus after PRID removal and before scheduled TAI, whereas heifers not observed in estrus received TAI as scheduled on d 8. Pregnancy diagnosis was performed using transrectal ultrasonography 32 and 67 d after AI. Data were analyzed by logistic regression using the GLIMMIX procedure of SAS. More (P < 0.01) PRID5 heifers received AI to estrus before scheduled TAI than PRID6 heifers (12% vs. 1%). Overall, P/AI did not differ between treatments 32 (P = 0.99, 55.7% vs 55.6% for PRID5 vs. PRID6 heifers, respectively) or 67 (P = 0.92, 54.8% vs. 54.3% for PRID5 vs. PRID6 heifers, respectively) d after AI. In addition, pregnancy loss between 32 and 67 d after AI did not differ (P = 0.65) between treatments (1.6% vs. 2.3% for PRID5 vs. PRID6 heifers, respectively). We conclude that delaying PRID removal by 24 h during a 5-d PRID-synch protocol decreased the incidence of estrus before scheduled TAI without affecting P/AI thereby decreasing the need for detection of estrus during the synchronization protocol. Supported by CEVA Santé Animale and USDA NIFA Hatch project 1006519

**Key Words:** dairy heifer, PRID, estrus

**T169** Differences in nerve growth factor-β concentrations in bull seminal plasma and its association with sire conception rate scores. J. S. Stewart1, I. F. Canisso1, J. C. Ferreira1, N. J. Sugai1, V. R. G. Mercadante2, and F. S. Lima*,1

University of Illinois, Urbana-Champaign, IL, 2Virginia Tech University, Blacksburg, VA.

Nerve growth factor-β (NGF) is a seminal plasma protein in bulls, and its expression on sperm head has been demonstrated to be positively and strongly correlated with sire conception rates (SCR). Although this correlation was speculated to be due to NGF effects on sperm motility, recent studies suggest that NGF in seminal plasma may play a beneficial role on conceptus development following insemination and may regulate the function of the bovine oviducts via its interactions with gonadotrophins. The objective of this study was to determine if seminal plasma NGF concentrations differed based on SCR deviations. Our hypothesis was that bulls with positive SCR deviations would have higher seminal plasma NGF concentrations than those with 0 or negative deviations. Semen aliquots (200 µL) were obtained from Holstein (n = 53) and Jersey (n = 7) bulls collected by artificial vagina during routine semen collection at a commercial stud operation (Select Sires, Inc., Plain City, OH). Individual samples were frozen at −80°C until processing. The SCR scores were provided for each bull by Select Sires. Bulls were allocated to 2 categories: (1) negative/0 SCR deviations (NEG); or (2) positive SCR deviations (POS). Upon thawing, samples were centrifuged at 12,000 × g for 10 min to harvest seminal plasma. Concentrations of NGF were measured using an enzyme-linked immunoassay (ELISA Duoset, #DY256; R&D Systems Inc.). Samples were processed and measured in duplicate according to the manufacturer instructions. Statistical analyses were performed using a Welch 2-sample t-test in R version 3.2.2. Intra-assay CV was measured to be ~7%. Bulls in the NEG category had lower seminal plasma NGF concentrations (4.1 ± 0.5 µg/mL) than those in the POS category (5.3 ± 0.4 µg/mL; P = 0.05). These results suggest that seminal plasma concentrations of NGF in bulls are associated with sire fertility deviations. Future studies are needed to determine if this is a causative relationship and whether adding NGF to semen extender could enhance semen freezing ability and AI conception rates in cattle.

**Key Words:** sire conception rates, nerve growth factor-β, bovine
T170 Effect of culling rates on profitability of dairy herds achieving the same pregnancy rate. G. M. Schuenemann*, K. N. Galvão, S. Borchardt, W. Heuwieser, and P. Federico, Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH. The objective was to assess the effect of each unit increment of culling (CULL; from 25 to 43%) on profitability of dairy herds achieving 20% pregnancy rate (PR) using a cow-based model. Same reproductive program was used, Presynch-Ovsynch with estrus detection (ED) and timed-artificial insemination (TAI) with a voluntary waiting period of 60 d in milk (DIM). Probability of involuntary CULL was set at 0.1% per d for the first 60 DIM, and 0.03% per d for the remaining of the lactation and dry period. Probability of death was set at 0.05% per d for the first 60 DIM, and 0.0076% per d for the remaining of the lactation and dry period. Herd size was set to 1,000 cows and each unit of CULL was modeled using the same TAI-ED to achieve 20% PR. Four factors affecting the economic benefits of dairy herds remain unchanged for the simulation: (1) milk price ($0.37 per kg), (2) feed prices for lactating ($0.25 per kg of dry matter [DM]) and dry cows ($0.15 per kg of DM), (3) cull cow price ($1.00/kg of live weight), and (4) replacement price ($1,600 per heifer). Conception to first service was set at 32% and then decreased by 2.6% for every subsequent service. Abortion was set at 9.3% for the first 90 DIM and at 1.7% for the remaining of gestation. Cows were not bred after 366 DIM and open cows were culled after 450 DIM. Simulation was performed until steady state was reached (3,000 d), and then average daily values for the subsequent 2,000 d were used to calculate profit/cow per yr. Net daily value was calculated by subtracting the costs (replacement, feeding, breeding, and other costs) from the daily income (milk sales, cow sales, and calf sales). Higher CULL reduced (P < 0.05) the proportion of cows with lactations ≥ 3, increased replacement costs, and reduced profit by $186 per cow/yr. For each unit increment of CULL, the proportion of first lactation cows increased by 0.66 percentage points and the proportion of net profit decreased by 1.09 percentage points. On average, it took 53 mo to reach the breakeven point considering the heifer replacement cost to first calving and the subsequent milk revenues over 3 consecutive lactations. Excessive CULL increased the proportion of first lactation cows and replacement costs; thus, reducing profitability.

Key Words: culling, economics, dairy

T171 Factor screening for prediction of retention-pay offs of dairy cows using standardized regression coefficients, random forests, and the method of elementary effects. A. Beyi* and A. De Vries, University of Florida, Gainesville, FL.

A dairy cow retention pay-off (RPO) of individual cows may be used to support culling decisions. Sensitivity analyses regarding herd inputs (factors) that affect RPOs are generally carried out with a one-factor-at-a-time design and typically include only the change in the average RPO. Our objective was therefore to carry out a formal, systematic sensitivity analysis of herd factors as recommended in the literature. RPOs were generated using a stochastic dynamic programming model (DairyVIP) using 15 herd inputs. Each combination of herd input variables resulted in 2,304 RPO for cow categories, a combination of lactation number, month in milk, and relative milk yield. We used Standardized Regression Coefficients (SRC), Random Forests (RF), and Morris’ method of Elementary Effects (EE) to investigate the importance of the 15 factors. Data for SRC and RF were calculated using independent Monte Carlo simulations of the 15 herd variables within preset ranges (n = 320 runs with DairyVIP). Data for EE were calculated with the “economical design” of the original Morris study using 4 levels per factor and 20 replicates (n = 320 runs). The 5 output variables were average RPO and change in herd profit, as well as Spearman’s rank correlation coefficient, root mean squared error, mean absolute error compared with the set of RPOs using default herd inputs. Results for the 5 rescaled variables were averaged. All 3 methods ranked heifer price as the most important factor. The methods did not agree on the rankings of most of the other factors. Replacement heifer price, milk price, and price per body weight of replaced cow were the 3 most important variables based on RF and EE. Using EE, the ranking of the 7 most important herd inputs was heifer price, milk price, price per live body weight, calf price, relative milk production, relative body weight, feed price. We concluded that the 3 methods may result in different rankings of herd input factors. The EE method is recommended in the literature and warrants wider use in sensitivity analysis in dairy sciences.

Key Words: elementary effect, sensitivity analysis


Dairy cow retention pay-offs (RPO) are typically calculated with a dynamic programming (DP) model. Alternatively, a large data set of pre-calculated RPO might be useful if it can predict the RPO of cows in new herds with sufficient accuracy. Objective was to investigate k-nearest neighbor (KNN) methods to predict RPO for new herds. Given a set of herd input variables, 2,304 RPO were calculated for non-pregnant cows varying by parity, month in milk, and relative level of milk yield with a DP model. We calculated the RPO for 500 sets of input variables which varied by heifer price, calf price, and body weight price. Mean of RPO in the 500 sets was $71 (min -$492, max $4,017). The data were divided into a training collection (450 sets) and a test collection (50 sets). The KNN method calculates similarity by (weighted) Euclidian distance between the inputs in the test collection and those in the training collection and selects those k = 5 training sets with the best similarity. The RPO for each test set were predicted by 3 variants of KNN: simple average of 5 RPO (KNNs), average of 5 RPO weighted by simple Euclidean distances (KNNw), and simple average of 5 RPO using weights from a linear regression of the 3 predictors (KNNr). Performances were assessed by similarity measures of the 2,304 RPO in the test set and the predicted RPO: root mean square error (RMSE), relative absolute error (RAE), and minimum and maximum prediction errors. Results are in the Table 1. Although average prediction errors were sufficiently small, some large prediction errors remained. In conclusion, K-Nearest Neighbors Method and a large RPO data set may produce sufficiently accurate RPO without the need of a DP model.

Key Words: data mining, k-nearest neighbors
Table 1 (abstract T172). Performance results of 50 test sets with the 3 k-nearest neighbors (KNN) methods

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T173  Sources of variation in feed conversion in commercial dairy farms of Argentina. R. A. Palladino*1, C. Magliola1, E. Giugge2, C. Chiavassa1, J. L. Monge1, M. P. Turiello1, and F. Bargo1, 1Universidad Buenos Aires, Buenos Aires, Argentina, 2Grupo Chia- vassa, Carlos Pellegrini, Santa Fe, Argentina, 3Universidad Nacional de Villa María, Villa María, Córdoba, Argentina, 4Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina.

Feed conversion (FC) is key to understand income over feed costs in dairy farms; however, FC is highly variable in commercial farms due to the multiple management factors involved. A 5-year (2012 to 2016) data set from 4 pens (early and mid-lactation multiparous cows, early and mid-lactation primiparous cows; n = 7300) from a commercial dairy farm (Chiavassa Dairy Farm, Argentina; –32° 02’ 60” S, –61° 47’ 59” W) was used to investigate which performance and feed management variables affect FC variability. Variables were recorded daily and included: milk yield (MY, kg/d), dry matter intake (DMI, kg/d), FC (kg milk/kg DM), and TMR DM content (%TMRDM). For the complete set of 5-year, coefficient of variation (CV) between days (i.e., from previous to current day or –1 d vs. 0 d) was then estimated for each of those variables. Individual MY was recorded by ALPROTM (DeLaval) and averaged by pen. DMI was estimated by difference between feed offered and refused, divided by the number of cows per pen. Offered TMR and orts DM content was determined in a forced-air oven for 2 h at 135°C. Holstein cows were milked 3x/d and fed 2x/d with a TMR (corn silage, alfalfa silage, alfalfa hay, corn grain, soybean meal, mineral premix; 49.8 ± 1.1% DM, 15.8 ± 0.9% CP, 29.9 ± 5.3% NDF, 3.9 ± 0.3% ether extract, and 2.89 ± 0.15 Mcal ME/kg DM; mean ± SD). Partial correlations (r, calculated using the MANOVA / PRINTE commands of PROC GLM of SAS version 9.3, SAS Institute Inc., Cary, NC) evaluated association between CV of FC and the other variables. The model included year, month, and pen. Coefficient of variation of FC was highly and positively correlated (P < 0.01) with CV of DMI (r = 0.90) and CV of MY (r = 0.34). It was also positively correlated (P < 0.01) but to a lesser extent with FC (r = 0.25), CV of %TMRDM (r = 0.08), and %TMRDM (r = 0.04). On the other hand, CV of FC was negatively correlated (P < 0.01) with DMI (r = -0.24) and MY (r = -0.07). Our data show that FC variability could be reduced by reducing daily variation in DMI and increasing FC.

Key Words: feed conversion, variability, feed bunk management

T174  Effect of stocking rate on feeding strategies and individual milk production of autumn calving grazing dairy cows. D. Custodio1, G. Ortega1, Y. Lopez1, T. Nuñez1, R. Mello1, and P. Chilibroste2, 1Agronomy Faculty, Animal Science Department CRCS, Progreso, Canelones, Uruguay, 2Agronomy Faculty, Animal Science Department, Grass Production and Utilization on Grazing Systems, EEMAC, Paysandú, Paysandú, Uruguay.

A farmlet study was conducted to determine the effect of stocking rate on feeding strategies and individual milk production of autumn calving grazing dairy cows. Four farmlets (2 per treatment) representing a pasture based dairy system in Uruguay, which combines annual with perennial pastures under a 4-yr rotation, were grazed either with 1.5 (MSR) or 2.0 (HSR) milking cows per hectare from June to December 2016. Ninety-six cows were randomized to the farmlets based on parity (3.3 ± 1.3), BW (500 ± 91) and BCS (2.95 ± 0.87). Every week, the number of daily grazing sessions (0, 1, or 2), the amount of roughage offered, the amount of concentrate feed in the milking parlor and the individual milk production and composition were recorded. Grazing rules were the same for the 4 farmlets, as well as the amount of concentrate feed to each individual cow. The amount of roughage offered and the addition or not of soybean hull as a diet corrector, were defined for each farmlet based on sward allowance and the number of grazing sessions. The data were analyzed with a mixed model that included stocking rate and month as fixed effects and farmlet as a random effect. The repeated measurement was week and an autoregressive covariance structure (order 1) was selected for all responsive variables. Differences were declared significant when P < 0.05. Individual milk production was not different between treatments (24.4 ± 0.6) though HSR cows ate more silage (2.7 vs 2.1 kgDM/d), hay (1.2 vs 0.8 kgDM/d) and soybean hull (1.2 vs 0.83 kgDM/d) than MSR cows. Mean concentrate intake was 5.5 ± 0.09 kgDM/d. MSR cows had higher herbage allowance (15.4 vs 13.4 kgDM/d) than HSR cows. Besides, MSR cows were able to graze a larger proportion (0.72 vs 0.65) and were forced to stay in the fed pad a shorter proportion (0.28 vs 0.35) of the total eating time. We concluded that under well-managed pastures, increasing stocking rate will affect diet composition and feeding strategy, which ultimately might have more negative side effects on long-term dairy system performance.

Key Words: stocking rate, milking cows, feeding strategies

T175  Effect of stocking rate at system level on produced and harvested forage. G. Ortega1, Y. Lopez1, T. Nuñez1, D. Custodio1, R. Mello1, and P. Chilibroste2, 1Agronomy Faculty, Animal Science Department CRCS, Progreso, Canelones, Uruguay, 2Agronomy Faculty, Animal Science Department, Grass Production and Utilization on Grazing Systems, EEMAC, Paysandú, Paysandú, Uruguay.

A farmlet study was being conducted to determine the effect of stocking rate on feeding strategies and individual milk production of autumn calving grazing dairy cows. Four farmlets (2 per treatment) representing a pasture based dairy system in Uruguay, which combines annual with perennial pastures under a 4-yr rotation, were grazed either with 1.5 (MSR) or 2.0 (HSR) milking cows per hectare from June to December 2016. Ninety-six cows were randomized to the farmlets based on parity (3.3 ± 1.3), BW (500 ± 91) and BCS (2.95 ± 0.87). Every week, the sward mass of each individual plot in each farmlet was assessed through the double sample technique (Haydock and Shaw, 1975). Based on these observations, mean growth rate (GR, kgDM/ha/day) for each paddock and the whole farmlet was estimated. The grazing area was adjusted weekly based on the GR registered for each treatment. Cows grazed a daily strip with a mean herbage allowance of 15.4 and 13.4 kgDM/d over 6 cm for MSR and HSR, respectively. Before and after grazing,
mean sward height and the phenologic stage of the pastures were registered in each individual grazing session. The data were analyzed with a mixed model that included stocking rate and month as fixed effects and farmlet as a random effect. The repeated measurement was week, and an autoregressive covariance structure (order 1) was selected. Differences were declared significant when \( P < 0.05 \). Cows on HSR grazed a larger amount of grass (825.3 vs 517.2 kg DM/month/ha) than MSR. Sward mass (1772 vs 1750 kg DM/ha) and GR (50.2 vs 53.2 kg DM/ha/day) did not differ significantly between treatments. Accumulated grass harvested was 4761 kg DM/ha for MSR (76% grazing: 24% haylage) and 5777 kg DM/ha for HSR (100% grazing). Accumulated milk production per hectare was different (7654 vs 10760 L for MSR and HSR, respectively). We concluded that when good grazing management practices are applied, increasing stocking rate will not affect mean stock of pasture or growth rate. However, according to stocking rate, the ratio between direct (grazing) and mechanical (mowing) harvesting will be affected.

**Key Words:** stocking rate, growth rate, milking cows

**T176  Milk yield and somatic cell score of northeastern United States organic dairy farms during the grazing and non-grazing seasons.** J. G. B. Galvao Jr.1, A. F. Brito2, A. H. N. Rangel3, J. B. A. Silva4, A. F. Benson5, A. N. Hafla6, H. M. Darby7, K. J. Soder6, and R. Kersbergen3, Instituto Federal de Educação, Ciencia e Tecnologia do Rio Grande do Norte, Ipanguaçu, RN, Brazil, 2University of New Hampshire, Durham, NH, 3Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil, 4University Federal do Semiariado, Mossoro, RN, Brazil, 5Cornell University Cooperative Extension, Cortland, NY, 6USDA-ARS, University Park, PA, 7University of Vermont, St. Albans, VT, 8University of Maine, Orono, ME.

The objective of this study was to evaluate milk yield and composition of organically-certified dairy herds during the grazing season (GS) and non-grazing season (NGS) in the Northeast region of the United States. Dairy Herd Improvement records of Holstein, Jersey, and Holstein-Jersey crossbred cows from May 2012 to June 2015 were obtained monthly from 14 herds in the states of New Hampshire (n = 3), Vermont (n = 3), Maine (n = 3), New York (n = 2), and Pennsylvania (n = 3). The length of GS averaged 160 d (May to November). A total of 14,246 observations including milk yield, 4% fat-corrected milk (FCM), concentrations of milk fat and true protein, and somatic cell score (SCS) were obtained. Seasonal effects (GS vs. NGS) were compared using the PROC GLM procedure of SAS. Herds averaged (mean ± SD) 38 ± 18 lactating cows, 21.3 ± 8.04 kg/d of milk, 21.7 ± 7.52 kg/d of FCM, 4.26 ± 0.98% milk fat, 3.28 ± 0.45% milk true protein, and 2.54 ± 1.08 SCS. Days in milk was similar and averaged 143 ± 89 (GS) and 152 ± 85 (NGS). All milk variables (mean ± SEM) analyzed herein were affected by season, except SCS, which averaged 2.50 ± 0.04 vs. 2.52 ± 0.03 for the GS and NGS, respectively. Milk yield (22.0 ± 0.15 kg/d vs. 20.8 ± 0.12 kg/d, FCM (22.1 ± 0.14 kg/d vs. 21.4 ± 0.12 kg/d), and concentration of milk true protein (3.32 ± 0.01% vs. 3.29 ± 0.01%) were greater during the GS than NGS, which may be explained by improved forage nutritional value when comparing pasture with conserved feeds. Milk fat concentration was greater in the NGS (4.35 ± 0.01% vs. GS (4.17 ± 0.02%). This increased milk fat concentration may have been caused by replacing pasture for conserved feeds with greater fiber content, or by a dilution effect caused by decreased milk volume (~1.20 kg of milk/d) during the NGS. Herd SCS suggest adequate year-round milking procedures and preventative mastitis protocols. However, farmers should adopt better supplementation strategies or implement forage-crop practices that improve conserved feed nutritional value to mitigate milk yield losses during the NGS.

**Key Words:** grazing season, milk somatic cell score, organic dairies

**T177  Dairy calf management—A comparison of practices and producer attitudes among conventional and organic herds.** J. Pempek*, G. Schuenemann, E. Holder, and G. Habing, The Ohio State University, Columbus, OH.

Dairy calves are at high risk for morbidity and mortality early in life. Understanding producer attitudes is important for the implementation of key management practices to improve calf health. The objectives of this study were to evaluate usage frequency and producer attitudes on key calf management practices between conventional and organic dairy operations. A cross-sectional survey was mailed to conventional and organic dairy producers in Ohio and Michigan, USA that included questions on cow-calf separation, colostrum management, and vaccination use. The overall survey response rate was 49% (727/1488); 449 and 172 conventional and organic producer respondents, respectively, were included in the final analysis. Binary, cumulative, and multinomial logistic regression models were used to test differences within and between herd types for management practices and producer attitudes. The majority of conventional (64%, 279/439) producers reported separating the calf from the dam 30 min to 6 h after birth. More organic (34%, 56/166) than conventional (18%, 80/439) producers reported separation 6 to 12 h after birth, and organic producers were more likely to agree that time before separation is beneficial. Few conventional (10%, 44/448) and organic (3%, 5/171) producers reported measuring colostrum quality. Most conventional producers (68%, 304/448) hand-fed the first feeding of colostrum, whereas the majority of organic producers (38%, 69/171) allowed calves to nurse colostrum. Lastly, 44% (188/430) of conventional producers reported vaccinating their calves for respiratory disease, compared with 14% (22/162) of organic producers; organic producers were more likely to perceive vaccines as ineffective and harmful to calf health. Thus, the usage frequency and perceived risks and benefits of calf management practices vary considerably between conventional and organic dairy producers. These findings provide helpful information to understand decision making at the herd-level regarding key calf management and health practices, regardless of production systems.

**Key Words:** calf health, cow-calf separation, colostrum

**T178  Milk yield distribution within pens in commercial dairy farms.** P. Turiello*1, C. Vissio1,2, S. Derado Mulleady1, F. Bargo3, A. Larrieta1, and A. Relling4, 1Universidad Nacional de La Plata, Argentina, 2CONICET, Buenos Aires, Argentina, 3Universidad de Buenos Aires, Buenos Aires, Argentina, 4Ohio State University, Wooster, OH.

The knowledge of milk yield distribution (average and standard deviation) is important in diet formulation to avoid the high-producing cows to be underfed. The objective of this experiment was to compare milk yield (MY) distribution using different measurement frequency. We characterized farm and pen MY distribution and compared distributions having monthly or daily records. Individual daily MY records from 2 commercial dairy farms in Argentina from year 2016 were used. Both farms had similar grouping criteria: hospital, fresh, first lactation, high and low producing cows. Hospital pens were excluded from the descriptive analysis. Day 15 of each month was selected arbitrary as the monthly record. Monthly MY were compared with daily MY records in a total of 8 pens (pens with less than 50 cows were not analyzed) from both dairy
farms for 12 mo using ANOVA of InfoStat. Descriptive statistics for each farm and each pen within farm are presented in Table 1. Although MY was not normally distributed, the median was in average within 0.4 and 0.5 kg of the mean for pens in farm A and B, respectively. Within pen coefficient of variation (CV) was lower than farm variation except for the fresh pens in both farms and the low producing pen for farm B. Pen CV ranged from 22.1 to 35.2% for farm A and from 23.4 to 37.1% for farm B. Pens with higher milk yields tended to have higher SD but lower CV (P = 0.08 and P = 0.1, respectively). We found differences (P < 0.05) between monthly and daily MY records 30.2% of the times analyzed (29 times, between 2 and 6 mo/year in every pen). Daily average MY was lower than monthly data in 34.5% of those comparisons. Further analysis of milk yield distribution is needed to improve grouping strategies and to make recommendations regarding data collection and diet adjustments frequency.

Table 1 (abstract T178). Milk yield (MY) distribution summarized by farm

<table>
<thead>
<tr>
<th>Farm</th>
<th>Milking cows</th>
<th>Pens</th>
<th>Mean MY, kg (SD)</th>
<th>Range in mean MY, kg</th>
<th>Range in pen SD, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>480</td>
<td>4</td>
<td>27.9 (8.74)</td>
<td>19.7–32.3</td>
<td>5.67–9.30</td>
</tr>
<tr>
<td>B</td>
<td>1,505</td>
<td>8</td>
<td>32.1 (8.02)</td>
<td>25.0–44.3</td>
<td>7.12–12.11</td>
</tr>
</tbody>
</table>

Key Words: milk distribution, diet formulation, grouping

T179 Using DHI electronic milk weights to improve farm management. H. Adams* and R. Fourdraine, CRI International Center for Biotechnology, Mt. Horeb, WI.

As the popularity of robotic milking continues to increase, so has the need to breed cows that exhibit traits that are directly related to maximizing the return of the robotic milking unit. Selection based on milk output includes not only high production, but also fast parlor throughput. However, previous research has associated faster milking cows with increased levels of clinical mastitis. Therefore, the key is understanding the relationships between milk output components and production and udder health to produce the most parlor-efficient cows. Since 2009 AgSource (Verona, WI) has utilized Tru-Test (Tru-Test Inc., Mineral Wells, TX) electronic milk meters (EMM). EMMs are calibrated and used to collect monthly DHI milk weights, milking durations and milk samples. Using actual measures of milking speed (MS) from the EMMs removes bias introduced by the subjective visual classification of cows into MS categories. To investigate the impact of MS on areas of production and health, test day records (n = 681,029) were extracted from the AgSource DHI database. Data were used from cows with complete individual and sire IDs, and if at least 2 records existed where milk duration was less than 20 min, and MS less than 9kg/min. When categorizing cows into MS classes by increments of 0.5 kg/min, SCC was high for slow and fast milkers, but lowest for those milking 2.5 kg/min. Correlation between MS and ME305 milk was 0.9963, and between MS and average somatic cell count (SSC) was −0.0986. An association study was conducted to identify potential markers associated with MS. Genotypes on cows within the AgSource database were imputed to 50K using BSLP by BEAGLE, with a final set of 52,890 genotypes for 1,326 Holstein cows available for analysis using the SNPpass package in R. One marker was identified as significantly (FDR-corrected P < 0.03) associated with MS. The marker, located on BTA28 within gene KCNMA1, is a key regulator of smooth muscle contractions, and has been previously associated with breast cancer proliferation in humans. This candidate gene could be potentially beneficial in marker-assisted selection schemes to identify cows ideal for an automatic milking system.

Key Words: milking speed, parlor efficiency, DHI milk recording


Automatic milking systems (AMS) are implemented in many different environments and situations around the world. To streamline management advice and recommendations to many producers at once, individual farming practices and challenges need to be identified. Benchmarking is often used in the dairy industry to compare farms’ performance by computing percentile ranks of the production values of groups of farms. Grouping of farms for conventional benchmarking is frequently limited to the use of a few common factors such as farms’ geographic location or breed of cattle. Tremblay et al. (2016; J. Dairy Sci. 99:5671–5680) showed that herds’ AMS production data and management information could be characterized in a meaningful way using cluster analysis and that this clustering approach yielded improved peer groups of farms than benchmarking methods based on criteria such as country, region, breed, or breed and region. The 6 clusters (i.e., peer groups) represent different management styles, unique goals or specific challenges and these peer groups could be used to distribute specialized advice to large groups of AMS producers at once (Tremblay et al., 2016). In addition, when using the cluster analysis peer groups, comparisons among farms lead to a more accurate representation of a farm’s strengths and weaknesses. For example, a cluster 5 farm with an average milk speed of 2.94 kg/min would be in the 90th percentile when compared with all 529 farms. This might give the producer an exaggerated sense of achievement but when compared with only cluster 5 farms, this farm would become in the 77th percentile, which could potentially motivate the farmer to set higher goals. Cluster analysis allows general recommendations to be produced for all farms within a cluster, and for individual farms to generate more appropriate goals by comparing themselves to farms within their own cluster.

Key Words: automatic milking systems; benchmarking; cluster analysis


Tremblay et al. (2016; J. Dairy Sci. 99:3824–3837) analyzed many data from North American dairy farms with automatic milking systems (AMS) for (risk) factors associated with increased milk production per cow and per robot per day. The final data set contained 54,065 observations from 529 farms collected from 2011 to 2014. The dependent variables of milk production per cow per day and milk production per robot per day were used in multivariable generalized mixed linear regression models with farm IDs as random effects and 2-way interactions selected using forward selection (Tremblay et al., 2016). Many variables and combinations of variables (interaction terms) were found to be significantly associated with the outcomes including traffic type (how cows are allowed to move among the AMS, feeding alley and
lying stalls) and the number of AMS robots per pen (Tremblay et al., 2016). On average, free traffic type produced 67.21 kg (CI: 48.6–86.0) more milk per robot per day than Guided/Select; having one AMS robot per pen produced 59.82 kg (CI: 50.4–68.8) less milk per robot per day than having 2 AMS robot per pen; a AMS farm will produce 20.5 kg (CI: 17.0–24.4) of milk per robot more after 4+ years since installation compared with within the first year start-up; Holsteins produce 216.71 kg (CI: 193.2–239.9) more milk per robot per day than Jerseys (not energy corrected) (Tremblay et al., 2016). Knowing how different factors affect milk production in AMS will help guide current and future AMS producers looking to maximize milk production and compare their performance to other AMS farms (Tremblay et al., 2016).

**Key Words:** automatic milking systems, milking robot, milk production

### T182 Estrus detected by activity monitors within 30 DIM is associated with estrus expression and fertility outcomes at first AI in lactating Holstein cows. A. M. L. Madureira1, L. B. Polsky1, B. F. Silper1, T. A. Burnett1, J. L. M. Vasconcelos2, and R. L. A. Cerri1, 1University of British Columbia, Vancouver, BC, Canada. 2São Paulo State University, Botucatu, SP, Brazil.

The aim of this study was to evaluate the association between estrus’ physical activity at 2 moments (before 30 DIM and at first AI) on ovulation rate and pregnancy per AI (P/AI). A total of 436 lactating Holstein cows were enrolled. Cows were monitored by a pedometer (Aftag, Afimilk). Ovulation was induced by a timed AI protocol based on estradiol and progesterone. Body condition score (BCS; 1 to 5 scale) was measured at the time of AI (d 0) and the ovaries were scanned on d+7 to check for the presence of a corpus luteum. Calving score and prevalence of endometritis were recorded. Estrus was determined as relative increase (RI) in activity >100% of the cow’s baseline activity, within the first 30 DIM(30D) and at AI. At estrus, physical activity was categorized as high (<300%RI) and low (<300%RI). Pregnancy was diagnosed at d+30. Data were analyzed using MIXED and GLIMMIX procedures of SAS. Relative increase in activity (mean ± SE) was 274.1 ± 97.3% at 30D estrus and 494.9 ± 159.6% at AI estrus. Low BCS (<2.75) tended to affect relative increase of activity at 30D (P = 0.09) and at AI (P = 0.12). Milk production was not correlated with increased physical activity (r = 0.06; P = 0.20). Multiparous had lower activity than primiparous at AI (479.8 ± 11.3% vs. 513.1 ± 12.3%; P = 0.04). Cows with endometritis and dystocia had lower activity at 30D estrus compared with those that were healthy or did not experience dystocia (204.3 ± 21.9% vs. 285.7 ± 8.9%; 213.8 ± 26.9% vs. 282.3 ± 13.0%). Cows that had one estrus by 30 DIM had greater P/AI (47.5% vs. 32.8%; P < 0.05) and greater relative increase in activity at AI (533.1 ± 14.8% vs. 477.7 ± 9.9%; P < 0.05) compared with cows with no 30D estrus. Cows with high estrus expression at AI had greater fertility (43.6% vs. 22.8%; P < 0.05) and greater ovulation rates (94.8% vs. 85.7%; P = 0.03). Cows with increased activity at both 30D and AI were more likely to ovulate (98.8% vs. 91.6%; P = 0.01) and had greater P/AI (52.7% vs. 32.9%; P < 0.01) compared with those that did not express estrus at either period. Increase in physical activity at 30D and at AI improved fertility and ovulation rates.

**Key Words:** rumination, feeding behavior, precision dairy monitoring

### T184 A case study of composting process establishment in a new compost bedded pack barn housing lactating dairy cattle. M. Borchers*, J. Taraba, and J. Bewley, University of Kentucky, Lexington, KY.

The objective of this study was to describe compost establishment factors in a new compost bedded pack barn. The barn had 2 bedded areas (Side 1: 557 m²; Side 2: 595 m²) bedded with kiln-dried sawdust at a 38.1 cm minimum depth. Three 6.1 m diameter high volume low speed fans hung above each bedded pack. The lactating herd was moved into the compost bedded pack barn on April 21, 2016. Bedded areas were tilled twice daily with a rototiller (45.7 cm depth) at milking time. Daily samples were collected at midday, until July 7, 2016. Bedding samples were collected from each pack at the center of 9 equally distributed sites using a 59.1 cm² scoop. Bedding temperatures were collected from these same areas using an infrared thermometer for surface temperatures and an analog probe thermometer for temperatures at 10.2 cm and 20.3 cm depth. Hourly weather data were collected from a university weather station. Descriptive statistics were prepared using SAS Version 9.3 (Table 1). Mean (mean ± SD) stocking density throughout the study was 11.8 ± 0.6 m² per cow for side 1 and 12.4 ± 0.7 m² per cow for side 2. Within 16 d of cattle being housed in the compost bedded pack barn, composting temperatures on both sides exceeded 43.3°C at 20.3 cm depth (accepted minimum compost temperature). Carbon content decreased and nitrogen...
content increased consistently throughout this same period, indicating sufficient composting of bedding and animal waste.

**Key Words:** compost bedded pack barn, compost, temperature

**T185** Variables associated with milk yield and rumination time of Holstein cows housed in compost bedded pack barns. J. L. Monge$^1$, G. Clemente$^1$, E. Clemente$^1$, M. L. Zingaretti$^1$, E. Giugge$^2$, C. Chiavassa$^3$, M. P. Turiello$^3$, A. Palladino$^4$, and F. Bargo$^4$, 1Universidad Nacional Villa María, Villa María, Córdoba, Argentina, 2Grupo Chivassava, Carlos Pellegrini, Santa Fe, Argentina, 3Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina, 4FAUBA, Buenos Aires, Argentina.

Our objective was to investigate which feed and housing management variables were associated with milk yield (MY) and rumination time (RT) in a compost bedded pack barn (CBPG). We used a data set from 2 pens of primiparous (PP) or multiparous (MP) Holstein cows from a commercial dairy farm in Argentina (Chiavassa Dairy Farm; −32° 02' 60″ S, 61° 47′ 59″ W). Variables evaluated included: DM intake (DMI), TMR DM content, feed bunk orts percentage (FBO), TMR particle size distribution (PSD), and cow stocking density (CSD, m$^2$/cow). All variables were recorded daily from May 2015 to December 2016. Milk yield (ALPRO, DeLaval) and RT (Heatime HR System, SCR) were averaged daily by pen and DMI was estimated by difference between feed offered and refused. The TMR PSD was measured using the 4-sieves PennState Particle Separator. The TMR DM content was determined with a forced-air oven for 2 h at 135°C. Cows were milked 3×/d and fed 2×/d with a TMR composed by corn silage, alfalfa silage, alfalfa hay, corn grain, soybean meal, and mineral premix that averaged 49.8 ± 1.1% DM, 15.8 ± 0.9% CP, 29.9 ± 5.3% NDF, and 2.89 ± 0.15 Mcal ME/kg DM (mean ± SD). We run Pearson correlations (r; R Core Team, www.r-project.org) to evaluate association between MY and RT, and the 5 variables. Milk yield was positively correlated ($P<0.01$) with DMI (r = 0.50 for PP, r = 0.49 for MP), TMR DM content (r = 0.27 for PP, r = 0.28 for MP), RT (r = 0.24 for PP, r = 0.38 for MP), and CSD (r = 0.08 for PP, r = 0.37 for MP). Rumination time was positively correlated ($P<0.01$) with 19 to 8 mm-sieve percentage (r = 0.45 for PP, r = 0.48 for MP), but it was negatively correlated ($P<0.01$) with 8 to 1.18 mm-sieve percentage (r = −0.32 for PP, r = −0.28 for MP). Our results show that milk yield of Holstein cows housed in CBPG was positively associated with key feeding management variables such as DMI and TMR DM content but also with housing management variables such as CSD. On the other hand, RT was affected more by TMR PSD due to feed mixing management.

**Key Words:** compost bedded-pack barn, milk yield, rumination time

**T186** Factors of cow comfort associated with herd-level reproductive outcomes on Canadian dairy farms. T. A. Burnett$^1$, R. Westin$^2$, E. Vasseur$^2$, D. Pellerin$^3$, D. B. Haley$^4$, A. M. de Passillé$^1$, J. Rushen$^1$, and R. L. A. Cerri$^1$, 1University of British Columbia, Vancouver, BC, Canada, 2McGill University, Sainte-Anne-de-Bellevue, QC, Canada, 3Université Laval, Quebec City, QC, Canada, 4University of Guelph, Guelph, ON, Canada.

We aimed to determine herd-level factors of cow comfort associated with reproductive outcomes on dairy farms. Animal (BCS, lameness, skin lesion and cleanliness) and environmental assessments were carried out on 130 freestall and 97 tiestall farms in Canada (AB, QC and ON) at a single visit to each farm. Herd reproductive variables were calculated from DHI records: calving interval (CI), number of inseminations per cow (NI), and conception rate (CR); days to first service was also calculated as a covariate. All categorized variables were classified by the median of each farm type. Mean (±SD) CI, NI, and CR was 417 ± 22d, 2.16 ± 0.34 inseminations (AI), and 34.1 ± 6.7% for freestall farms, and 427 ± 21d, 2.30 ± 0.37 AI, and 29.9 ± 6.4% for tiestall farms. On freestall farms, knee lesion prevalence had negative relationships with all reproductive outcomes: farms with fewer knee lesions tended to have shorter CI (415 ± 2.5 vs. 422 ± 2.5d; $P<0.07$), and had lower NI (2.04 ± 0.04 vs. 2.16 ± 0.04; $P<0.01$) had shorter CI; CI was also shorter in herds producing more milk (P = 0.01). Knee lesions and insufficient stall length tended to be related to NI, where younger herds had lower NI (2.04 ± 0.04 vs. 2.16 ± 0.04; $P<0.03$). Milk production per cow per year was related to CI and CR: farms producing more milk had better reproductive outcomes. On tiestall farms, CI was related to the proportion of older cows and power in electric trainers, younger herds (420 ± 2.5 vs. 431 ± 2.7d; $P<0.01$) and those that did not power electric trainers (420 ± 2.7 vs. 430 ± 2.8d; $P = 0.01$) had shorter CI; CI was also shorter in herds producing more milk (P = 0.01). Knee lesions and insufficient stall length tended to be related to NI, where farms with more stalls having insufficient length for their cows (2.43 ± 0.06 vs. 2.31 ± 0.06 AI; $P = 0.09$) and more knee lesions (2.44 ± 0.06 vs. 2.30 ± 0.06 AI; $P<0.07$) required more NI. Lameness prevalence was only related to CR on tiestall farms; farms with less lameness had higher CR ($P=0.01$). Herd-level BCS and cleanliness were not associated with reproductive outcome on either farm type. In conclusion, farms with poorer reproductive outcomes were...
characterized by a higher prevalence of knee lesions, higher proportion of older cows and produced less milk.

Key Words: fertility, injury, reproductive outcomes

T187  Relationship between cow cleanliness, locomotion, and bulk tank somatic cell count in southeastern United States dairy farms. G. Mazon*1, J. Guinn1, D. Nolan1, P. Krawczel2, C. Petersen-Wolfe3, G. Pighetti2, A. Stone1,2, S. Ward1,4, M. Marcondes5, and J. Bewley1, 1University of Kentucky, Lexington, KY, 2University of Tennessee, Knoxville, TN, 3Virginia Polytechnic Institute, Blacksburg, VA, 4Mississippi State University, Starkville, MS, 5North Carolina State University, Raleigh, NC, 6Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

The objective of this study was to evaluate udder hygiene score (UHS), locomotion score (LS), and their relationships to bulk tank somatic cell count (BTSCC) group and bulk tank somatic cell score (BTSCS). Data from 4,837 cows were obtained from 94 Kentucky dairy herds participating in the Southeast Quality Milk Initiative project (USDA-NIFA-AFRI grant no. 2013–68004–20424). Each herd was visited once between June 2014, and June 2015. Herds were divided into 3 BTSCC groups: low = mean BTSCC ≤210,000 cells/mL (n = 28), medium = 210,000 < BTSCC <380,000 cells/mL (n = 50), and high = BTSCC ≥380,000 cells/mL (n = 10). Herd size ranged from 3 to 2,500 lactating cows. Animals from each herd were scored for LS and UHS once. A 3-point scale was used for LS where 1 = sound cow, 2 = moderately lame cow, and 3 = severely lame cow. Udder hygiene was scored using a 4-point scale where 0 = less than 50% of the udder covered with fresh splashes of manure, and 3 = entire udder covered with dry manure. Yearly mean BTSCS was calculated from monthly milk processor BTSCC data where BTSCS = log2 (BTSCC/100,000) + 3. The FREQ procedure of SAS (Version 9.3 SAS Institute, Inc., Cary, NC) and a Chi-squared analysis were used to examine the relationship between BTSCC group and UHS or LS. The MIXED procedure of SAS was used to determine the relationship of UHS, LS, and their interaction to BTSCS. In the Chi-squared analysis, a greater percentage of the lactating herd with a UHS = 0 occurred in low BTSCC herds (68%) compared with medium (60%) and high (58%) BTSCC herds (P < 0.01). A greater percentage of the lactating herd with a LS = 1 occurred in low BTSCC (77%) compared with medium (69%) and high (67%) BTSCC herds (P < 0.01). In the mixed model, no significant interaction between UHS and LS was found (P = 0.28). As LS decreased BTSCS decreased (P < 0.02), whereas UHS had no significant relationship to BTSCS (P = 0.28). The results of this study suggested lower lameness and cleaner udders were associated with lower BTSCC and BTSCS.

Key Words: bulk tank somatic cell count, lameness, hygiene

T188  Evaluation of four on-farm culture plates to identify pathogens associated with mastitis in dairy cows. J. C. Ferreira*1, M. S. Gomes, E. C. R. Bonsaglia, I. C. Canisso, E. F. Garrett, and F. S. Lima, University of Illinois, Champaign-Urbana, IL.

A precise cow-side point care system for the diagnosis of mastitis is critical for targeted antimicrobial therapy. Recently, several multiple-media culture systems became commercially available for on-farm identification of mastitis pathogens. However, the accuracy of these systems has not been thoroughly and independently validated against microbiological evaluations performed by referral laboratories. Therefore, the purpose of the present study was to evaluate the effectiveness of popular commercially available culture plates (Accumast, Minnesota Easy System, SSGN and SSGNC Quad plates) to identify pathogens associated with clinical mastitis in dairy cows. Milk samples from the affected quarter with clinical mastitis were collected aseptically. Samples were aerobically cultured with the on-farm culture systems and by 2 reference laboratories. Agreeing results from both reference laboratories were denoted as gold standard (GS). Accuracy (Ac), sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV, respectively), and Cohen’s kappa coefficient (k) of on-farm plates were determined based on the GS culture of 211 milk samples. All 4 plates correctly identified ≥84.9% of milk samples with no bacterial growth. Accumast had greater values for all predictive factors and a substantial agreement (k = 0.79) with GS (Table 1). The inter-rater agreements of Minnesota, SSGN, and SSGNC with GS were moderate (0.45 ≤ k ≤0.55). Only Accumast correctly identified Staphylococcus aureus (Ac = 66.7% and Se = 100%). Likewise, Accumast had greater Ac for Streptococcus sp. (55%) than the remaining plate systems (33.3% ≤ AC ≤38.9%). Our findings suggest that Accumast was the most accurate on-farm culture system for identification of mastitis pathogens.

Table 1 (abstract T188). Predictive factors for identifying mastitis-related pathogens using four culture plates (n = 211)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Accumast</th>
<th>Minnesota</th>
<th>SSGN</th>
<th>SSGNC</th>
</tr>
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<tbody>
<tr>
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<tr>
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</tbody>
</table>

Key Words: on-farm culture, mastitis-associated pathogens, antibiotics

T189  Abortion lactation curves. M. Piccardi*1,4, A. C. Funes2, G. Bo3, and M. Balzarini4,1, 1Facultad de Ciencias Agropecuarias de la Universidad Nacional de Córdoba, Córdoba, Argentina, 2Dairy-Tech S.R.L., Rosario, Santa Fe, Argentina, 3Instituto de Reproducción Bovina Córdoba, Córdoba, Argentina, 4CONICET, Córdoba, Argentina.

The aim of this study was to establish if there is an association between the type of lactation (gestation at term vs. abortion) and the parity period (warm vs. cool) for each animal category (primiparous cows and multiparous cows). Data were analyzed through a Pearson’s chi-squared test using the statistical software InfoStat. In addition, to compare the lactation curves started with abortions versus lactation started with gestations at term, a nonlinear model (MilkBot) was adjusted considering the subject-specific effect given by the cow. Lactations were classified by parity period and partitioned by animal category (primiparous cows and multiparous cows). We used PROC NLMIXED from SAS to estimate the cumulative milk yield at 305 d by parity period and partitioned by animal category. We used PROC NLMIXED from SAS to estimate the cumulative milk yield at 305 d by parity period and partitioned by animal category (primiparous cows and multiparous cows). We used PROC NLMIXED from SAS to estimate the cumulative milk yield at 305 d by parity period and partitioned by animal category (primiparous cows and multiparous cows). We used PROC NLMIXED from SAS to estimate the cumulative milk yield at 305 d by parity period and partitioned by animal category (primiparous cows and multiparous cows). We used PROC NLMIXED from SAS to estimate the cumulative milk yield at 305 d by parity period and partitioned by animal category (primiparous cows and multiparous cows). We used PROC NLMIXED from SAS to estimate the cumulative milk yield at 305 d by parity period and partitioned by animal category (primiparous cows and multiparous cows). We used PROC NLMIXED from SAS to estimate the cumulative milk yield at 305 d by parity period and partitioned by animal category (primiparous cows and multiparous cows). We used PROC NLMIXED from SAS to estimate the cumulative milk yield at 305 d by parity period and partitioned by animal category (primiparous cows and multiparous cows). We used PROC NLMIXED from SAS to estimate the cumulative milk yield at 305 d by parity period and partitioned by animal category (primiparous cows and multiparous cows).
versus in cool period \( (P \leq 0.001) \). Within multiparous cows, the odds ratio of starting a lactation with an abortion were 1.52 times higher in warmer period versus in cool period \( (P \leq 0.001) \). For primiparous and multiparous cows 305dMY and PMY were significantly higher in cows that had gestation at term than cows that had an abortion \( \text{Differences in 305dMY: primiparous: 869 L; } P \leq 0.001, \text{ multiparous: 2,062 L; } P \leq 0.001 \); Differences in PMY: primiparous: 4 L/d; \( P \leq 0.001 \), multiparous: 8 L/d; \( P \leq 0.001 \). The effect of parity period and the interaction with type of lactation depends on animal category. For primiparous cows, the 305dMY and PMY were less in warmer period in both type of lactation. Whereas for multiparous cows, 305dMY and PMY were less in warmer period in cows who had calved in term, but were greater in cows that had experienced an abortion.

**Key Words:** no linear models, milk test, cumulative milk yield

### T190 Relationship between body condition score and serum plasma insulin-to-glucose ratio on embryo production in lactating dairy cows. T. Leiva*, R. F. Cooke2, R. D. Bertin1, A. C. Fonseca1, R. P. Rodrigues1, and J. L. M. Vasconcelos1, 1Sao Paulo State University, Botucatu, Brazil, 2Oregon State University-EOARC Burns, OR, 3University of Missouri, Columbia, MO.

The aim was to evaluate oocyte and embryo production (EP) in lactating dairy cows of low and high body condition score (BCS) presenting different plasma insulin-to-glucose ratio \( (I:G) \). Follicles of 290 non-pregnant lactating dairy cows between 50 to 280 d in milk (DIM) were aspirated in random days of the estrus cycle for EP. At the time of follicle aspiration, cows were scored for body condition, and blood was collected for determination of plasma concentrations of glucose and insulin. Data were analyzed as a \( 2 \times 4 \) factorial design, having BCS and I:G ratio as main factors. Animals were classified as low or high \((\leq 3.25; \text{LS})\) or high \( (>3.25; \text{HS}) \) BCS, and for I:G using its quartile distribution \(( \text{low – LL, low intermediate – LI, high intermediate – HI, and high quartiles - HH}) \). Insulin-to-glucose ratio increased as I:G quartile distribution increased \((0.02, 0.08, 0.15, 0.37 \pm 0.01 \text{ for LL, LI, HI, and HH, respectively; } P < 0.001) \), but no differences were observed for BCS or BCS \( \times I:G \). Similarly, insulin followed previous patterns. On the other hand, glucose was higher \((P < 0.05) \) in HS compared with LS or in LL compared with LI, HI, HH. For BCS main effect, milk yield, DIM, and oocyte and EP were higher in HS compared with LS cows; embryo produced per oocyte collected did not differ between BCS groups. For I:G quartiles main effect, EP and embryo produced per oocyte collected were the highest for HI. There were no significant effects of BCS \( \times I:G \) quartiles for milk yield, oocyte production, and embryo produced per oocyte collected. Furthermore, DIM and EP were not different between I:G quartiles when BCS \( \leq 3.25 \). However, when BCS \( >3.25 \), DIM was higher for LI but only differed from HI \((213 \text{ vs. } 169 \pm 15 \text{ d, respectively; } P < 0.04) \); additionally, HI produced the most number of embryos, and LI the least number \((0.9, 3.1, 5.7, \text{ and } 1.6 \pm 0.6 \text{ embryos for LL, LI, HI, and HH, respectively; } P < 0.001) \). These data suggest that mid-to-high plasma insulin-to-glucose ratio enhances EP in lactating dairy cows with BCS \( >3.25 \) without affecting oocytes, mostly through insulin-dependent mechanisms.

**Key Words:** oocyte, metabolite, reproduction

### T191 Does a 500-ohm shunt resistor accurately characterize the electrical resistance of adult dairy cattle? R. Norell*, I. J. Spencer2, A. Ahmadzadeh2, M. E. de Haro Martí2, and M. Chahine3, 1University of Idaho, Idaho Falls, ID, 2University of Idaho, Moscow, ID, 3University of Idaho, Gooding, ID, 4University of Idaho, Twin Falls, ID.

By Idaho statute, a 500-ohm shunt resistor must be used when investigating stray voltage on dairies and represents a “worst case” body resistance \( \text{BODYR} \) value for adult dairy cows \( \text{USDA stray voltage handbook 696, 1992)} \). \text{BODYR} \ data from the 80s were typically collected from cows in tie stall barns and may not represent modern open lot and free-stall dairies. Our study objectives were to compare: \( \text{a) BODYR of 6 cow pathways, } \text{b) effect of wet versus dry haircoats, and } \text{c) 3 electrical connections to the mouth area.} \text{BODYR data were collected from 42 cows on a commercial open lot dairy in Idaho. Pathways were: front to rear hooves (FR), neck to all hooves (NALL), rump to all hooves (RALL), mouth to all hooves (MALL), mouth to front hooves (MF), and mouth to rear hooves (MR). NALL and RALL were tested with wet and dry haircoats and data were analyzed as a \( 2 \times 2 \) factorial model in SAS. MALL, MF, and MR were evaluated with 3 mouth area connections (nose tongs (NT), metal bit (MT), and metal basket (MB)) and data were analyzed as a \( 3 \times 3 \) factorial model in SAS. \text{BODYR} \ varied significantly between pathways and ranking median \text{BODYR} \ (ohms) from lowest to highest value yields: MALL \( (255), \text{ RALL-wet \( (314) \), MF \( (360), \text{ MR \( (361), \text{ FR \( (469), \text{ NALL-wet \( (544), \text{ RALL-dry \( (9,185) \) and NALL-dry \( (820,000). \text{BODYR} \ was significantly lower for rump versus neck location, for wet versus dry haircoats, and exhibited a significant location by haircoat status interaction due to significantly lower \text{BODYR} \ with dry rump versus dry neck. Cows were measured immediately after milking and the rump or udder may have received water spray or contacted wet pipework during milking. \text{BODYR} \ with the mouth connections and pathways were significantly lower for MALL than MF or MR; for NT versus MT and MB; and exhibited a significant interaction between mouth connections and hoof pathways. The percentage of measurements below 500 ohms varies by pathway: MALL \( (98%), \text{ MF \( (98%), \text{ MR \( (96%), \text{ RALL-wet \( (82%), \text{ FR \( (69%), \text{ NALL-wet \( (34%), \text{ RALL-dry \( (5%) \) and NALL-dry \( (0%) \). A 500-ohm shunt resistor overestimates \text{BODYR} \ and underestimates electrical current flow at a given voltage for MALL, MF, MR, RALL-wet, and FR pathway. Contact method and haircoat condition influence measured \text{BODYR} \ of cows.

**Key Words:** resistance, stray voltage

### T192 Survey of work processes on German dairy farms. A. Hesse*, S. Bertulat1, and W. Heuwers1, 1Clinic for Animal Reproduction, College of Veterinary Medicine, Universitaet Berlin, Berlin, Germany, 2Department of Population Medicine and Diagnostic Sciences, Cornell University, College of Veterinary Medicine, Ithaca, NY.

Current information about continuing education, prevalence and utilization of standard operating procedures (SOP), and challenges related to training of the work force implemented on commercial dairy farms is rare. Transparency and consistency of production processes, however, are important to assure trust by the consumers and satisfy their expectations. Therefore, the objective of this study was to conduct a survey to gain insight into the organization of work processes on commercial German dairy farms analyzing the use of SOPs. A questionnaire was developed that consisted of 16 questions and 9 statements focusing on general farm data, the generation, implementation and handling of SOP as well as an assessment of challenges in handling work processes on the farm. The questionnaire was distributed using 3 convenience samples (i.e., 2 workshops, 1 mailing). A total of 250 survey forms were returned and 248 could be used for final analysis. The existence of SOP was indicated by 82% of all respondents, but only 54% stated...
that these were written down. On only 30% of the participating dairy farms SOP were available for trainees. Existence of SOP correlated with farm size such that larger farms were more likely to implement SOP than smaller farms ($P = 0.007$). However, many farmers lacked the time (41%) or ability (42%) to create SOP to provide the employees with detailed instructions on how to perform a specific task. Sixty-six per cent of the participants agreed or strongly agreed with the statements that various employees handled the same tasks differently. Eighty-one per cent of the managers indicated that they “sometimes get annoyed about employees not completing tasks the way they consider right.” However, 86% of respondents considered a consistent work performance as a benefit; another 49% (87/179) and 39% (70/179), respectively, regarded monitoring of work processes and improvement of animal health as beneficial. The majority of respondents (59%) were interested in using ready-made SOP that could be adjusted to their farm. On 68% of the farms continuing education for employees was neglected. There was an obvious discrepancy between the motivation of the farmers to improve the performance on their farm and the expertise in realizing these goals and intentions.

**Key Words:** survey, standard operating procedure, training
energy partitioning and to increase insulin and glucose plasma concentra-
tions in early lactation. The present study evaluated the effect of these
supplements on the response of fed-restricted cows to an intravenous
intravenous glucose tolerance test.

Key Words: insulin, folic acid, vitamin B12
larger pore size allowed more material to leave the bag or more access for bacteria; the effect of pore size was not observed on SBM ID: 97.8% with 10 μm vs. 97.8% with 20 μm pore size. SEM = 0.7. Overall, this study showed that the reason for the underestimation of MP supply by NRC model (2001) in dairy ration including CM is likely not related to an underestimation of the ID of the RUP fraction of the CM.

Key Words: labelled canola meal, intestinal digestibility, amino acids

T196  Effects of straw processing and pen stocking density on Holstein dairy heifers: 1. Growth and sorting behaviors. W. K. Cobeltz*,1, M. S. Akins2, N. M. Esser2, and R. K. Ogden1, 1US Dairy FORAGE Research Center, Marshfield, WI, 2University of Wisconsin, Marshfield, WI.

Our objectives were to evaluate effects of pen-stocking density (freestall housing) and straw processing on heifer growth performance and feedbunk sorting behaviors by Holstein dairy heifers. A 2 × 3 factorial arrangement of straw-processing (GOOD or POOR) and pen-stocking-density [100 (CONTROL), 125, or 150% of capacity] treatments was evaluated with 240 Holstein dairy heifers (410 ± 56.3 kg) that were blocked by weight, and assigned to 24 pens with 4 pens/interactive treatment. For 91 d, heifers were offered TMR diets containing wheat straw either processed through a 7.5-cm screen (GOOD; 46.0% NDF, 12.9% CP, 60.7% TDN) or not processed (POOR; 46.5% NDF, 12.6% CP, 60.0% TDN) before mixing. Diets were dispersed at 1000 h daily, and bunks were sampled throughout the day. Diets were offered for ad-libitum intake, but with minimal orts (<3%); as such, sorting factors were calculated as bunk value/initial value. For POOR straw, sorting factors for large particles increased linearly from 1.26 to 2.82 across sampling times (P = 0.001), differing (P = 0.004) from GOOD straw diets at 2200, 0100, 0600, and 0900 h (orts). Sorting factors for diets blended with GOOD straw also increased linearly (P < 0.001) across sampling times, but sorting was less severe (1.27 to 1.97). Overall, physically effective fiber (pEF) exhibited responses similar to those for large particles, except that sorting factors for pEF particles were limited to narrower ranges for both GOOD (1.04 to 1.14) and POOR (1.03 to 1.26) diets. Despite these differences in sorting behaviors, daily DMI was not affected by treatment (mean = 9.64 kg DM/d; P = 0.38), nor was dry matter intake of TDN (5.92 kg TDN/d; P = 0.164). For GOOD straw processing, heifers housed within overstocked pens exhibited reduced ADG compared with CONTROL (0.92 vs. 0.99 kg/d; P = 0.009). With POOR straw processing, ADG differed between the 125 and 150% stocking rates (0.96 vs. 0.88 kg/d; P = 0.005), as did the within-pen CV for ADG (10.7 vs. 18.6%; P = 0.025). These within-pen CV effects were not observed (P = 0.317) with GOOD straw processing, suggesting that within-pen variability in ADG may be exacerbated by overstocking when straw is processed poorly.

Key Words: dairy heifer, growth, sorting

T197  Effects of straw processing and pen stocking density on Holstein dairy heifers: 2. Behavior and hygiene. W. K. Cobeltz*,1, M. S. Akins2, N. M. Esser2, and R. K. Ogden1, 1US Dairy FORAGE Research Center, Marshfield, WI, 2University of Wisconsin, Marshfield, WI.

The effects of pen-stocking density and straw processing on the daily behavior traits and hygiene of Holstein dairy heifers housed in a freestall system are not understood. Our objective was to evaluate these factors in a trial with a 2 × 3 factorial arrangement of straw-processing (GOOD or POOR) and pen-stocking-density [100 (CONTROL), 125, or 150% of capacity] treatments. A total of 240 Holstein dairy heifers (410 ± 56.3 kg) were blocked by weight, and assigned to 24 experimental units (pens) with 4 pens/interactive treatment. Heifers were offered (ad-libitum) a TMR diet for 91 d that was comprised of alfalfa haylage, corn silage, and wheat straw; wheat straw was either processed through a 7.5-cm screen (GOOD; 46.0% NDF, 12.9% CP, 60.7% TDN) or not processed (POOR; 46.5% NDF, 12.6% CP, 60.0% TDN) before loading into the TMR mixer. Feed was dispersed at 1000 h daily, and simple pen counts were taken at 1300, 1600, 1900, 2200, 0100, and 0600 h. The percentage of heifers lying in freestalls within overstocked pens was greater than observed for CONTROL at 1900 h (43.0 vs. 31.3%; P < 0.001), but was greater (P = 0.012) for CONTROL at all subsequent evaluation times. Furthermore, the percentage of heifers lying in stalls was greater for the 125% compared with the 150% stocking rate at 0100 (68.8 vs. 61.3%; P = 0.009) and 0600 h (74.0 vs. 63.6%; P < 0.001). Heifers lying in alleys differed only at 0600 h, when overstocked pens were greater than CONTROL (6.8 vs. 0.1%; P < 0.001), and 150% was greater than 125% (9.8 vs. 3.8%; P < 0.001). The percentage of heifers eating differed only at 1900 h for comparisons of overstocked pens with CONTROL (35.0 vs. 43.6%; P < 0.001). Heifers inactively standing were greater (P = 0.005) for overstocked pens at 2200, 0100, and 0600 h, and greater (P ≤ 0.022) at 150% compared with 125% at 0100 and 0600 h. Hygiene scores (1 = clean, 5 = badly soiled) for legs (2.1 to 2.3) and flanks (1.6 to 1.9) indicated heifers stayed acceptably clean, but the within-pen CV for these measures was greater (P ≤ 0.045) for overstocked pens compared with CONTROL, suggesting hygiene scores were more variable without a freestall for each heifer.

Key Words: dairy heifer, behavior, hygiene

T198  Effect of feeding increasing amounts of beet pulp on weaned calf performance and digestion. T. S. Dennis*,1, F. X. Suarez-Mena1, G. J. Lascano2, T. M. Hill1, J. D. Quigley1, W. Hu1, and R. L. Schlotterbeck1, 1Nurture Research Center, Provinci North America, Brookville, OH, 2Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC.

Soluble fiber sources in calf feeds are commonly included to reduce costs compared with using traditional cereal grains as energy sources. Beet pulp (BP) contains relatively high concentrations of soluble fiber and pectin compared with other fibrous feed ingredients and has been shown as an acceptable replacement for corn in adult cow diets. However, limited information is available on BP digestibility and growth performance in young weaned calves. In this study, 48 male Holstein calves (60 d of age, 77 ± 2.2 kg initial BW) were group-fed (4 calves/pen) 95% concentrate, 5% chopped grass hay diets for 56 d. Pens were randomly assigned to 1 of 3 dietary treatments using textured grower mixes containing 0, 17, or 34% BP with 18% CP (DM basis). Body weights (BW), hip widths (HW), and body condition scores (BCS) were assessed at the beginning of the study and at 84 and 112 d of age. Dry matter intakes and refusals were recorded daily by pen. Digestion coefficients (DC) of the diets and microbial protein flows were estimated when calves were approximately 84 d of age. Fecal samples were collected daily from pen floors over a 7 d period to estimate apparent dC using acid-insoluble ash as a marker. Urine samples were collected from 2 calves/pen over 2 d and analyzed for purine derivatives to estimate microbial protein flow. Data were analyzed as a completely randomized design using the MIXED procedure of SAS with linear and quadratic polynomial contrasts and pen as the experimental unit. Average daily BW gain and HW change decreased linearly (P ≤ 0.01) and final HW decreased quadratically (P = 0.03) with increasing BP. Dry matter intake, feed efficiency (BW gain/DM intake), and BCS were similar among...
The objectives of this study were to evaluate calf performance and diet digestibility post-weaning of calves previously fed moderate (MOD) or high (HIGH) milk replacer (MR) feeding programs and weaned at 2 ages. Male Holstein calves (n = 96; 79 ± 1.3 kg of BW) were previously fed a common MR (25% CP, 17% fat on DM basis) using 4 feeding programs. Program MOD6 was 0.66 kg/d MR weaned at 42 d, HIGH6 was up to 1.09 kg/d MR weaned at 42 d, HIGH8 was up to 1.09 kg/d MR weaned at 53 d, and GRAD8 was up to 1.09 kg/d and gradually weaned from d 35 to 53. Calves were grouped by MR program (4 calves/pen) at 56 d of age and fed a common diet with 95% textured starter (20% CP, 37% starch on DM basis) and 5% chopped grass hay (7% CP, 64% NDF). Calf BW, hip widths (HW), and body condition scores (BCS) were measured on d 56, 84, and 112 of age. Fecal samples were collected from d 80–84 and 108–112 from each pen floor and composted by pen to estimate diet digestibility coefficients (dC) using acid-insoluble ash. Data were analyzed as a randomized complete block design with repeated measures. Pre-planned contrasts of MOD6 vs. others, HIGH6 vs. HIGH8, and HIGH8 vs. STEP8 were used to separate the means. Prior to beginning this study, ADG was greatest for HIGH8 and GRAD8 but frame growth was similar among treatments. Final BW were similar among treatments, but calves fed MOD6 gained 0.6 cm more HW vs. other treatments (P = 0.01). Calves fed MOD6 had greater ADG (P = 0.01) and tended to be 9% more feed efficient (P = 0.07) vs. other treatments as DM intakes were similar. At 84 d, dC of DM, OM, CP, NDF, ADF, and starch were greatest for calves fed MOD6 (P ≤ 0.05). Additionally, calves fed HIGH6 and GRAD8 had greater dC of NDF and ADF compared with HIGH8 (P ≤ 0.05) at 84 d. At 112 d, dC were similar among treatments with the exception of starch which was least for calves fed GRAD8 (P < 0.01). In this study, calves previously fed a MOD MR rate gained more BW and frame post-weaning to 112 d of age, likely due to improved diet digestibility. Additionally, gradually weaning calves fed a HIGH MR rate can improve fiber digestibility post-weaning.

Key Words: Calf, post-weaning digestion

The aim of this study was to evaluate the effect of dietary Acacia mearnsii tannin extract (TE) and managing the grazing schedule on N partitioning in lactating dairy cows. C. A. Pozo1, G. V. Kozloski1, C. Cajarville2, A. R. Sprunck2, Y. A. Ketenjian3, M. Cuffia3, and J. L. Repetto2. 1Departamento de Zootecnia, Universidad Federal de Santa Maria, Santa Maria, RS, Brazil, 2Facultad de Veterinaria, Universidad de la República, San José, Uruguay, 3Facultad de Agronomía, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina.

The the aim of this study was to evaluate the effect of dietary Acacia mearnsii tannin extract (TE) and managing the grazing schedule on N partitioning. The trial was conducted with 9 Holstein cows averaging 197 ± 12 d in milk, in a triplicate 3 × 3 Latin square design, through 32 22 d experimental periods. Cows were fed a ryegrass pasture (12.8% CP) and a total mixed ration (TMR, 16.4% CP). The 3 treatments consisted of morning grazing and afternoon TMR (AM), morning grazing and afternoon TMR added with 15 g/kg of TE (AMt), or morning TMR and afternoon grazing (PM). Individual dry matter intake was measured during 5 d of each period. Milk samples were collected during 2 d of each period for N analysis. Spot fecal and urine samples were collected twice daily during 3 d of each period. Fecal output was estimated using indigestible NDF as an internal marker. Urinary output was estimated assuming a daily creatinine excretion rate of 25.5 mg/kg of body weight. Statistical analysis was carried out using the PROC MIXED of SAS, where treatments were compared through contrast analysis. The treatments did not affect N intake or milk N excretion. Tannins did not affect fecal N excretion, but increased the fecal ADIN excretion (P < 0.01), and decreased N-excretion. A high dietary N supply tended to decrease N excretion (P < 0.05). Afternoon grazing increased fecal N excretion (P < 0.05), tended to decrease urinary N excretion (P < 0.001), and decreased urinary N-urea excretion (P < 0.05). Although the treatments did not affect the
T202  Lactational performance and energy partitioning of dairy cows fed with N-acetyl-l-methionine as a source of rumen-protected methionine during mid to late lactation. T. G. Grisenti1, S. Sharp1, S. Y. Yang1, J.-S. Eun*1, J. O. Hall1, J. S. Park2, and J. O. Moon2, 1Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT, 2CJ CheilJedang Research Institute of Biotechnology, Suwon, South Korea.

The N-acetyl-l-methionine (NALM) is one of methionine (Met) derivatives produced via protection of l-Met α-amino group with an N-acetyl group and has been shown to be bioavailable and capable of replacing the dietary requirement for Met in animals and humans. The current experiment was conducted to test a hypothesis that lactating dairy cows fed with NALM would increase milk production by increasing N and energy utilization efficiencies in a dose dependent manner. Eight multiparous Holstein cows that were mid lactation (124 ± 13 d-in-milk) and manure during mid to late lactation, were used as the supplemental source of rumen-protected Met in the present study. Four dietary treatments included 0 g (control), 15 g, 30 g, and 45 g/d/cow of NALM supplementation. Supplementing NALM sizably increased dry matter intake (linear effect; P < 0.01), while milk yield tended to increase quadratically (P = 0.07). A linear decrease in milk fat concentration was seen due to NALM treatments relative to the control (P = 0.02). However, milk fat yield was similar across treatments. A trend toward an increase in milk protein yield was observed between the control and the 45 g NALM (1.18 vs. 1.21 kg/d; P = 0.10). There were no differences in energy-corrected or 3.5% fat-corrected milk yields in response to treatments. It is likely that the supplementation of NALM to mid to late lactating dairy cows may have shifted nutrient and energy utilization toward tissue gain rather than lactation, which resulted in a decrease in feed efficiency for lactation (P = 0.02). Overall results from the present study suggest that supplementing NALM to mid to late lactating cows can increase milk yield in a dose dependent manner with a shift of net energy partitioning toward milk production and body weight gain. In addition, supplementing NALM increased milk N output without affecting urinary N excretion.

Key Words: N-acetyl-l-methionine, milk production, net energy partition

Table 1 (abstract T201). Effect of tannin and grazing schedule on N utilization and manure excretion

<table>
<thead>
<tr>
<th>Item, g/d</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>AMt</td>
</tr>
<tr>
<td>N intake</td>
<td>465</td>
<td>467</td>
</tr>
<tr>
<td>Milk N</td>
<td>119</td>
<td>116</td>
</tr>
<tr>
<td>Fecal N</td>
<td>168</td>
<td>182</td>
</tr>
<tr>
<td>Fecal ADIN</td>
<td>7.1</td>
<td>17.6</td>
</tr>
<tr>
<td>Urine N</td>
<td>214</td>
<td>196</td>
</tr>
<tr>
<td>Urine urea-N</td>
<td>123</td>
<td>105</td>
</tr>
<tr>
<td>Manure N</td>
<td>381</td>
<td>377</td>
</tr>
<tr>
<td>Urinary N/Fecal N</td>
<td>1.35</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Key Words: tannin, grazing schedule, nitrogen


Drying of milk, urine or feces for nitrogen (N) analysis can result in N losses; therefore, this study examined the effect of drying method on N concentration in those samples. Twelve lactating cows assigned to 2 groups were fed 4 treatment diets for 21 d with total collection of milk, urine, and feces on the last 4 d. Daily subsamples of feces, urine, and milk were composited by cow and analyzed for N by Kjeldahl or with an elemental analyzer (EA). Fresh feces, milk, and urine were directly applied to Kjeldahl (no drying). For EA analysis, milk and urine were pipetted into tin cups, lyophilized for 8 h or oven-dried at 55°C for 1 h and then applied to EA; fecal samples were lyophilized for 120 h or oven-dried at 55°C for 72 h and analyzed for total N in EA. All data were analyzed using the MIXED procedure of SAS with fixed effects of dietary treatment, method, and treatment by method and the random effects of group and group by treatment. No interaction of dietary treatment and method was observed. Because Kjeldahl analysis occurred without drying samples, this was used as a reference to compare dried samples to. Results of N concentration, secretion, and excretion from different drying methods are shown in Table 1. Milk, urine, and fecal N concentrations by EA after lyophilizing or oven drying did not differ. However, fecal N concentration decreased about 5% when analyzed with EA compared with Kjeldahl. In addition, N concentrations of milk obtained by EA was 10% greater than Kjeldahl, indicating that Kjeldahl likely under predicts milk N concentration and therefore secretion.

Key Words: nitrogen determination, drying method

Table 1 (abstract T203). Nitrogen concentration and partitioning calculated from different drying methods

<table>
<thead>
<tr>
<th>Item</th>
<th>Kjeldahl Wet</th>
<th>EA Lyophilizing</th>
<th>Oven drying</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk N, g/L</td>
<td>4.98</td>
<td>5.45</td>
<td>5.55</td>
<td>0.264</td>
<td>0.04</td>
</tr>
<tr>
<td>Milk N, g/d</td>
<td>200.6</td>
<td>218.0</td>
<td>223.0</td>
<td>8.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Urine N, g/L</td>
<td>6.57</td>
<td>6.53</td>
<td>6.74</td>
<td>0.245</td>
<td>0.80</td>
</tr>
<tr>
<td>Urine N, g/d</td>
<td>213.1</td>
<td>212.0</td>
<td>218.8</td>
<td>15.19</td>
<td>0.72</td>
</tr>
<tr>
<td>Fecal N, % of DM</td>
<td>2.94</td>
<td>2.78</td>
<td>2.80</td>
<td>0.107</td>
<td>0.003</td>
</tr>
<tr>
<td>Fecal N, g/d</td>
<td>240.3</td>
<td>227.3</td>
<td>228.1</td>
<td>6.74</td>
<td>0.054</td>
</tr>
</tbody>
</table>

P-value: Kj vs. EA, Kjeldahl vs. EA (lyophilizing and oven drying); EA = lyophilizing vs. oven drying.
T204  Effect of top dressing plant extracts in early lactating Holstein cow: milk yield, milk composition, plasma lipomobilization indicators and body condition score. D. K. Kumprechtova1, B. C. Cadudal2, and F. J. Jancik1, 1Institute of Animal Science Prague, Prague, Czech Republic, 2Phytosynthese, Mozaic, France.

Experimental objective was to evaluate the effect of supplementation with plant extracts (Ruminolac, Phytosynthese, France) on lactation performance of dairy cows. Early lactation Holstein cows, from 15 to 75 d in milk, were housed in a free-stall barn, milked 3 times daily, fed a basal diet formulated to 16.5% CP and 6.8 MJ NEL (DM basis) as TMR. Secondary objectives were to investigate the effect on plasma parameters and body condition score. Throughout the trial period (6 mo), the experimental group (n = 117) received 20g of Ruminolac/cow/day, given as top dressing on TMR in the feed bunk with all cows of the experimental pen having an even access to it. The Control group (n = 110) received the basal TMR. The cows were evenly distributed by parity (primiparous and multiparous cows) and days in milk between the 2 pens. Measurements under study included: daily milk yield, milk values (fat %, protein %, SCC, milk urea), dung consistency estimation, serum lipomobilization indicators (NEFA, BHBA), reproduction results (days open, insemination index), body condition scoring, health. Data were analyzed using the MIXED procedure of SAS, with treatment, parity and their interaction as fixed effects. The cow was an experimental unit. For multiparous cows, average daily milk yield between d 46 and 60 was significantly higher in the Ruminolac group (46.98 kg vs. 44.43 kg, P < 0.05). In primiparous cows statistical trend toward significance was achieved between d 46 and 60 (37.09 kg vs. 35.08 kg, P < 0.1). Serum NEFA levels were generally within the physiological standards, but slightly lower (P < 0.1) in the experimental groups on d 40 and 60. Moreover a significantly smaller average body condition decrease was observed for Experimental cows than Control cows (−0.32 vs. −0.43). In conclusion, the dietary supplementation with plant extracts resulted in 5.4% improvement of milk yield in peak lactation without a negative impact on energy metabolism and body condition of the cows.

Key Words: plant extract, dairy cattle, milk production

T205  Effects of lactose and sucrose with varying starch and rumen degradable protein concentrations on ruminal fermentation in vitro. E. L. Sorge* and R. D. Shaver, University of Wisconsin-Madison, Madison, WI.

Study objective was to determine effects of supplementing sucrose and lactose, with varying starch and rumen degradable protein (RDP) concentrations, on ruminal in vitro pH, volatile fatty acids (VFA), and forage neutral detergent fiber digestibility (ivFNDFD). The in vitro substrate in a concentrate mixture formulated for (DM basis) 21, 25 or 29% starch, 0, 2.5 or 5% of each sucrose and lactose and 10 or 12% RDP (3 × 3 × 2 factorial), placed in the media, and a 70:30 corn silage: alfalfa haylage mixture placed in an Ankom F57 bag and then the media. Two ruminally-cannulated lactating Holstein cows were fed a diet with (DM basis) 25% starch, 2.5% sucrose and 2.5% lactose to adapt the microbial population. The ivFNDFD procedure was the Combs-Goessel Assay with incubations of 24, 30 and 48 h. Each treatment was replicated 3 times and data were analyzed using PROC MIXED in SAS 9.4; model included fixed effects of RDP, starch, sucrose, lactose and incubation time, and the random effect of run. No differences were observed for pH, or effects of starch, RDP or sugar concentrations on ivFNDFD, but there was a tendency (P = 0.08) for a sucrose × lactose interaction, suggesting that sucrose and lactose may affect fiber digestibility differently. Increasing starch decreased acetate molar % (55.3, 54.4 and 53.4 ± 0.84% for 21, 25 and 29% starch, respectively; P < 0.0001) and increasing sucrose decreased acetate molar % (55.0, 54.8 and 54.1 ± 1.2% for 0, 2.5 and 5% sucrose, respectively; P = 0.003), but lactose had no effect. Starch increased butyrate molar % (14.1, 14.7 and 15.6 ± 0.40% for 21, 25 and 29% starch, respectively; P < 0.0001). Sucrose increased butyrate molar % (14.1, 14.7 and 15.2 ± 0.40% for 0, 2.5, and 5% sucrose, respectively; P = 0.0006), but lactose had no effect. Effects on molar proportions of acetate and butyrate in response to increasing starch and sucrose, but not lactose, suggests that sucrose and lactose may affect the ruminal fermentation differently. These results warrant further research exploring the potential interactions between sucrose and lactose in dairy rations.

Key Words: lactose, starch, sucrose

T206  In vitro disappearance fails to predict extent of ruminal and total tract digestion of NDF and starch from corn silage diets by growing Holstein heifers. L. Nuzback1, R. A. Zinn2, and F. N. Owens*, 1DuPont Pioneer, Johnston, IA, 2University of California, Imperial Valley, C.

The in vitro incubation time intervals selected to predict in vivo digestion of feeds at commercial laboratories have been based on ruminal retention time of undigested particles. To determine the specific in vitro incubation time that numerically matched and correlated best with ruminal and total-tract digestion of NDF and starch, site of digestion of 7 non-BMR corn silages ensiled for 4 mo was measured in 6 periods using 12 growing Holstein heifers (300 kg) equipped with duodenal cannulas in a 6 by 12 Youden square. Diets contained 90% of DM from corn silage plus a supplement with chromic oxide, an indigestible marker. For each corn silage, in vitro disappearance of NDF and starch also was measured at a commercial laboratory at multiple time points up to 240 and 48 h, respectively. In vivo ruminal and total-tract digestion of NDF ranged from 16 to 34% and from 26 to 43% numerically matching mean in vitro disappearance at 12 to 20 h and at 18 to 28 h, but the in vitro to in vivo correlations at these times were low (R2 = 0.07 to 0.20). Total-tract NDF digestion was correlated best with in vitro NDF disappearance at 240 h (R2 = 0.48). Ruminal and total-tract starch digestion ranged from 78 to 95 and from 95.5 to 99% numerically matching mean in vitro starch disappearance at 12 to 18 h and at 24 h. The incubation time best correlated with ruminal starch digestion was 7 h (R2 = 0.36). Ruminal NDF disappearance of individual corn silages was overestimated by 6 to 139 percentage points by in vitro incubation for 24 h while ruminal starch digestion was underestimated by 48 to 72% by in vitro incubation for 7 h. Correlations with in vivo ruminal digestion for NDF and starch digestion at these incubation times were 0.19 and 0.36. None of the incubation times tested gave disappearance values that correlated well with ruminal or total-tract digestion of starch or NDF from corn silage by growing heifers.

Key Words: in vitro, incubation time, in vivo

T207  Could diet composition modulate concentration of vitamin B12 in milk? M. Duplessis*,1 R. Robichaud2, L. Falud-Pacheco3, D. Pellerin2, D. E. Santchi3, and C. L. Girard1, 1Département des sciences animales, Québec, QC, Canada, 2Département des sciences animales, Québec, QC, Canada, 3Valacta, Sainte-Anne-de-Bellevue, QC, Canada.

Milk is an excellent source of vitamin B12 for humans. Previous studies showed that its concentration is variable among herds. The purpose of this study was to identify diet characteristics affecting milk vitamin B12 concentration (B12) and to verify if maximizing milk B12 could be
achieved without affecting income over feed cost (IOFC). A total of 4,440 Holstein cows located in 100 herds participated in the study. One milk sample per cow was collected during the morning milking and analyzed for B12. A sample of each feed ingredient given to the cows within a herd was collected, analyzed by wet chemistry and then nutrient composition of the total mixed ration was calculated. Cost of each ingredient was recorded. Milk B12 was averaged by herd taking into account individual milk production. Proc CLUSTER of SAS was used to divide herds in groups according to milk B12, and IOFC; 4 groups were identified: (1) High B12 (4.4 ng/g) + high IOFC ($18.65 CAN/cow/d); (2) Low B12 (3.9 ng/g) + low IOFC ($12.57 CAN/cow/d); (3) Low B12 (3.5 ng/g) + high IOFC ($16.43 CAN/cow/d); and, (4) High B12 (5.3 ng/g) + low IOFC ($14.55 CAN/cow/d). Proc GLIMMIX of SAS and orthogonal contrasts (1 vs. 4; 1 vs. 3 and; 1+4 vs. 2+3) were used to analyze data. On a herd basis, milk B12 averaged 4.1 ng/g (min-max: 2.6–5.9 ng/g). When comparing herds with high B12, IOFC was higher when diets contained more non-fiber carbohydrates (+3.3% dry matter [DM]; NFC) and less acid (−2.5%; ADF) and neutral detergent fibers (−3.2% DM; NDF) and less grass (−11.2% DM) and less hay (−8.9% DM; NDF; P < 0.02). In higher IOFC herds, B12 of milk was compared with 38.9, 22.9, 39.4% on a DM basis for herds with lower B12 (P = 0.0002). Moreover, diets of herds with higher B12 contained 14.8% DM less hay and physically effective NDF, respectively (P < 0.02). In line with previous results, regardless of IOFC, high B12 and lower B12, herds were characterized with diets containing more NFC (+2.7% DM), less ADF and NDF (−1.7 and −3.2% DM, respectively), more grass silage (+11.2% DM) and less hay (−8.9% DM; P < 0.04) than low B12 herds; maximizing milk B12 can be achieved without decreasing IOFC.

**Key Words:** vitamin B12, milk, cow

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**T208 Effects of protein and forage sources on milk production, rumen parameters and intestinal digestibility in lactating dairy cows.** C. E. Galindo1, D. R. Ouellet2, G. Maxim2, R. Martineau2, D. Pellerin1, and H. Lapierre*1, 1Université Laval, Québec, QC, Canada, 2Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.

The effects of protein source (PS), soybean meal (SBM) vs. canola meal, CNM, in corn silage-based diets, and of forage source (FS), corn silage vs. grass silage, in CM-based diets, were examined in 9 rumen-cannulated cows (103 ± 28 DIM, 704 ± 65 kg BW), in 3 3 × 3 Latin squares (LS; 21-d periods). The 3 isoenergetic (1.60 Mcal/kg) and iso-N (10.4% CP) diets were: SC = SBM (17.7% DM)+corn silage; CC = CM (20.6% DM)+corn silage; and CG = CM (27.4% DM)+corn silage; and CG = CM (20.6% DM)+grass silage. Milk yield and composition, and DMI were monitored on d 19–21; on d 21, 2 samples of rumen fluid were collected. On d 12 of period 2, the PS of that period was incubated for 16 h in the rumen of each cow to prepare 16-h residues. After the completion of the LS, cows continued to receive their period-3 ration for 1 wk. To determine CP intestinal digestibility, small nylon bags (2 × 2 cm) containing 80 mg of the 16-h residues of the PS of that period were inserted into the abomasum of each cow and recovered in the feces (10 bags recovered per cow). Data of the LS were analyzed using the Mixed procedure of SAS, using contrasts to test the effect of PS and FS. Results are presented in Table 1; ns = 0.20. Intestinal digestibility of CP was higher (P < 0.01) for CM vs. CM, averaging 94.3 vs. 74.4 ± 1.2%. Substituting SBM by CM in a corn-based diet tended to increase milk yield whereas a positive effect of CM inclusion was larger when the cows were fed a grass-based compared with a corn-based diet.

**Key Words:** canola meal, soybean meal, digestibility

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**Table 1 (abstract T208).**

<table>
<thead>
<tr>
<th>Item</th>
<th>SC</th>
<th>CC</th>
<th>CG</th>
<th>SEM</th>
<th>SC vs. CC</th>
<th>CC vs. CG</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>26.0</td>
<td>27.5</td>
<td>25.9</td>
<td>0.5</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>N intake, g/d</td>
<td>672</td>
<td>696</td>
<td>681</td>
<td>18</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>43.7</td>
<td>46.0</td>
<td>48.9</td>
<td>0.8</td>
<td>0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>True protein yield, g/d</td>
<td>1,301</td>
<td>1,330</td>
<td>1,389</td>
<td>36</td>
<td>NS</td>
<td>0.20</td>
</tr>
<tr>
<td>Fat yield, g/d</td>
<td>1,540</td>
<td>1,639</td>
<td>1,753</td>
<td>33</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>Lactose yield, g/d</td>
<td>1,942</td>
<td>2,003</td>
<td>2,187</td>
<td>46</td>
<td>NS</td>
<td>0.02</td>
</tr>
<tr>
<td>BW change, kg/21d</td>
<td>−0.4</td>
<td>−1.4</td>
<td>12.0</td>
<td>5.2</td>
<td>NS</td>
<td>0.09</td>
</tr>
<tr>
<td>Rumen pH</td>
<td>5.90</td>
<td>5.87</td>
<td>6.23</td>
<td>0.12</td>
<td>NS</td>
<td>0.06</td>
</tr>
<tr>
<td>Rumen ammonia, mM</td>
<td>10.5</td>
<td>7.9</td>
<td>8.0</td>
<td>0.7</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Rumen total VFA, mM</td>
<td>109.4</td>
<td>99.2</td>
<td>95.5</td>
<td>3.2</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Rumen acetate:propionate</td>
<td>2.1</td>
<td>2.2</td>
<td>2.9</td>
<td>0.1</td>
<td>NS</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Bacteroidetes and Firmicutes, accounting for 49% and 39% of total sequences, respectively. The bacterial community compositions in both of liquid and solid fractions of effluent digesta were changed (P < 0.01) by dietary CC but not by dietary fat levels. Including CC in the diets decreased (P < 0.05) the relative abundance of Ruminococcus flavefaciens, Ruminococcus albus, Fibrobacter spp., and Butyrivibrio spp. The most abundant genus, across treatments, Prevotella, was decreased (P < 0.05) by high dietary fat levels while Megasphaera was increased (P < 0.01) by CC in the liquid fraction. Correlatively, the concentration of acetate was decreased (P < 0.01) than propionate was increased (P = 0.01); saturated fatty acid (C16:0 and C18:0) were decreased (P < 0.01) and PUFA, especially C18:2 n-6 and C18:3 n-3 were increased (P < 0.01) by dietary CC. Based on the correlation analysis between genus and metabolites, this study revealed that CC could be energetically beneficial to dairy cows and useful at suppressing ruminal bacteria associated with biohydrogenation; however, attention should be given to avoid negative effects of CC on suppressing cellulolytic bacteria.

**Key Words:** PUFs, microbiome, biohydrogenation

**T210 First-lactation performance of Holstein cows fed milk replacer or pasteurized or raw non-saleable milk as preweaning feeders.** M. Garcia*, S. R. Montgomery, L. E. Hulbert, and B. J. Bradford, Kansas State University, Manhattan, KS.

A growing body of research has shown that strategic feeding and nutritional management of preweaning heifers can have life-long effects on their performance. Our objective was to assess the performance of prewean- lation heifers that were fed either accelerated milk replacer (MR; 3.98% CP, 2.56% fat) or non-saleable milk (3.59 ± 0.28% true protein; 4.12 ± 0.37% fat) that was either pasteurized (PM) or raw (RM). Heifers were randomly assigned to feeding treatments after birth and were fed 3 times daily either 1.4 L (calves <36.3 kg, until the target weight was achieved) or 1.9 L (calves ≥36.3 kg). After weaning, all heifers were managed uniformly. Monthly test day data were used to generate DHIJA estimates of 305-d mature equivalent milk (MEM), fat (MFF), and protein (MEF) yields; predicted transmitted ability (PTA) estimates for these traits were also collected. Data were analyzed using mixed, regression, and logistical procedures of SAS and significance declared at P ≤ 0.05. The model included the main effect of milk treatments, amount fed daily at enrollment, and their interaction. The corresponding PTA was used as covariate for milk yield variables. The sample size (n = 152) provided sufficient power (80%) to detect statistical differences of 950 kg MEM between treatments before accounting for the covariate. Treatments did not influence (P > 0.10) the number of inseminations at first calving, age at first calving, days open, or retention in the herd by 36 mo of age (left/enrolled: 18/50, 18/50, 16/52 for MR, PM, and RM, respectively). Heifers fed 5.7 L/d produced more MEM (P = 0.04; +695 kg), tended (P = 0.07) to produce more MFF (+26 kg), and produced more MEF (P = 0.05; +18 kg) compared with heifers fed 4.2 L/d, which may be related to either prenatal factors causing low BW or to the level of nutrition early in life. An interaction of treatment and feeding rate was observed for MEM and MFF (P = 0.07); within the RM-treatment, low-BW heifers produced less MEM and MFF compared with high-BW heifers (~1.705 kg MEM and ~68 kg MFF, both P < 0.01). Although no overall treatment effects on first-lactation performance were observed, feeding RM may impair first lactation performance of low-BW heifers.

**Key Words:** heifer, pasteurized milk, programming

**T211 Enriching bovine milk fat with α-linolenic acid, an n-3 fatty acid, through feeding of a rumen-protected flax-based supplement.** H. Peterson*, R. Day, J. E. Williams, W. J. Price, B. Shaffi, and M. A. McGuire, University of Idaho, Moscow, ID.

The objective of this study was to increase the α-linolenic acid (ALA) concentration in milk fat by feeding a rumen protected flax-based (RPF) supplement. Eight lactating Holstein cows at 194 ± 16.3 DIM were randomly assigned to 4 treatment sequences in replicated 4 × 4 Latin squares with 16-d periods. The 4 treatments were 0, 0.9, 1.8, and 2.7 kg of RPF (26.6% protein and 31.9% lipid, of which 49.9% was ALA) added to a base ration (3% lipid DM basis) daily. Milk samples were collected on d 15 and 16 of each period. Milk components were assessed by near-infrared analysis and fatty acids by gas chromatography. Data were analyzed using a generalized linear mixed model with cow nested within period as the random effect and treatment, period, and treatment by period interactions as the fixed effects. A β distribution was assumed for the proportion fatty acid data and a normal distribution was assumed for dry matter intake, milk yield, and milk components. RPF did not affect dry matter intake, milk yield, or most milk components. Milk fat concentration tended to increase (P = 0.02) from 3.75% to 3.91% and milk urea nitrogen concentration tended to decrease (P = 0.08) from 14.2 to 11.9 mg/dL as the amount of RPF supplement added to the diet increased. Many fatty acids in milk were altered by the RPF dose showing a generally decreasing trend in short- and medium-chain saturated fatty acids and increasing trends in long-chain fatty acids. Compared with the diet with no added RPF (ALA concentration 0.53 g/100 g total fatty acids), RPF added at 0.9, 1.8, and 2.7 kg/d increased (P = 0.0001) the concentration of ALA in milk to 1.43, 2.14, and 2.77 g/100 g total fatty acids, respectively. The n-6-to-n-3 fatty acid ratio was lowered (6.6 to 1.3) as the amount of RPF supplement added to the diet increased. This novel rumen protected ALA source enriched milk ALA greater than any previous method.

**Key Words:** lipid supplement, milk fatty acid, α-linolenic acid


The objective of this study was to estimate bioavailability of the third generation of a rumen-protected Lys (RPL) product, AjiPro-L (A3G; Ajinomoto Heartland Inc.) using the currently marketed AjiPro-L (A2G; Ajinomoto Heartland Inc.) after products were exposed to TMR. Fourteen multiparous lactating Holstein cows (114 ± 8 d in milk) housed in tie stalls were used in a replicated 7 × 7 Latin square design with 7-d periods. A common basal diet adequate in Lys was prepared 1 × /d and fed proportionately at 0500 h, 1300 h, and 2100 h. Treatments included Lys supplemented at 0, 75, 112.5 and 150 g/d prepared 1 × /d and fed proportionately at 0500 h, 1300 h, and 2100 h. Treatments were fed 3 × /d 1 h before feeding on d 2 through 7 of each period in amounts proportional to feed offered. Four blood samples were obtained from cows on d 6 and 7 of each period from the tail vein at 2-h intervals starting at 0600 h. Plasma, pooled by day, was analyzed for AA concentrations. Data were reduced to period mean for each cow and analyzed using the MIXED procedure of SAS. The REG procedure was used to generate linear regression models for each RPL product using the values of Lys (µmol/L) and Lys as a percent of total

**Key Words:** heifer, pasteurized milk, programming
AA (µmol/L basis) to determine the degree of elevation of plasma Lys in response to treatment. Relative to A2G, estimated bioavailability of A3G was determined using the slope-ratio assay technique. Plasma Lys was greatest ($P \leq 0.05$) for 150 g/d A2G (119.8 ± 3.2 µmol/L) and 150 g/d A3G (123.8 ± 3.5 µmol/L) when compared with 0 g/d Lys (110.7 ± 3.3 µmol/L). The slope for A3G treatment was numerically greater (0.009; $r^2 = 0.92$) when compared with the slope for A2G treatment (0.006; $r^2 = 0.93$) when expressing the concentration of plasma Lys from A2G or A3G particles in feces. Amounts of Lys excreted in the feces were homogenized with hot water to extract free Lys was released during the following 18 h. These results demonstrate that handling properties of A3G are not compromised regardless of reduction in the particle size.

**Key Words:** rumen-protected lysine, handling, stability

T215  Impact of tannins and grazing schedule on ruminal inoculum activity of dairy cows: Evaluation using the in vitro gas-production technique. C. A. Pozo1, J. L. Repetto2, G. V. Kozloski1, M. Cuffa1, A. Ramírez2, and C. Cajarville2, 1Departamento de Zootecnia, Universidad Federal de Santa Maria, Santa Maria, RS, Brazil, 2Facultad de Veterinaria, Universidad de la República, San José, Uruguay, 3Facultad de Agronomía, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina.

The aim of this study was to evaluate the effects of dietary Acacia mearnsii tannin extract (TE) and managing the grazing schedule, on in vitro fermentative activity of the rumen inoculum (RI) collected from dairy cows. The trial was conducted with 9 Holstein cows averaging 197 ± 12 d in milk, in a triplicate $3 \times 3$ Latin square design, through three 22-d experimental periods. The treatments consisted of morning grazing and afternoon TMR (AM), morning grazing and afternoon TMR added with 15 g/kg of TE (AMt), or morning TMR and afternoon grazing (PM). Cows were fed twice a day at 0700 and 1600 h, having access to TMR or pasture during 5 h. After 20 d of adaptation, RI of each cow was collected twice a day, 4 h after the morning and afternoon meal. The pH was measured and the fermentative activity of the RI was estimated through a 96-h in vitro gas-production assay. The substrates used were 2 whole-crop oat silages incubated in triplicate. Data were fitted to a simple exponential model with lag. Statistical analysis was carried out using the PROC MIXED of SAS, where treatments were compared for statistical differences ($P < 0.05$). Assuming...
through contrast analysis. The contrast tested were AM vs. AMt and AM vs. PM. There was not any significant interaction between treatments and time of sampling. The pH of the RI collected in the morning was lower in PM than AM (6.0 vs. 6.4; P < 0.01). For this RI, the treatments did not affect the volume of gas production (a = 243 mL/g of OM) or the rate of gas production (kd = 3.2%/h). The pH of RI collected in the afternoon was similar for all treatments (6.1). This RI collected from AMt and PM resulted in lower a compared with AM (234 and 232 vs. 246 mL/g of OM; P < 0.01), whereas no effect of treatments on the kd was observed (4.2%/h). Independently of treatments, the RI collected in the afternoon presented lower pH (P < 0.05) and higher kd (P < 0.01) than RI collected in the morning (6.1 vs. 6.3 and 4.2 vs. 3.2%/h respectively). It was concluded that tannins and afternoon grazing affects the fermentative activity of RI collected from dairy cows. The effects of sampling schedule on pH and rate of gas production suggest a higher fermentative activity of RI collected in the afternoon.

Key Words: tannin, grazing schedule, fermentative activity

T218  Evaluation of starter intake in Holstein calves during weaning using blood β-hydroxybutyrate concentrations measured with a handheld meter. R. A. Molano* and M. E. Van Amburgh, Department of Animal Science, Cornell University, Ithaca, NY.

Rumen development is stimulated by the fermentation of carbohydrates and production of volatile fatty acids such as butyrate and propionate. Blood β-hydroxybutyrate (BHB) is well associated with feed intake (FI) and rumen development. Recently, the Nova Vet meter has been validated for BHB monitoring in transition dairy cows. The aim of this study was to evaluate the ketone Nova Vet meter and its ability to detect the production of BHB associated with starter intake during the weaning period. Sixty-one Holstein calves were offered up to 1.6 kg DM/d of milk replacer (28% CP, 15% fat at 150g/L) 3 times/d. Starter grain (25.6% CP, 2.5 Mcal ME/kg) was offered ad libitum and FI was measured daily. Weaning occurred during wk 8 and 9 (i.e., d 49 to 63). Blood was collected from wks 5 to 13, 4.65 ± 0.01 h after the morning feeding and blood BHB was determined using the Ketone Nova Vet kit (NVK). Another sample of plasma was frozen and stored until laboratory analysis using the BHB LiQuiColor assay. The NVK BHB measurements were plotted against laboratory values and the average FI of the 3 previous days, including the day of blood collection. Plotted data were fitted with a linear regression. The optimal cut-point for NVK BHB measure was determined using a receiver operating characteristic curve (ROC), considering FI ≥ 1 kg DM/d as a positive event. Data were analyzed for the entire period of study (EP, n = 540) and the weaning period only (WP, n = 121). Overall, NVK BHB measurements were reasonably correlated with laboratory assay values (r = 0.86, P < 0.001) and FI (r = 0.74, P < 0.001), but correlations were lower during the WP (r = 0.67 and 0.49, P < 0.001). From both EP and WP ROC (AUC = 0.92 and 0.74), the best combination of sensitivity (85 and 58%) and specificity (90 and 82%) using the NVK was found at 0.3 mmol/L BHB. The Nova Vet meter performed well for the period of study. However, since the potential interest of using this tool is determining the appropriate FI for more efficient weaning, data obtained suggests this tool might not be sensitive for this purpose.

Key Words: calf, starter, β-hydroxybutyrate

T217  Effect of cinnamaldehyde on feed intake, rumen fermentation, nutrient digestibility, and milk components in lactating dairy cows. C. Chapman*1, S. Ort2, K. Aragona3, R. Cabral4, and P. Erickson5, 1Penn State Extension-Bradford County, Towanda, PA,

Essential oils, such as cinnamaldehyde, are secondary metabolites obtained from plants that appear to be natural alternatives to antibiotics and function similarly to ionophores. The objective of this study was to evaluate the effect of the essential oil, cinnamaldehyde, on feed intake, rumen fermentation, nutrient digestibility, and milk components in lactating dairy cows. Six lactating Holstein dairy cows (3 ruminally cannulated and 3 noncannulated) averaging (mean ± SD) 263 ± 41 d in milk (DIM) and 754 ± 45 kg of body weight (BW) at the beginning of the study were selected. Cows were randomly assigned to 1 of 3 treatments in a replicated 3 × 3 Latin square design with 19 d periods (14 d for diet adaptation and 5 d for sample collection). Treatments were 0, 2, or 4 mg/kg of BW of the essential oil, cinnamaldehyde. Cinnamaldehyde was mixed with 40 g of corn meal and top-dressed onto the total mixed ration (TMR). Diet was fed as a TMR and contained 37% corn silage, 18.5% mixed-mostly grass silage, 24.5% energy mix, 16.5% protein mix, and 3.5% vitamin and mineral mix on a dry mater (DM) basis. The dietary nutrient composition averaged 15.1% crude protein (CP), 37.8% neutral detergent fiber (NDF), and 24.7% acid detergent fiber (ADF), for all 3 periods. Cows were fed and milked twice daily. No significant differences were observed for dry matter intake (DMI; mean = 42.6 kg/d), milk yield (mean = 28.4 kg/d), 3.5% fat-corrected milk (FCM; mean = 30.6 kg/d), and 3.5% energy-corrected milk (ECM; mean = 30.7 kg/d). Dose of cinnamaldehyde did not have any effect on milk components or rumen fermentation and pH (P > 0.05). There were no differences in nutrient digestibility, but there was a trend for a quadratic effect for DM digestibility: 74.4%, 76.3%, and 73.7% for treatments 0, 2, and 4 mg/kg of BW of cinnamaldehyde, respectively (P = 0.09). Overall, supplementing lactating dairy cows with the essential oil, cinnamaldehyde had no effect on feed intake, rumen fermentation, nutrient digestibility, or milk components.

Key Words: dairy cow, cinnamaldehyde, essential oil

T218  Gene expression of some hepatic gluconeogenic and fatty acid metabolism in early lactation dairy cows as affected by dietary starch and monensin supplementation. M. M. McCarthy1,3, G. D. Mechör1, and T. R. Overton2, 1Elanco Animal Health, Greensfield, IN, 2Department of Animal Science, Cornell University, Ithaca, NY.

The objective of this study was to evaluate the impact of dietary starch level and monensin on the gene expression of some key enzymes related to hepatic gluconeogenesis and fatty acid metabolism during early lactation. Prior to parturition primiparous (primi; n = 16) and multiparous (multi; n = 33) Holstein cows were fed a common controlled energy diet adapted for diet adaptation and 5 d for sample collection). Treatments were 0, 2, or 4 mg/kg of BW of cinnamaldehyde, respectively (P = 0.09). Overall, supplementing lactating dairy cows with the essential oil, cinnamaldehyde had no effect on feed intake, rumen fermentation, nutrient digestibility, or milk components.

Key Words: dairy cow, cinnamaldehyde, essential oil
Mon × parity interactions for PC (P = 0.13) and PCK1 (P = 0.09) such that multi cows fed Mon tended to have increased expression for both PC and PCK1. Correlation analysis was performed for all gene expression variables. Overall, relationships were similar in directionality and magnitude between cows fed HS and LS and Con and Mon. However, for cows fed Con there was a positive relationship between HMGCS2 and PC (r = 0.44; P = 0.02) and HMGCS2 and PCK1 (r = 0.78; P < 0.01), whereas cows fed Mon there was no relationship (r = 0.08; P = 0.72 and r = 0.34; P = 0.10, respectively). Overall, cows fed diets of different starch content in early lactation and monensin throughout the transition period had some alterations in hepatic expression of genes related to glucose and fatty acid metabolism.

**Key Words:** early lactation, starch, monensin

### T219  Effect of dry matter intake (DMI) on N metabolism and urea kinetics in lactating dairy cows.

S. H. Lee¹, H. Lapierre², and D. R. Ouellet², ¹Gyeongsangnam Livestock Promotion Institute, Sancheong, South Korea, ²R&D Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.

To study DMI effect on N metabolism and urea kinetics, 4 dairy cows were used in an incomplete replicated 3 × 3 Latin square (21-d periods). Three diets were formulated to provide similar supply of NE₃ (34.7 Mcal/d) and MP (2300 g/d) through 3 DMI levels: 74% (Low), 86% (Med), and 98% (High) of ad libitum intake. Providing similar supply of MP and NE₃ required adjusting RUP to estimated microbial-N, thus affecting N intake. On d 13, cows were infused with [¹⁵N¹⁵N]-urea (0.5 mmol/h) in a jugular vein for 3 d. Enrichments of [¹⁵N¹⁵N]- and [¹⁵N¹⁴N]-urea in urine and total ¹⁵N in feces were measured to estimate urea kinetics (Lobley et al., 2000, BJN 84: 459). Urine and feces were totally collected from d 14 to 19. Data were analyzed using MIXED procedure of SAS, evaluating DMI effect with polynomial contrasts. The LSM are given for Low, Med, High ± SEM, respectively. Despite a similar MP supply, increased DMI (16.5, 19.2 and 21.8 ± 0.2 kg/d) linearly increased N intake, fecal excretion, and balance but decreased urinary N. Although urea entry rate (UER) was not affected by DMI, urinary urea-N (UUN) linearly decreased with increased DMI, as did the ratio UUN/UER (P = 0.04: 0.53, 0.43, 0.36 ± 0.04). Urea recycled to the gut (GER) tended to linearly increase with DMI, as did the ratio of GER/UER (P = 0.04: 0.47, 0.57, 0.64 ± 0.04). The proportion of GER returned to ornithine cycle (ROC) was unaffected. Urea-N to feces (UFE) linearly increased with increased DMI, as did the ratio of UFE/GER (P = 0.01: 0.07, 0.09, 0.12 ± 0.01). Although GER was elevated with increased DMI, 25% of this increment was lost in the feces. One third of the increment in fecal N with increased DMI was related to the increased UFE: other sources of incremental fecal N could be non-digested RUP or MCP, and endogenous N secretion.

### Table 1 (abstract T219).

<table>
<thead>
<tr>
<th>Item (g/d)</th>
<th>DMI levels</th>
<th>Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Med</td>
</tr>
<tr>
<td>N intake</td>
<td>514.5</td>
<td>550.4</td>
</tr>
<tr>
<td>Urinary N</td>
<td>213.4</td>
<td>186.2</td>
</tr>
<tr>
<td>Fecal N</td>
<td>118.2</td>
<td>145.2</td>
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<tr>
<td>Milk N</td>
<td>145.9</td>
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</tr>
<tr>
<td>N balance</td>
<td>37.1</td>
<td>71.3</td>
</tr>
<tr>
<td>UER</td>
<td>332.2</td>
<td>341.6</td>
</tr>
<tr>
<td>UUN</td>
<td>173.9</td>
<td>145.6</td>
</tr>
<tr>
<td>GER</td>
<td>158.3</td>
<td>196.1</td>
</tr>
<tr>
<td>ROC</td>
<td>55.7</td>
<td>64.8</td>
</tr>
<tr>
<td>UFE</td>
<td>10.7</td>
<td>16.5</td>
</tr>
<tr>
<td>Anabolism</td>
<td>91.9</td>
<td>114.8</td>
</tr>
</tbody>
</table>

**Key Words:** urea kinetics, recycling, dry matter intake

### T220  Effects of forage level and site of starch digestion on N utilization and in vitro urea flux across the ovine ruminal, duodenal, and cecal epithelia.

K. Scott*, G. B. Penner, and T. Mutsvangwa, Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.

Urea recycling to the gut allows ruminants to maintain a positive N balance as recycled urea can support ruminal microbial growth. Our objective was to determine the effects of forage level and site of starch digestion on N utilization and in vitro serosal-to-mucosal urea flux (Jsm-urea) across ruminal, duodenal and cecal epithelia using sheep as a model. Wether lambs (32) were blocked by BW into groups of 4 and randomly assigned (within blocks) to 1 of 4 isoproteic (10.7% CP) diets (n = 8) in a 2 × 2 factorial design. Diet factors were forage level (30% [LF] vs. 70% [HF]) and corn grain processing (whole-shelled [WS] vs. steam-flaked [SF]). Feeding SF was expected to shift starch digestion from post-ruminal sites to the rumen when compared with WS. Four blocks of lambs (n = 4) were used to determine N balance. Lambs were killed after 23 d of diet adaptation. Ruminal, duodenal and cecal epithelia were collected and mounted in Ussing chambers to determine Jsm-urea fluxes were unaffected by diet. Duodenal Jsm-urea fluxes were greater in lambs fed HF compared with LF (77.5 vs. 57.2 nmol·(cm²)⁻¹·h⁻¹; P <
T221  Effect of prepartum nonesterified fatty acids on milk yield and first postpartum ovulation in multiparous Holstein dairy cows.  E. Miqueo1, A. Chiarle2, M. J. Giuliodori2, and A. E. Relling3*. 1Department of Animal Sciences, The Ohio State University, Wooster, OH, 2Fac. Cs Veterinarias, UNLP, La Plata, Buenos Aires Argentina.

The objective of this study was to assess the effect of prepartum nonesterified fatty acids (NEFA) concentration on first postpartum ovulation, milk yield and composition. In a completely randomized block design (3 visits to the farm), 92 prepartum multiparous Holstein dairy cows were screened for plasma NEFA concentration and top and bottom quartile were enrolled in the experiment (n = 54) and considered as high (H-NEFA, plasma NEFA concentration 294 ± 14.52 mM) and low (L-NEFA plasma NEFA concentration 122 ± 14.52 mM) plasma NEFA concentration cows. Blood samples were taken from the coccygeal vein from at a target –6 (range from –21 to 0) days related to calving to measure NEFA. Also, strip milk samples were collected twice a week after the second of 3 daily milkings, from 15 through 56 DIM or until milk progesterone concentration was ≥ 3 ng/mL (indicative of ovulation). Milk yield, milk composition, and progesterone concentration were recorded for each cow. The effect of plasma prepartum NEFA concentration on milk yield and composition were analyzed with Proc Mixed and the hazard of first postpartum ovulation was analyzed with Proc PHReg. The models included the random effect of visit and the fixed effect of NEFA (H- vs. L-NEFA). Sampling week was also included as random effect in the model for milk. NEFA had no effect on milk yield (H-NEFA = 16.45 ± 0.4 vs. L-NEFA = 16.64 ± 0.42; P = 0.738), or milk composition (fat, protein and fat:protein ratio; P > 0.1). Cows having H-NEFA had lower hazard of ovulation than herd mates having L-NEFA (HR = 0.44, 95%CI = 0.19 – 1.02, P = 0.055). Therefore, plasma NEFA prepartum might predict delayed postpartum ovulations but not milk yield or milk composition.

Key Words: NEFA, postpartum ovulation, milk yield

T222  Effects of different physical starter forms on health, growth, rumen parameters and selected blood metabolites in dairy calves.  C. Du1,2, Y. G. Zhen2, L. Ma1,4, A. F. Kertz3, and D. P. Bu*1,5. 1State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2Jilin Agricultural University, Changchun,Jilin, China, 3ANDHIL LLC, Louis, MO, 4CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China, 5Hunan Co-Innovation Center of Safety Animal Production, CICSP, ChangSha, Hunan, China.

The study was conducted to determine whether starter physical form would influence performance, rumen fermentation parameters and selected blood metabolites in dairy calves. Sixty-three Holstein female calves (41.3 ± 2.8 kg at birth) were randomly assigned to 1 of the 3 starter diets differing in physical form: (1) texturized starter (TS; n = 21), (2) pelleted starter with the same formula as TS (PSA; n = 21) and (3) commercially available pelleted starter (PSB; n = 21). All calves were fed 4 L colostrum within 1 h after birth and housed in individual hunches. All calves were offered pasteurized whole milk twice daily 2 L for wk 1, 3 L for wk 2 to 6, and 2 L for wk 7 before full weaning at the end of wk 7 of age. The study finished at the end of wk 9. Calves were fed starter beginning at d 5 of age, and had free access to water and starter. Twelve male calves were randomly assigned to the same 3 treatments for a digestion trial using indigestible NDF and acid insoluble ash as internal marker. Data were analyzed with mixed models using SAS. Starter DMI, gain-to-feed ratios and ADG were not different within the first 6 wk among treatments. During wk 7, DMI of TS (1.23 kg) and PSA (1.24 kg) were not different, but increased more (P < 0.05) than PSB (1.00 kg). For 2 wk after fully weaned, both TS (0.81 kg) and PSA (0.74 kg) had greater DMI (P < 0.05) than PSB (0.63 kg). DMI during wk 7 was lower (P < 0.05) than previous for PSB, and ADG of TS (0.60 kg) and PSA (0.60 kg) were higher (P < 0.05) than PSB (0.53 kg). Observations and photos showed more flies were attracted to TS than for either PSA or PSB during summer mo of this study. During this 9 wk study, PSB had lower (P < 0.05) ADG than the other 2 treatments, but there were no differences in gain-to-feed ratio. TS and PSA had greater (P < 0.05) final BW than PSB. Serum parameters were not different among 3 treatments. PSB had greater butyrate concentration in rumen fluid than PSA and TS (P < 0.05). Also PSB had greater acetate to propionate ratio than PSA and TS. The TS treatment had the lowest digestibility for DM, CP, and ADF (P < 0.05). Hardness of corn kernels appeared to be a factor in lower TS digestibility.

Key Words: calf, texturized starter, pelleted starter

T223  Comparative effects of different forms of flax seed and oil on milk yield and composition in dairy cows: A meta-analysis.  M. Leduc*, M.-P. Létourneau-Montminy, R. Gervais, and P. Y. Chouinard, Département des sciences animales, Université Laval, Québec. QC, Canada.

Numerous trials were conducted during the past decades to evaluate the nutritive value of flax seed and oil for lactating dairy cows. The current meta-analysis was undertaken to assess the overall impact of different forms of flax oil, seed or fractions of seed on production performance and transfer efficiency of its constituent 18:3 n-3 to milk. The database was composed of 85 research papers published between 1998 and 2015 for a total of 158 dietary treatments. Supplements were classified as (1) free oil, (2) intact untreated whole seed (WS), (3) mechanically processed WS (rolling or grinding), (4) extruded WS, (5) protected flax (oil or WS; encapsulation, xyllose or formaldehyde treatments, Ca salts), and (6) flax hulls. A Wilcoxon nonparametric test was used to compare these 6 categories and significance was declared at P < 0.05. Feeding mechanically processed WS (31.7 kg/d) increased the yield of energy-corrected milk (ECM) as compared with intact WS (26.2 kg/d), extruded WS (28.1 kg/d), flax hulls (25.5 kg/d), and free oil (25.4 kg/d), whereas intermediate ECM was observed with protected flax (28.9 kg/d). The feed efficiency (ECM/DMI) was improved by feeding mechanically processed WS (1.55 kg/kgd) as compared with all other oil forms. Among these remaining categories, feed efficiency was greater for extruded WS (1.35 kg/kgd) and free oil (1.35 kg/kgd), intermediate with intact WS (1.28 kg/kgd) and protected flax (1.39 kg/kgd), and lower with flax hulls (1.24 kg/kgd). The transfer efficiency of 18:3 n-3 from diet to milk was, from the lowest to the highest, of 1.96%, 3.63%, 4.17%, 4.79%, 5.80%, and 6.50% for free oil, intact WS, extruded WS, flax hulls, mechanically processed WS, and protected flax, respectively. To conclude, mechanically processed WS was the most suitable treatment, among the 6 flax forms evaluated, to combine optimum lactation performance and protection of 18:3 n-3 to increase its transfer into milk fat.

Key Words: α-linolenic acid, fatty acid transfer efficiency, linseed
Table 1 (abstract T225).

<table>
<thead>
<tr>
<th>Item</th>
<th>AHD</th>
<th>ATD</th>
<th>THD</th>
<th>SEM</th>
<th>P-value</th>
</tr>
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<tr>
<td>Performance data (kg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>(n = 62 cows per pen)</td>
<td></td>
<td></td>
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<tr>
<td>DMI</td>
<td>21.1</td>
<td>20.5</td>
<td>20.6</td>
<td>0.81</td>
<td>0.42</td>
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<tr>
<td>NDF intake</td>
<td>5.9b</td>
<td>6.2a</td>
<td>6.3a</td>
<td>0.24</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Milk yield</td>
<td>37.7a</td>
<td>37.2a</td>
<td>35.9b</td>
<td>0.68</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Milk fat yield</td>
<td>1.44a</td>
<td>1.44a</td>
<td>1.41b</td>
<td>0.10</td>
<td>0.06</td>
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<tr>
<td>Milk protein yield</td>
<td>1.22a</td>
<td>1.19b</td>
<td>1.15c</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N balance (g/d)</td>
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<td>(n = 8 cows per pen)</td>
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<tr>
<td>N intake</td>
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<td>598ab</td>
<td>593b</td>
<td>18.5</td>
<td>0.09</td>
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<tr>
<td>Milk N</td>
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<td>186a</td>
<td>180b</td>
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<tr>
<td>Urinary N</td>
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<td>225</td>
<td>221</td>
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<td>Fecal N</td>
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<td>192</td>
<td>192</td>
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<td>Unaccounted N</td>
<td>12.8</td>
<td>-6.2</td>
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<td>23.4</td>
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tT224 Effect of replacing alfalfa hay with triticale hay on milk production and nitrogen metabolism in dairy cows. O. Santana*1, J. Olmos-Colmenero2, and M. Wattiaux1, 1University of Wisconsin-Madison, Madison, WI, 2Centro Universitario de los Altos, Universidad de Guadalajara, Tepatitlán, Jalisco, México.

Triticale is a less water-intensive crop than alfalfa and could become an alternative source of high crude protein (CP) forage on dairy farms located in water shortage areas. Our objective was to evaluate the effect of replacing alfalfa hay (AH) with triticale hay (TH) on milk production and N metabolism in mid-lactation Holstein cows. The study was conducted on a commercial farm in Jalisco, Mexico, where 12 pens (experimental units) were allocated to 3 treatments in 4 3 × 3 Latin Square where each square was a separate barn. Each pen had 62 cows (54 primiparous and 8 multiparous; 124 ± 34 d in milk and 578 ± 21 kg body weight). Diets (17.4% CP, 1.68 Mcal/kg, and 29.1% neutral detergent fiber (NDF), dry matter (DM) basis) included 25.6% corn silage and either 16.4% AH (AHD), 9.0% AH and 7.6% TH (ATD), or 16.4% TH (THD). Pen values for performance data were from all cows in each pen but N metabolism data were from 8 cows per pen. Nitrogen balance was based on fecal and urine spot sampling. Results in Table indicated no differences in DM intake but higher NDF intake and lower milk protein yield with TH in the diet. Milk and milk fat yields declined only with the highest level of TH in the diet. No difference was detected for milk urea-N (12.4 ± 0.19 mg/dL; P = 0.56) or N use efficiency (milk N / intake N; 30.9 ± 1.03%, P = 0.32). The N balance data showed similar N intake and milk N for cows fed AHD and ATD, but depressed values for cows fed THD. There were no differences for fecal N and urinary N. Findings suggested similar efficiency of N utilization, but restriction in energy supply associated with rumen fill when TH replaced AH in the diet.

Key Words: alfalfa, triticale, dairy

Table 1 (abstract T225).

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>MON</th>
<th>VIRG</th>
<th>CHI</th>
<th>SEM</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
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<tbody>
<tr>
<td>Intake (kg/d)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DM</td>
<td>5.89</td>
<td>4.53</td>
<td>5.63</td>
<td>5.64</td>
<td>0.33</td>
<td>0.050</td>
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<td>CP</td>
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<td>0.584</td>
<td>0.656</td>
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<td>0.009</td>
<td>0.232</td>
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<td>NDF</td>
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<td>1.76</td>
<td>2.22</td>
<td>2.26</td>
<td>0.14</td>
<td>0.043</td>
<td>0.595</td>
<td>0.135</td>
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<td>Relative intake (%BW)</td>
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<tr>
<td>DM</td>
<td>3.24</td>
<td>2.49</td>
<td>3.05</td>
<td>3.13</td>
<td>0.10</td>
<td>0.082</td>
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<td>0.97</td>
<td>1.21</td>
<td>1.25</td>
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<td>0.098</td>
<td>0.685</td>
<td>0.096</td>
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<td></td>
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<tr>
<td>DM</td>
<td>55.8</td>
<td>53.9</td>
<td>55.2</td>
<td>55.5</td>
<td>0.01</td>
<td>0.545</td>
<td>0.751</td>
<td>0.754</td>
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<tr>
<td>CP</td>
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<td>50.0</td>
<td>56.9</td>
<td>63.6</td>
<td>0.02</td>
<td>0.033</td>
<td>0.933</td>
<td>0.014</td>
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<tr>
<td>NDF</td>
<td>54.2</td>
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<td>52.7</td>
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<td>0.01</td>
<td>0.753</td>
<td>0.985</td>
<td>0.681</td>
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</tbody>
</table>

a,bMeans with different superscript differ (P ≤ 0.05).

T225 Comparative effects of chitosan supplementation on nutrient intake and digestibility of Holstein steers. T. Lemos1, M. Ferreira1, A. Pause*1, I. Franco1, G. Rodrigues1, H. Araki1, A. Santos1, C. Takiya2, A. Gabriel1, E. Oliveira1, and J. Gandra1, 1Universidade Federal da Grande Dourados, Dourados, Brazil, 2Kansas State University, Manhattan, KS.

Chitosan, a derivative from chitin presented in the exoskeleton of crustaceans, has antimicrobial properties that influence nutrient utilization in ruminants. This study aimed to compare the effects of monensin (MON) and virginiamycin (VIR) with chitosan (CHI) on nutrient digestibility of Holstein steers. Eight Holstein steers (7 ± 1.5 mo age and 159 ± 6 kg
Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows. C. Villot*1, B. Meunier1, J. Bodin2, C. Martin1, and M. Silberberg1, 1Institut national de la recherche agronomique, UMR1213 Herbivores, Saint-Genès-Champanelle, France, 2BR3 Consultants, Lyon, France.

Subacute ruminal acidosis (SARA) in dairy cows is a nutritional disease difficult to diagnose on field due to a lack of specific clinical signs. Abnormal and intermittent drops in rumen pH remain relevant observations to characterize SARA. The aim of this study was to assess the ability of different pH indicators calculated from rumen pH kinetics to detect SARA. Eleven primiparous dairy cows were submitted into a long-term feed challenge to experimentally induce SARA. Animals were consecutively fed 2 different diets: a low starch control diet (LSD; 13% starch, 4 weeks), a high-starch acidic diet (HSD; 32% starch, 4 weeks), and again the LSD (3 weeks). Rumen pH was monitored continuously using a reticulo-rumen sensor (eCow bolus). Commonly used pH indicators of SARA were daily average, area under the curve (AUC), time spent under pH < 5.8 and pH < 6.0. Rumen pH was also processed to remove sensor drift (8 weeks filter) and noise (48h filter) and normalized on 0 to suppress inter-individual variability. Relative pH indicators such as normalized AUC, times spent under pH < −0.3 and pH < −0.5 (drop of 0.3 or 0.5 pH unit regarding normalized pH on 0), pH range and pH standard deviation were calculated and compared with commonly used pH indicators. SARA syndrome induced by HSD was confirmed by consistent expected changes in animal performances and rumen conditions. Due to high variability in animals (initial rumen pH ranged from 6.1 to 6.7) and sensor drifts and noises, absolute pH indicators appeared less relevant than relative pH indicators for SARA detection during HSD. The observed AUC of receiver operating characteristic (ROC) curves for absolute pH indicators (from 0.48 to 0.71) were lower than those of relative pH indicators (0.78 to 0.91). Moreover AUC of ROC curve of relative pH indicators were statistically higher than time spent under pH < 6.0 (DeLong’s test for 2 correlated ROC curves, P < 0.05). This work shows that relative pH indicators can take into account drift and noise of pH sensors and animal variability. Relative pH indicators appear relevant and robust for SARA detection in a herd and to compare SARA situations among studies.

Key Words: subacute ruminal acidosis (SARA), reticulo-rumen pH sensor, relative pH indicators

Omnigen-AF improves milk yield and composition of grazing dairy cows under a semi-intensive management. N. Orbach1, C. Pedrini1, R. Santos1, B. Alem1, R. Barbosa1, G. Barreto3, L. Barbosa1, C. Takiya2, A. Pause*1, E. Oliveira1, and J. Gandra1, 1Universidade Federal da Grande Dourados, Dourados, Brazil, 2Kansas State University, Manhattan, KS, 3Phibro Animal Health Corporation, Teaneck, NJ

Omnigen-AF (OMN; Phibro Animal Health, Teaneck, NJ) is an immune stimulating feed additive composed of a blend of ingredients including silicon dioxide, aluminosilicate, brewers dehydrated yeast, dried Trichoderma longibrachiatum formation products, and B-complex vitamins. This experiment aimed to determine whether OMN influence milk

Table 1 (abstract T227).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>OMN</td>
<td>Trt</td>
</tr>
<tr>
<td>Production (kg/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>20.3</td>
<td>21.0</td>
<td>0.38</td>
</tr>
<tr>
<td>3.5% FCM</td>
<td>19.6</td>
<td>21.4</td>
<td>0.37</td>
</tr>
<tr>
<td>Fat</td>
<td>0.67</td>
<td>0.74</td>
<td>0.01</td>
</tr>
<tr>
<td>CP</td>
<td>0.67</td>
<td>0.68</td>
<td>0.01</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.91</td>
<td>0.95</td>
<td>0.01</td>
</tr>
<tr>
<td>Casein</td>
<td>0.50</td>
<td>0.53</td>
<td>0.01</td>
</tr>
<tr>
<td>Content (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>3.32</td>
<td>3.53</td>
<td>0.03</td>
</tr>
<tr>
<td>Protein</td>
<td>3.28</td>
<td>3.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.51</td>
<td>4.50</td>
<td>0.01</td>
</tr>
<tr>
<td>Casein</td>
<td>2.47</td>
<td>2.51</td>
<td>0.02</td>
</tr>
<tr>
<td>MUN (mg/dL)</td>
<td>18.5</td>
<td>17.9</td>
<td>0.42</td>
</tr>
<tr>
<td>SCC (=1,000/mL)</td>
<td>119</td>
<td>92</td>
<td>6.65</td>
</tr>
</tbody>
</table>
yield and composition of cows under a semi-intensive management. The experiment was conducted in a commercial farm in Brazil (77.8 temperature-humidity index). Thirty-six multiparous Holstein cows (136 ± 52 DIM, 23.8 ± 4.96 kg/d, and 512 ± 65 BW, at the start of experiment) were used in a completely randomized experiment, being assigned to control diet or top dressed OMG (50 g/d, equal amounts in each meal) during 84 d. Cows were maintained in pasture of Panicum maximum (cv. Mombasa), excepted for periods of milking and meals (4 h/d). Cows received feed in individual bunks consisting of fixed amounts of corn silage (6.2 kg/d DM basis), and grain mixture adjusted according to NRC (2001). Cows were milked and fed twice daily (0500 h and 1600 h), being the milk weight registered and samples taken weekly. Milk samples were analyzed to contents of fat, protein, lactose, casein, urea N, and somatic cell count (SCC) at Clinica do Leite (University of Sao Paulo, Piracicaba, Brazil). Milk urea N concentration was determined by enzymatic-colorimetric method. Data were analyzed as repeated measures using the PROC MIXED of SAS 9.3. OMN-fed cows had lower milk fat content, heat stress, pasture transitions cow, somatic cell count, NEFA-BHB.

Key Words: fat milk content, heat stress, pasture

T228 Yeast-based supplements as an efficient peri-parturient solution on performance and health status of dairy cows. C. Julien1, J. P. Marden1, Y. Huang*1, and D. Kumprechtova2, 1Phileo Lesaffre Animal Care, Marcq-en-Baroeul, France, 2Institute of Animal Science Prague, Prague, Czech Republic.

The objective was to evaluate the effect of live yeast (Saccharomyces cerevisiae Sc47-CNMC1-4407, 1010 cfu/g DM, Phileo Lesaffre Animal Care, France) and yeast derivatives (YP, 30 g/h/d) distributed to a total of 156 Holstein peripartum dairy cows (top dressing on TMR) on performances and health status. Cows were assigned randomly into 3 groups (C, YPP, YTP and YPP) according to their calving date: C received basal close-up (pre-partum) and early lactation (postpartum) diets; YPP received the basal diets supplemented with yeast-based products from −21 d to 0d pre-partum; YTP received the basal diets supplemented with yeast-based products from −21 d to +21 d around calving. Milk yield was individually and daily measured. Milk composition was recorded every 2 weeks over the first 10 weeks of lactation. Somatic cells counts (SCC) linear score was determined using the 0 to 9 scale. Blood samples were collected −7d, 3d and 20d around calving for NEFA, BHB and IGF1 analysis. Milk production, quality and metabolic parameters were analyzed as repeated measures by using the general mixed model including fixed effects of treatment, week, parity, treatment x parity, treatment x week and random effect of cow. SCC linear score was significantly lower for YPP and YTP than C: 3.46 vs. 2.92 on average (P < 0.01). Blood NEFA and BHB contents were significantly lower for YPP (0.64 mM/L and 0.35 mM/L, respectively) than control (0.80 mM/L and 0.45 mM/L, respectively). Blood IGF1 content was significantly increased: 118.2 ng/mL and 98.5 ng/mL with respect to YPP and C. Milk production over the first 15 weeks of lactation was significantly affected by treatments (P < 0.001): 35.8, 36.5 and 37.6 L/d for C, YPP and YTP, respectively. Milk yield of YPP tended to differ than C (P = 0.081) whereas C and YTP and YPP differed significantly. In conclusion, (i) YP given during the close-up period resulted in decreasing SCC in milk and alleviating negative energetic balance and (ii) in addition, supplementing YP during the entire transition period highly improve milk yield.

Key Words: transition cow, somatic cell count, NEFA-BHB

T229 The effect of inclusion of soybean meal or canola meal on an excess of rumen-degradable protein on N metabolism in dairy cows fed grass silage-based diets. C. Roy*1,2, D. R. Oueller2, D. Pellerin1, and H. Lapiere2, 1Department of Animal Science, Université Laval, Québec, Canada, 2Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada.

The effect of inclusion of soybean meal (SBM), canola meal (CM), or an excess of RDP on N metabolism was determined on 6 multiparous Holstein cows (663 ± 25 kg BW) using a replicated 3 x 3 Latin square, balanced for residual effects, with 3 21-d periods. The cows were fed grass silage-based diets, including the following protein sources 1) SBM (14.5% DM), 2) CM (21.1% DM) and 3) CM plus 317 g of urea (CUM). The SBM and CM diets were planned to be isoenetic (1.60 Mcal/kg) and isonitrogenous (16.5% CP), and balanced to meet RDP requirements according to NRC (2001). On the last 4 d of each experimental period, DMI, and milk production and composition were determined. On the last 3 d (2 cows per d), 2 rumen samples were collected to determine NH3 concentrations and 3 spot samples of urine were collected. Creatinine and purine derivatives concentrations were analyzed in urine to estimate urine volume and duodenal microbial nitrogen flow (MNF). Data were analyzed using the Mixed procedure of SAS (treatment, and period as fixed effects, cow as random effect). The multiple comparison test Tukey-Kramer was used to compare the treatment means. Despite lower estimated MP supply with CM vs SBM diets (2504 vs. 2765 g/d), milk and milk protein yield were not affected by protein source. Inclusion of CM in the diet numerically decreased MUN and the proportion of urinary urea-N compared with SBM but did not affect rumen ammonia concentration. Excess RDP supply did not penalize milk and milk protein yields, but decreased the efficiency of N utilization, increased MUN, the proportion of urinary urea-N, and rumen ammonia concentration. Estimated MNF was not affected by treatments.

Table 1 (abstract T229).

<table>
<thead>
<tr>
<th>Item</th>
<th>SBM</th>
<th>CM</th>
<th>CU</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N intake, g/d</td>
<td>651a</td>
<td>635a</td>
<td>799b</td>
<td>24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>36.9</td>
<td>37.6</td>
<td>38.2</td>
<td>1.5</td>
<td>0.18</td>
</tr>
<tr>
<td>True protein yield, g/d</td>
<td>1,040</td>
<td>1,091</td>
<td>1,106</td>
<td>55</td>
<td>0.39</td>
</tr>
<tr>
<td>Milk N / N intake, %</td>
<td>26.4b</td>
<td>28.7b</td>
<td>24.2b</td>
<td>1.1</td>
<td>0.02</td>
</tr>
<tr>
<td>MUN, mg N/dL</td>
<td>13.0b</td>
<td>12.0a</td>
<td>23.0b</td>
<td>0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary urea/total N, %</td>
<td>65.8a</td>
<td>59.9a</td>
<td>72.6b</td>
<td>2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rumen ammonia, mg/100 mL</td>
<td>18.7a</td>
<td>18.6a</td>
<td>42.9b</td>
<td>2.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Means within rows with different superscripts differ (P < 0.05).

Key Words: urea, nitrogen metabolism, rumen degradable protein

T230 Health, milk yield and milk quality records evaluated in 76 European dairy farms before and during OmniGen-AF supplementation to dry and lactating cows. R. García-González*1, P. Bozzi1, M. Corsini1, A. Dekker2, W. Germis2, E. Hoogland1, J. Chapman1, and L. Ely2, 1Phileo Animal Health Corp., Teaneck, NJ, 2University of Georgia, Athens, GA.

Strategies are being implemented in European countries to reduce the use of antimicrobials in farm animals. In this context, innovative husbandry practices that support animal health and productivity will likely play...
a key role in the near future. OmniGen-AF® (Phibro Animal Health, Teaneck, NJ) is a feed product that has been shown to influence key markers of immune function in cattle. The objective of this study was to evaluate the effects of feeding OmniGen-AF on health and milk records in European dairy farms. Seventy-six dairy farms from Belgium, Italy, and the Netherlands were enrolled in this study in 2016. Farm records were collected for the 120 or 150 d before (Pre-OG), and during feeding 55 g/hd/d of OmniGen-AF to all dry and lactating cows (OG). Metrics recorded by number of farms included: cases of mastitis (n = 44), retained placenta (n = 49), metritis (n = 47), and culled cows (n = 17); milk yield (n = 70) and bulk tank SCC (n = 74). Incidence of mastitis and culled cows was expressed as % total cows, while incidence of retained placenta and metritis as % fresh cows. Farm was the statistical unit, OG vs. Pre-OG data were analyzed using a paired t-test, and statistical significance was declared when P < 0.05 and trends when P < 0.10.

Average farm size was 154 cows/farm; 24% of farms were less than 100 cows, 59% had 100 to 200 cows, and 17% were greater than 200 cows. Overall, the incidence of mastitis, retained placenta and metritis all decreased (P = 0.001) during the OG vs. Pre-OG period: −27% mastitis (14.7% Pre-OG vs. 10.7% OG), −38% retained placenta (16.7% vs. 10.3%), and −32% metritis (24.5% vs. 16.7%); no significant difference was detected in number of culled cows. Bulk tank SCC decreased (P = 0.001) an average of −55,000 cells/mL during the OG period (309k vs. 254k cells/mL) while milk yield tended to increase (P = 0.057): 31.3 vs. 31.8 kg/hd/d. These data suggest that feeding OmniGen-AF to dry and lactating cows along with sound nutritional and management practices can have a positive influence on health parameters, milk yield and milk quality in European dairies.

Key Words: health, production, OmniGen-AF

T231 Effect of dietary supplementation of two forms of B-vitamins on growth and efficiency of Holstein calves from 3 to 13 weeks of age. R. A. Molano*, 1 C. L. Girard2, and M. E. Van Amburgh1, 1Department of Animal Science, Cornell University, Ithaca, NY, 2Agriculture & Agri-Food Canada, Sherbrooke, QC, Canada.

Weaning involves upregulation of rumen function and B-vitamin (B-vit) supplementation has been considered unnecessary due to their production by rumen microbes. Literature data are equivocal concerning the need for supplemental dietary B-vit through the transition phase. The hypothesis was that B-vit are limiting during the transition period and to be effective, the vitamins must be fed in a protected form. To evaluate the effect of supplementing B-vitamins on calf performance, sixty-one 3-week-old Holstein calves were individually housed from birth to 13 wk of age. Milk replacer (MR; 28% CP, 15% fat) was offered up to 1.6 kg DM/d at 15% solids, 3 times/d. Starter grain (SG; 25.5% CP, 2.5 Mcal ME/kg) with no added B-vit was offered ad libitum. Calves were randomly assigned among 3 treatments: rumen protected B-vit blend (RPBV, n = 20); a 30:70 mix of non-protected B-vit blend and fat (UPBV, n = 22); and fat only (CTRL, n = 19). Treatments were fed daily at levels corresponding to 0.39 ± 0.001% and 0.28 ± 0.001% (mean ± SEM) of the SG intake, for the B-vit and fat, respectively. The treatments were weighed into gel caps of varying weights and administered once a day to each calf based on the previous day SG intake. Weaning was from d 49 to d 63. Body growth was measured weekly. Blood was collected at wk 3, 7, 9 and 13, and analyzed for hematocrit (Hem), Plasma Urea Nitrogen (PUN), folates (Fol) and vitamin B12. Data were analyzed as a completely randomized design with a mixed-effects model, using fixed effects of treatment, time, and their interaction, and initial body weight as covariate. Overall ADG (0.99 ± 0.01 kg/d), DMI (1.90 ± 0.02 kg DM/d) and feed efficiency (0.52 ± 0.01) were not different (P ≥ 0.4). In general, plasma B12 levels were not different between RPBV and UPBV (P ≥ 0.5) and a tendency was detected for supplemented vs control at weaning (P = 0.09). No differences were observed for Hem, PUN, or Fol (P ≥ 0.2). Thus, both forms of supplemented B-vit tended to improve vitamin B12 status through weaning. Under the conditions of this study, supplementary vitamins did not improve calf performance.

Key Words: calf, weaning, B-vitamins

T232 Original XPC and NutriTek increase volatile fatty acid production in an in vitro rumen microbial model using TMR diets from various US regions. T. Kwan*, C. Reedy, T. Werner, J. Butler, and I. Yoon, Diamond V, Cedar Rapids, IA.

The objective of this study was to determine the effects of 2 Saccharomyces cerevisiae fermentation products—Diamond V Original XPC (XPC) and NutriTek (NutriTek)—on in vitro VFA production on TMR from various US regions. Six TMR representing the Midwest (2), South Central (1), Southwest (1), Northeast (1), and Southeast (1) were evaluated. Serum bottles (100 mL) containing 0.3 g TMR and treatment (Control, XPC, or NutriTek) were inoculated with buffered rumen fluid and incubated for 12 and 24 h. Three replicate experiments were performed to generate 9 replicates per treatment, diet, and time point. Data were analyzed using the GLM model of JMP. Significance was defined as P ≤ 0.05. Averaged across diets, XPC resulted in higher (P < 0.001) VFA concentrations than Control, while NutriTek further increased (P < 0.001) VFA concentrations over XPC (Table 1). Within individual TMR diets, XPC increased acetate (4.0 to 7.6%), propionate (4.8 to 16.9%), butyrate (4.6 to 6.7%) or total VFA (2.9 to 11.0%) over Control (P ≤ 0.01) on all TMR diets at 12 h or 24 h. NutriTek increased acetate (4.1 to 12.6%), propionate (9.5 to 24.9%), butyrate (7.9 to 18.5%) or total VFA (6.8 to 16.6%) over Control (P ≤ 0.03) for all TMR diets at 12 and 24 h. There were no diet by treatment interactions (P ≥ 0.17). In conclusion, XPC and NutriTek were able to enhance VFA production on a range of TMR diets in vitro. NutriTek further enhanced ruminal VFA production over XPC on multiple diets tested.

Key Words: XPC, NutriTek, TMR

Table 1 (abstract T232). In vitro VFA production by rumen microbiota incubated with two Saccharomyces cerevisiae fermentation products averaged across six TMR diets.

<table>
<thead>
<tr>
<th>Time/Treatment</th>
<th>Acetate (mM)</th>
<th>Propionate (mM)</th>
<th>Butyrate (mM)</th>
<th>Total (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16.8±</td>
<td>11.3±</td>
<td>2.6±</td>
<td>31.2±</td>
</tr>
<tr>
<td>XPC</td>
<td>17.8±</td>
<td>12.4±</td>
<td>2.7±</td>
<td>33.5±</td>
</tr>
<tr>
<td>NutriTek</td>
<td>18.5±</td>
<td>13.2±</td>
<td>2.9±</td>
<td>35.2±</td>
</tr>
<tr>
<td>SEM</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>24.7±</td>
<td>15.9±</td>
<td>3.9±</td>
<td>45.4±</td>
</tr>
<tr>
<td>XPC</td>
<td>25.6±</td>
<td>16.5±</td>
<td>4.1±</td>
<td>47.2±</td>
</tr>
<tr>
<td>NutriTek</td>
<td>26.7±</td>
<td>17.0±</td>
<td>4.3±</td>
<td>49.9±</td>
</tr>
<tr>
<td>SEM</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*Means without a common superscript differ (P ≤ 0.05).

Key Words: XPC, NutriTek, TMR

T233 Monitoring ketosis in a commercial Holstein and Jersey herd. K. E. Mitchell* and H. A. Rossow, University of California, Davis, Davis, CA.
Subclinical ketosis, a common metabolic issue in the transition period, is estimated to cost $78 per case due to decreased milk production, reduced fertility, displaced abomasum, and other health issues (Geisshauer et al., 2001). Considering the potential loss of profit to a producer, this study focused on identifying the most effective monitoring program to identify at risk cows. Holstein (n = 54) and Jersey (n = 52) multiparous cows at a commercial dairy were enrolled during the prepartum period and then followed to approximately 21 DIM. Weekly blood samples were analyzed using Nova Max (Nova Diabetes Care Inc., Billerica, MA) for Glucose (Glu, mg/dL) and β-hydroxybutyric acid (BHB, mmol/L). Previous research has shown the meter to have a sensitivity of 74.4% and specificity of 100%. Weekly milk tests were taken in first 21 DIM and compared with BHB and Glu. Both breeds experienced a decrease in Glu and an increase in BHB from prepartment to postpartum (P < 0.0001). However, Jerseys were lower than Holsteins for both during early lactation with values of 2.45 mg/dL and 0.13 mmol/L, respectively (P = 0.061, P < 0.0001). Due to this difference, breeds were separated for the rest of analysis. Blood categories were assigned as 1 (less than) or 2 (greater than) for multiple thresholds for both BHBA and Glu levels. Initially, blood parameters were compared with health issue risk and milk production level individually, but neither alone yielded any significant results. Due to a low incidence of hyperketonemia, Jerseys did not have enough samples available for analysis; only 5 cows had >1.0 mmol/L BHB versus 25 Holstein cows. For Holsteins, using both parameters as markers were more accurate for identifying suppressed milk production. They had increased risk of health issues and a decrease of 5.44 kg/d of milk when BHB >1.0 mmol/L and Glu <50 mg/dL (P = 0.099). The highest incidence of ketosis for both breeds was in the first week of production. Therefore, a monitoring program within the first week of lactation would be more beneficial for Holsteins. Based on these results, both BHBA and Glu testing is recommended. Because Glu strips are less expensive, Glu can be used to prescreen for hypoglycemia first, results, both BHB and Glu testing is recommended. Because Glu strips are less expensive, Glu can be used to prescreen for hypoglycemia first.

Key Words: BHB, glucose, ketosis

T234  Effects of spray-dried plasma product on transition and early lactation dairy cows. C. Lee1, A. Tebbe1, J. M. Campbell2, and W. P. Weiss1, 1Department of Animal Sciences, OARDC, The Ohio State University, Wooster; OH, 2APC Inc., Ankeny, IA.

The objective of the experiment was to investigate the effects of feeding a spray-dried plasma (SDP) product on production and blood profile in dairy cows during the transition and early lactation period. Seventy-two Holstein cows at 14 d before calving were used in a randomized block design. During the prepartum period, cows were fed a typical gestation diet containing milk meal (BM; 0.8% in dietary DM; Control, n = 24) or SDP (0.8% in dietary DM; n = 48). After calving, cows on control were fed a typical lactation diet containing 0.6% BM (DM basis). Cows on SDP prepartum were fed a lactation diet containing 0.6 (LowSDP; n = 24) or 2.4% SDP (HighSDP; n = 24) on a DM basis where SDP replaced BM or BM and soybean products. All data were analyzed using the MIXED procedure of SAS as a randomized block design. Prepartum supplementation of SDP had no effect on blood NEFA and BHBA (2 d before calving). After parturition, plasma NEFA (255 ± 29 µEq/mL) and BHBA (675 ± 70 µmol/L; 8 and 14 d of lactation) were not affected (P ≥ 0.61) by SDP in the diet. Cows fed SDP (Low and HighSDP vs. Control) did not affect DMI but increased or tended to increase (P ≤ 0.10) milk fat, protein, and lactose contents for 16 wks after parturition. High inclusion of SDP in the diet increased (P ≤ 0.07) milk yield (42 vs. 39 kg), ECM (44 vs. 41 kg), ECM per kg DMI, and yields of milk fat (1.60 vs. 1.48 kg/d), protein (1.21 vs. 1.16 kg/d), and lactose (2.07 vs. 1.97 kg) compared with LowSDP. Body weight losses were lower (P = 0.05) for SDP compared with the control without a difference between LowSDP and HighSDP. Plasma histidine concentration (d 14 of lactation) was lower (P = 0.01) for SDP compared with the control. In addition, plasma 1-methyl-L-histidine tended to be lower (P = 0.09) as inclusion rate of SDP increased. In conclusion, SDP at high inclusion rate increased milk and milk component yields without an increase in feed intake when milk meal was replaced in the diet. Studies evaluating effects of functional and active compounds in SDP on gut microbiome, gut health, and immune functions may be needed to determine mode of action.

Key Words: spray-dried plasma, transition, early lactation

T235  Effect of source and pelleting on protein degradation of dried distillers grains with solubles. A. Carpenter1,2, J. F. Rivera1, C. Ylioja1, K. Herrick4, and B. Bradford1, 1Kansas State University, Department of Animal Sciences and Industry, Manhattan, KS, 2University of Guelph, Department of Animal Biosciences, Ridgetown, ON, Canada, 3Universidad Zamorano, Francisco Morazan, Honduras, 4POET Nutrition, Sioux Falls, SD.

Two experiments were performed to determine whether source of dried distillers grains with solubles (DDGS) or pelleting of DDGS impact ruminal and intestinal digestibility of CP and DM. In Experiment 1, treatments consisted of unprocessed Dakota Gold DDGS (DAK; POET Nutrition, Sioux Falls, SD) or commodity DDGS (CON). In Experiment 2, treatments consisted of DAK subjected to various pelleting parameters, and statistical contrasts were used to test differences within each phase. Phase 1 evaluated pelleting at 49 or 82°C; phase 2 treatments were pelleted at 66°C, either using a 4- × 22.5-mm die or a 19- × 114.3-mm die; and phase 3 evaluated pelleting at a feeder rate of 1,360 or 3,175 kg/h. Approximately 0.5 g of dried and ground (2mm) substrate was sealed into Dacron bags and suspended in the rumens of 4 heifers for 0 (washout), 2, 8, 16, 24, or 48 h, with 4 replicates per time point by heifer. A subset of each treatment was exposed to 16 h of in situ rumen degradation followed by the Three-Step Procedure of Calsamiglia and Stern (1995) to estimate intestinal digestibility. In Experiment 1, DAK had decreased soluble N (15.5 vs. 33.2 ± 4.3%) and a tendency for decreased N residue after 48 h of ruminal incubation (5.8 vs. 3.5 ± 0.7%). Using an estimated pass rate of 10%/h and observed intestinal digestibility, digestible RUP (dRUP) was greater for DAK than CON (43.4 vs. 34.8 ± 2.0% of CP). In Experiment 2, pelleting of DAK increased DM and N solubility and tended to increase intestinal digestibility compared with unprocessed DAK, resulting in an increase in RDP and a decrease in dRUP (17.9 vs. 27.2 ± 1.7%). Dry matter solubility was increased (65.9 vs. 63.0 ± 0.7%; P < 0.01) and there was a tendency for greater N solubility (72.9 vs. 66.5 ± 3.4%; P < 0.10) when the larger die size was used. Additionally, increased feeder rate tended to increase DM solubility (P < 0.10). No other treatment effects were observed. In conclusion, unprocessed DAK provided more dRUP than CON, whereas pelleting of DDGS shifted protein digestion to the rumen, with minimal effects of pelleting protocol except for die size.

Key Words: protein, distillers grains, digestion

T236  Effects of spray-dried plasma product supplementation on transition and lactation on milk production and reproduction in dairy cows. A. Bach1, J. Polc1, J. M. Campbell1, M. E. de Haro Martí2, and M. Chahine5, 1Department of Ruminant Production, IRTA, Spain, 2AECI, Institució Catalana de Recerca i Estudis Avançats, Spain, 3Department of Ruminant Production, IRTA, Spain, 4APC Inc., Ankeny, IA, 5University of Idaho Extension, 314 J. Dairy Sci. Vol. 100, Suppl. 2
T238  Consequences of supplying methyl donors during pregnancy on the methyome of the offspring from lactating and non-lactating dairy cattle. A. Bach*1,2, A. Aris2, A. Pinto1, and I. Guasch3, 1ICREA, Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain, 2Department of Ruminant Production, IRTA, Caldes de Montbui, Spain, 3Blanca from the Pyrenees, Lleida, Spain.

To assess the potential effects of vitamin B12 (B12) and folic acid (FA) supplementation during early stages of pregnancy on the methylome of the offspring from either lactating cows or heifers, 20 Holstein cows, 10 nulliparous (612 ± 46 kg of BW) and 10 multiparous (677 ± 58 kg of BW), once confirmed pregnant (35.7 ± 3.86 d pregnant), were blocked by parity and randomly assigned to either an i.m. weekly injections of a placebo (CTRL) or a solution containing methyl donors (MET). Between pregnancy diagnosis until cows reached 120 d of pregnancy, MET nulliparous cows received 10 mg of B12 and 1,000 mg of FA, whereas MET multiparous cows received 20 mg of B12 and 2,000 mg of FA. After calving, 5 calves randomly selected from each treatment (2 born to primiparous and 3 to multiparous dams) were blood-sampled to isolate peripheral blood mononuclear cells, extract DNA and determine their full methylome. A differentially methylated region (DMR) analysis of CpG islands was performed. Only significant DMR with changes >30% and P < 0.01 were considered. There were more than 2,000 CpG differentially (P < 0.001) methylated between calves born to CTRL and calves born to MET. Within these CpG, 32 and 587 CpG at the promoter and gene regions, respectively, were heavily hypomethylated in MET calves compared with CTRL calves. Likewise, 27 and 437 CpG at the promoter and gene regions, respectively, were heavily hypomethylated in MET calves compared with CTRL calves. These changes were more (P < 0.05) marked in calves born to lactating cows than to heifers. Offspring from MET lactating cows had 63 and 464 CpG hypermethylated in the promoter and gene regions, respectively, and 47 and 438 CpG hypomethylated in the promoter and gene regions, respectively. However, supplementing pregnant heifers induced only an hypermethylation of 30 and 161 CpGs in the promoter and gene regions, respectively, and an hypomethylation of 34 and 157 GpG in the promoter and gene regions, respectively. It is concluded that current nutritional recommendations for FA and B12 may be underestimated, and that supplementation of methyl donors early in gestation has important effects on the methylome, especially in lactating dams.

Key Words: epigenetics, gestation, methylation

T237  Performance response of dairy cattle supplemented with a fungal-derived extract from Trichoderma reesei. I. Guasch¹, G. Elcós¹, M. S. Gómez-Conde², N. D. Walker³, G. Cordero², and A. Bach*⁴,⁵, ¹Blanca, Hostalets de Tost, Lleida, Spain, ²SETNA SAU, Madrid, Spain, ³ABVista, Marlborough, UK, ⁴ICREA, Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain, ⁵Department of Ruminant Production, IRTA, Caldes de Montbui, Spain.

Fifty lactating Holstein dairy cows (679 ± 75 kg of BW; 164 ± 84 DIM) were enrolled in a study following a randomized design to assess the effects of a fermentation product derived from Trichoderma reesei strains RF8055 and RF 5427 (VistaPre-T, ABVista, UK) on efficiency of milk production. Cows were blocked by parity (26 primiparous and 24 multiparous) and allocated to either a Control ration (no supplementation) or ration (VIST) containing 750 mL/ton (DM basis) of VistaPre-T for 2 mo. The ration provided 15.4% CP, 33.8% NDF, 2.8% fat, and 1.56 Mcal of NEl/kg (DM basis). Individual feed intake, milk production and fat and protein content, and BW were monitored daily. Data were summarized by week and analyzed using a mixed-effects model for repeated measures accounting for the fixed effects of treatment, week, parity and their 2- and 3-way interactions. Multiparous (24.8 ± 0.68 kg/d) cows consumed more (P < 0.05) feed than primiparous cows (21.6 ± 0.65 kg/d) but there were no differences in DMI between treatments (23.2 ± 0.39 kg/d). There was interaction between treatment and week (P < 0.05), treatment and parity (P < 0.05), and among treatment, week, and parity (P < 0.01). Primiparous cows on VIST produced more milk (33.5 ± 1.46 kg/d) than primiparous cows on Control (30.6 ± 1.34 kg/d) after about 3 wk of exposure to treatments. Milk fat (3.71 ± 0.08%) and protein (3.31 ± 0.03%) contents were unaffected by treatment, but fat was greater (P < 0.05) in multiparous cows after 4 wk on VIT than in multiparous cows on Control, with no differences in primiparous cows. Body weight was also affected by an interaction among treatment, week, and parity (P < 0.05), with primiparous cows on VIST gaining more weight than primiparous cows on Control. Feed efficiency (kg of milk/kg of DMI) increased in VIST cows also after about 3 wk of exposure, and the increase was more (P < 0.01) pronounced in primiparous (+0.21 increase; P < 0.001) than in multiparous (+0.02 increase; not significant) cows. In conclusion, supplementation of fungal-derived extracts from Trichoderma reesei improves milk yield and feed efficiency in first lactation animals in mid lactation and milk fat content in multiparous cows.

Key Words: lactation, milk, reproduction

T239  Relationship of NDFD24 and uNDF240 to NDF components and the impact of maturity on predicted digestible energy of corn plants harvested at silage maturity. B. Powel-Smith, M.
Components of NDF (hemicellulose, cellulose, and lignin) differ in digestibility. How plant maturity alters subcomponents and changes in vitro NDF digestibility and uNDF240 interests plant breeders. Silage producers need more data on the impact of plant maturation on NDF components, starch content, digestibility, and yield. One commercial Pioneer silage hybrid was grown on 6 farms in the upper Midwest. Six plants from 2 diverse locations within each farm were harvested at silage maturity (26 to 38% plant DM). Divided into ear and non-ear fractions, plants were dried and assayed or proportionally recombined and assayed at a commercial lab. Based on within-farm replicate silages, differences among farms were detected (P < 0.05) in whole plant dry weight and in NDF, ADF, starch, cellulose, and lignin contents. Farms did not differ significantly in NDFD24 despite a range of 52 to 62%. Covaried by plant DM content, only lignin remained different (P < 0.03) among farms as greater (P < 0.01) starch diluted (P < 0.05) NDF, ADF, potentially digested NDF (1 – uNDF240), and cellulose. uNDFD24 was related (P < 0.01) negatively to uNDF240 but positively to hemicellulose as a fraction of NDF; NDFD240 was related (P < 0.01) negatively to uNDF but positively to digestible NDF (NDFD24 x NDF). With components expressed as a fraction of NDF, hemicellulose increased whereas cellulose and lignin decreased (P < 0.03) as plant DM increased reflecting NDF compositions of ear and non-ear plant portions. By multiple regression, uNDFD24 and NDFD240 were more responsive to hemicellulose than to cellulose or lignin as a fraction of NDF (slopes of 0.93, 0.33, and 0.03; 0.90, 0.66, and 0.42) reflecting digestibility differences. With regressions forced through zero, uNDFD24 increased by 2.1, 6.6, and 4.6 percentage points for each 1% increased plant lignin. Based on constant NDFD24, decreased NDF, and increased starch content (assuming 94% starch digestibility), digestible DM increased (P < 0.02) 0.42% per kg and 1.84% per hectare for each 1% increase in corn plant DM. Delayed harvest within the corn silage DM window increases available energy content of and yields from corn plants.

Key Words: corn plant maturity, NDF digestion, NDF components

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**T240**  The time of wheat straw inclusion affects weaning weight and average daily gain in Holstein calves. A. Gahremani1, E. Mahjoubi2, M. Chamani1, M. H. Yazdi3, R. A. Patton*3, and M. Bahrami1

Forage and straw inclusion in calf grain starter has beneficial effects on rumen development, average daily gain (ADG), and dry matter intake of dairy calves. However, the optimum age when roughage should be included in the starter diet has not yet been defined. To determine optimum time of straw inclusion, 40 Holstein calves (36.7 ± 5.0 kg) were assigned at 3 d of age to 1 of 4 treatments in a completely randomized block design lasting until weaning at 60 d. Treatments (% of dry matter) were: control starter without wheat straw (WS) until d 21 of age; 90% calf starter concentrate (CSC) + 10% chopped WS, and 90% calf starter concentrate (CSC) + 10% chopped WS offered from d 21 to d 60 (WS21); 90% CSC + 10% chopped WS offered from d 21 to d 60 (WS21); 90% CSC + 10% chopped WS offered from d 21 to d 60 (WS21). All WS was mixed with the starter, and all calves received 8 to 2 L of whole milk daily in a step up-step down manner according to farm management protocol. Total feed consumption and body weight (BW) gain were monitored until weaning at d 60. Effect of diet was analyzed within specific time periods using the JMP version of SAS with the effect of WS evaluated as an orthogonal contrast (WS versus no WS) at each period. Solid food consumption at d 60 (827, 947, 1064 and 1151 ± 24 g/d for CON, WS21, WS21, and WS41, respectively, was different among treatments (P < 0.05) with inclusion of WS positive (P < 0.001). The 60 d ADG (0.74, 0.78, 1.00 and 0.94 ± 0.03 kg/d for the respective treatments with CON and WS being lower than WS21 and WS41 (P < 0.05) and with a positive WS effect (P < 0.001). The 60 d BW followed a similar pattern (69.8, 69.9, 79.8, and 73.2 ± 1.9 kg/d for treatments CON, WS3, WS21 and WS41, respectively (P < 0.05) and inclusion of WS still positive (P < 0.05). Rumen β-hydroxy butyric acid was not affected by treatments. The days that calves experienced a negative health event were also not affected by treatment. In conclusion, WS inclusion at d 21 of age is recommended based on weaning BW,DMI and ADG.

Key Words: calf, wheat straw, growth

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**T241**  Effect of diet supplementation with probiotic *Pedio -

Forage and straw inclusion in calf grain starter has beneficial effects on rumen development, average daily gain (ADG), and dry matter intake of dairy calves. However, the optimum age when roughage should be included in the starter diet has not yet been defined. To determine optimum time of straw inclusion, 40 Holstein calves (121 ± 22 DIM) were blocked by milk yield in a randomized complete block design. All treatments consisted of a basal TMR with top-dressed supplements: (1) control (CON) with no probiotics; (2) PED fed at 1 × 1010 cfu/d; (3) BAC fed at 1 × 1010 cfu/d; and (4) basal TMR supplemented with a combination of *Enterococcus faecium* 3 at 1 × 1010 cfu/d and yeast (PRO). Cows were housed in a free stall barn with individual feeding gates, milked thrice a day and fed twice daily for 105 d; daily feed intake and milk yield data were averaged weekly. Eight rumen fistulated cows, 2 per treatment, were used for rumen pH measurements every 2 h during a 24-h period on d 105. Data were analyzed using a mixed model with week, treatment and their interaction as fixed effects with pre-trial milk yield as a covariate; block and cow were considered random effects. Dry matter intake was similar (P = 0.22) across treatments averaging 24.3 ± 0.76 kg/d. Milk yield was similar across treatments (P = 0.82), values were 37.2, 37.7, 36.5 and 37.9 ± 1.36 kg/d for CON, PRO, BAC and PED, respectively. Concentration of milk fat and protein were similar (P ≥ 0.69) across treatments averaging 3.63 ± 0.16% and 3.05 ± 0.06%, respectively. Overall yield of milk fat, protein and milk urea nitrogen were similar (P ≥ 0.34) across treatments and averaged 1.33 ± 0.08 kg/d, 1.11 ± 0.06 kg/d, and 12.6 ± 0.39 mg/dL, respectively. Cows consuming CON and PED tended (P = 0.12) be 28.5 kg heavier compared with the PRO and BAC. Mean daily rumen pH was similar across treatments (P = 0.29) and averaged 5.69 ± 0.05. These results demonstrate that supplementation with *P. acidilactici* 1 or *B. subtilis* 2 as probiotics for dairy cows do not have negative effects on rumen pH and that milk production and composition is maintained.

Key Words: direct-fed microbial, lactic acid bacteria

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**T242**  Amount of milk replacer offered to female Holstein calves during first eight weeks of life: Effect on ruminal pH and diet digestibility in pre and post weaning weeks. G. Antúnez1, C. Cajaville*1, C. M. Fernández1, J. E. Dayuto1, L. M. Artús1, M. Fernández1, L. Hornos1, F. Correa1, R. Biasiolo2, A. Vicente3, and J. L. Repetto1, Instituto de Producción Animal de Veterinaria, Facultad de Zootecnia, Universidad de Chile, Santiago, Chile.

The objective of this study was to test the effects of *Pediococcus acidilactici* 1 (PED) isolated from bovine cecum wall or *Bacillus subtilis* 2 (BAC) isolated from porcine feces as probiotics in the diet of lactating dairy cows on lactation performance and ruminal pH. To do so, 48 multiparous Holstein cows (121 ± 22 DIM) were blocked by milk yield in a randomized complete block design. All treatments consisted of a basal TMR with top-dressed supplements: (1) control (CON) with no probiotics; (2) PED fed at 1 × 1010 cfu/d; (3) BAC fed at 1 × 1010 cfu/d; and (4) basal TMR supplemented with a combination of *Enterococcus faecium* 3 at 1 × 1010 cfu/d and yeast (PRO). Cows were housed in a free stall barn with individual feeding gates, milked thrice a day and fed twice daily for 105 d; daily feed intake and milk yield data were averaged weekly. Eight rumen fistulated cows, 2 per treatment, were used for rumen pH measurements every 2 h during a 24-h period on d 105. Data were analyzed using a mixed model with week, treatment and their interaction as fixed effects with pre-trial milk yield as a covariate; block and cow were considered random effects. Dry matter intake was similar (P = 0.22) across treatments averaging 24.3 ± 0.76 kg/d. Milk yield was similar across treatments (P = 0.82), values were 37.2, 37.7, 36.5 and 37.9 ± 1.36 kg/d for CON, PRO, BAC and PED, respectively. Concentration of milk fat and protein were similar (P ≥ 0.69) across treatments averaging 3.63 ± 0.16% and 3.05 ± 0.06%, respectively. Overall yield of milk fat, protein and milk urea nitrogen were similar (P ≥ 0.34) across treatments and averaged 1.33 ± 0.08 kg/d, 1.11 ± 0.06 kg/d, and 12.6 ± 0.39 mg/dL, respectively. Cows consuming CON and PED tended (P = 0.12) be 28.5 kg heavier compared with the PRO and BAC. Mean daily rumen pH was similar across treatments (P = 0.29) and averaged 5.69 ± 0.05. These results demonstrate that supplementation with *P. acidilactici* 1 or *B. subtilis* 2 as probiotics for dairy cows do not have negative effects on rumen pH and that milk production and composition is maintained.

Key Words: direct-fed microbial, lactic acid bacteria
The aim of this study was to evaluate whether amount of milk replacer (MR) offered to female Holstein calves during first 8 weeks of life affects ruminal pH and diet digestibility in pre and post weaning weeks. Thirty-two newborn female Holstein calves (41 ± 4.3 kg BW) were housed individually. From 2nd to 7th week of life, calves were distributed in 2 feeding programs: 24 g (AR, n = 16) or 12 g (CR, n = 16) of MR powder per kg of initial BW (25% CP, 20% fat, prepared at 12.5% solid and offered at 0800 and 1630 h). In both treatments, calves had free access to a pelleted starter (PS, 21% CP, 15% NDF) and water. Weaning process was during 8th weeks and in this period calves received decreasing amounts of MR, free access to PS and 0.2 kg of chopped hay (CH, 18% CP, 40% NDF) in a separated bucket. In 9th week, all calves received 2.0 kg of PS and 0.4 kg of CH per day. In 7th and 9th week feed intake was recorded daily and feces were collected during 6 consecutive days to determine diet dry matter digestibility ([g DM intake - g DM excreted]/g DM intake). In the same weeks ruminal fluid samples was taken through a stomach tube at 0800, 1200, 1630 and 2000 h and ruminal pH was measured. Feed intake, digestibility and daily average ruminal pH were analyzed using a mixed model including the fixed effect of treatment and the random effect of block. During the 7th week, calves fed the AR treatment had a lower intake of PS (695 vs. 1160 ± 86.0 g DM/d, P < 0.01) but digestible dry matter intake was higher (1592 vs. 1198 ± 73.7 g DM/d, P < 0.01). Their digestibility and daily average ruminal pH were higher (0.92 vs. 0.87 ± 0.01 g DM, P < 0.01 and 5.56 vs. 5.38 ± 0.06, P = 0.04, respectively). However, during the week after weaning (9th week) no significant differences were found in diet dry matter digestibility, digestible dry matter intake or ruminal pH (P > 0.05). In conclusion, offering greater amounts of MR affected feed intake, diet digestibility and ruminal pH before weaning, but did not affect intake and digestion immediately after weaning.

**Key Words:** dairy calf, digestibility, ruminal pH

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Feeding supplements to grazing dairy cows as a partial mixed ration (PMR) can have milk production benefits over feeding equivalent amounts of ME as cereal grain in the parlor and conserved forage in the field, especially if the PMR contains canola meal. The aim of this experiment was to see if similar milk responses to PMR could result from feeding identical components as a grain mix (GM) in the parlor, with hay in the field. The importance of canola in the PMR to the milk response was assessed at 2 pasture allowances. Three supplementation strategies were used: PMR, comprising 7.1 kg DM wheat, 2.3 kg DM corn grain and 2.6 kg DM oaten hay, mixed and offered twice daily on a feedpad; PMR+C, same as PMR except 2.6 kg DM of wheat was replaced with the same amount of canola meal; and GM, a mix of the same ingredients as PMR+C was fed in the parlor at milking but the hay was fed in the field. Total amount of supplement offered was 12 kg DM/cow per d. There were also 2 pasture allowances of 15 and 25 kg DM/cow per d (to ground level), making 6 combinations of supplement strategy and pasture allowance, each of which was allocated to 3 groups of 6 Holstein-Friesian cows (a total of 108 cows). Intake and milk was measured during the final 14 d of a 28 d experiment. There were no differences in yields of energy-corrected milk (ECM), fat or protein between cows offered PMR+C and GM at either pasture allowance, while cows offered supplements as the PMR produced less ECM (ECM yields were 31.7, 34.3 and 34.7 kg/cow per d for cows offered the PMR, PMR+C and GM diets, respectively, at the high pasture allowance, and 30.2, 31.4, and 32.7 kg/cow per d at the low allowance; s.e.d. = 0.622, P = 0.002). The GM cows achieved the same yields as PMR+C cows with reduced intake of pasture and supplement. These data show that a response similar to that achieved feeding PMR+C could be achieved by feeding the components in the parlor using existing feeding infrastructure, making the strategy more relevant to the 80% of Australian...

The objective of this study was to evaluate the effect of virginiamycin (VM) combined with crude glycerin (CG) on dry matter intake (DMI, % BW), average daily gain (ADG), and fatty acid (FA) profile (g/100 of FA) of Longissimus dorsi muscle of Nellore bulls. Forty-eight bulls with initial BW (408.4 ± 22.2 kg) were individually fed with feedlot finishing diets without virginiamycin (VM-) or with 25 mg/kg of VM in DM (VM+) combined with diets without crude glycerin (CG-) or with 100 g/kg of CG (80% glycerol) in DM (CG+). The sugar cane bagasse was used as the exclusive roughage in the proportion of 20% in DM of diet and crude glycerin replace corn in the diet formulation. After 81 d of feeding bulls were weighed and slaughtered. After 24 h of cooling carcasses, samples of Longissimus muscle (LM) between the 12th and 13th ribs were taken and freeze-dried. The fatty acids were quantified by gas chromatography. The data of animal performance and fatty acid profile were analyzed as a completely block randomized design arranged in a 2 × 2 factorial, virginiamycin and crude glycerin. Statistical model included the fixed effect of virginiamycin (1 degree of freedom, DF), crude glycerin (1DF) and all interactions. Random effects were block (1DF) and residual error. The data were analyzed using PROC MIXED of SAS. There were no interactions for the variables evaluated (P > 0.05). The DMI, % BW was greater for CG+ diets than CG-, independent of VM addition (1.95 ± 2.06%; P < 0.05). ADG over 81 d was similar (1.30 kg/d) for CG or VM diets (P < 0.05). The G: F were statistically similar (0.12) between diets (P > 0.05). Total saturated fatty acids (SFA) in LM decreased in CG+ than CG- diets (45.64 vs. 42.64%; P < 0.05). Total monounsaturated fatty acids (MUFA) was greater in CG+ than CG- (42.57 vs. 40.33%; P < 0.05). Total unsaturated fatty acids (UFA) was greater in VM+ than VM- diets (50.28 vs. 50.38%; P < 0.05) and n-6/n-3 ratio did not change with CG (P > 0.05) or VM inclusion (P > 0.05). CG+ diets had greater UFA/SFA than CG- (1.05 vs. 1.15; P < 0.05). The G: F were statistically similar (0.12) between diets (P > 0.05). The results showed that the average daily gain (ADG) of calves was significantly higher but the incidence of diarrhea was lower in calves fed different ratio of dietary neutral detergent fiber (NDF): starch. Eight primiparous dairy cows were assigned to 4 total mixed rations (TMR) with NDF to starch ratios of 0.86, 1.18, 1.63, and 2.34 in a replicated 4 × 4 Latin square design. The duration of each period was 21 d including a 14 d for adaptation and 7 d for sampling. At the beginning of the study, milk production and days in milk were 35.1 ± 0.5 kg/day and 146 ± 21.6 d, respectively. The NDF to starch ratios were achieved by altering the proportions of forage (corn silage and oat hay) and concentrate (corn), with the same composition and inclusion proportion of other dietary ingredients. Plasma, urine, milk, rumen fluid, and feces were collected once daily on the last 3 d of each period. The metabolic profiles were analyzed using HPLC Q-TOF MS, and the identified metabolites were confirmed by both retention time and mass spectra. All data were processed using the Agilent MassHunter Qualitative Analysis and Mass Profiler Professional software. A student t-test was used to search for compounds with a fold change greater than 2 and a P-value less than 0.05. Metabolites were identified using the METLIN and Human metabolome database. 31 different metabolic profiles in feces, 5 in rumen fluid, 3 in plasma, and 6 in urine were detected, including leukotriene increased (P < 0.05) in rumen fluid, fatty acids increased (P < 0.05) in plasma, vitamin D3 increased (P < 0.05) in plasma, glucuronide decreased (P < 0.05) in urine, isovalerate decreased and then increased (P < 0.05) in urine and L-isoleucyl-L-proline increased (P < 0.05) in feces. All the different metabolic profiles were related to several metabolic pathways, including fatty acid biosynthesis, the metabolism of vitamin D3, amino acids, protein and alkaloids, bile secretion, and inflammatory mediator regulation. Taken together, different NDF: starch ratio in diets can affect metabolism inside and outside of the rumen of dairy cows, and some of these metabolic responses may be attributable to the observed effects of NDF: starch ratio on animal productivity and health.

Key Words: dairy cow, NDF:starch ratio, metabolic

T247  Effects of different sources of dietary zinc on growth performance and incidence of diarrhea of newborn calves. L. Y. Hao1, P. Sun1, J. Wang1, and D. P. Bu1, 2, 3 State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China, 3Hunan Co-Innovation Center of Safety Animal Production, CICSAP, Changsha, Hunan, China.

Zinc is an important trace element which is involved in protein synthesis and cellular metabolism. Zinc has been used to treat and prevent diarrhea in infants and children effectively. The objective of this trial was to investigate the effects of zinc methionine and zinc oxide on performance and diarrhea of newborn calves. Thirty-six Chinese Holstein newborn calves were randomly assigned to 3 groups of 12 calves. The treatments were as follows, group 1: no addition (control group), group 2: 457 mg of zinc methionine (equivalent to 80 mg of zinc) was added per day, and group 3: 104 mg of zinc oxide (equivalent to 80 mg of zinc) per day was added. Zinc methionine and zinc oxide were mixed with milk and fed directly to the calves after birth. The whole experiment lasted for 14 d when blood samples were taken from all the calves. Starter diet was supplemented to all calves on the fourth day after birth. Milk and starter intake was recorded every 2 d. Calves were measured for body height, body length, bust, and weighed at the beginning and end of the trial. Incidents of calves with diarrhea was observed twice daily (morning and evening). All data were processed using SAS systems. The results showed that the average daily gain (ADG) of calves was significantly higher but the incidence of diarrhea was lower in calves fed different ratio of dietary zinc.
from group 2 than in the group 1 ($P < 0.05$). No significant difference was observed in average daily feed intake and calf size (body height, body length, bust) among different groups. Compared with the control group, zinc oxide numerically increased ADG and reduced diarrhea with no dramatic differences. In conclusion, zinc supplementation in the diets for newborn calves promoted growth and effectively relieved diarrhea.

Key Words: calf, zinc, diarrhea

T248  Effects of limit-feeding forage to concentrate ratios on nutrients intake, rumination, ruminal fermentation, digestibility, and growth in Holstein heifers. J. Zhang, H. Shi, Z. Cao, S. Li, S. Ji*, and Y. Wang, State Key Laboratory of Animal Nutrition, Beijing Engineering Technology Research Center of Raw Milk Quality and Safety Control, College of Animal Science and Technology, China Agricultural University, Beijing, China.

Dairy heifers commonly receive diets in which the majority of nutrients come from forages instead of concentrates in China. The objective of this study was to evaluate the effects of limit-feeding of different levels of concentrate diets on nutrients intake, rumination, ruminal fermentation, digestibility, and animal growth in Holstein heifers. Twenty-four Holstein heifers (8–10 mo and 253 ± 29 kg of BW) were randomly allocated into 4 groups for a 28 d period fed with one of 4 diets containing different levels of concentrate (20%, 40%, 60%, and 80%, namely C20, C40, C60, and C80, respectively). Feed intakes were adjusted for heifers to obtain similar ADG at 0.8 kg/d. Individual rumination loggers were used to monitor rumination time. DMI was recorded daily, and BW, body measurements, and BCS were recorded weekly. Rumen liquid samples were taken at d 28 for detecting fermentation parameters. Data were analyzed using the PROC MIXED procedure of SAS. Contrast was used to test the linear and quadratic changes affected by increasing amount of dietary concentrate. DMI (5.32 vs. 4.97 vs. 4.69 vs. 4.42 for C20, C40, C60, and C80, respectively), intakes of OM, NDF and ADF, total rumination time, and ruminal pH were linearly decreased with increasing amount of concentrate in diets ($P < 0.01$). However, intakes of NFC and starch, rumination time per kg of NDF and ADF, and concentrations of NH$_3$-N, propionate, and butyrate were linearly increased with increasing amount of concentrate in diets ($P < 0.01$). Digestibilities of DM, OM, and CP were significantly higher in C80 group than in C40 and C20 groups ($P < 0.01$). Dietary concentrate levels had no significant effects on BCS, feed efficiency (0.19 vs. 0.18 vs. 0.18 vs. 0.18 for C20, C40, C60, and C80, respectively; ADG/DMI; FE), and most of body measurements expect for paunch girth. What is worth mentioning, the results of ADG and FE might be eliminated or even reversed when gastrointestinal tract digesta was removed. In conclusion, limit-feeding of high concentrate diet gave heifers similar body growth as low concentrate diet feeding, and had the potential to come from forages instead of concentrates in China. The objective of this study was to evaluate the activity of glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC) and superoxide dismutase (SOD) in the serum compared with SS-0.3 ($P < 0.05$). Furthermore, HMSeBA supplementation linearly improved the activity of serum GSH-Px and SOD, but decreased the content of MDA. Compared with the SS-0.3, the SO-0.3 significantly increased the concentrations of total Se in milk (22.6 vs. 50.95 µg/kg, $P < 0.01$) and plasma (82.87 vs. 101.75 µg/kg, $P < 0.01$), and total Se concentration ratio between milk and plasma ($P < 0.01$). In addition, increasing doses of HMSeBA elevated the concentrations of total selenium in the milk and plasma linearly ($P < 0.01$). The current study demonstrated that HMSeBA improved the antioxidant status and elevated the milk and plasma selenium concentrations more effectively than SS, indicating HMSeBA is an effective organic Se source for lactating dairy cows.

Key Words: organic selenium, antioxidant status, selenium concentration

T250  Supplementation of lactating dairy cows with strains of live yeast during summer. K. T. Silva$^{1,2}$, F. F. Cardoso$^{1}$, E. F. Barbosa$^{1}$, J. C. Silva$^{1}$, L. J. Lara$^{1}$, E. A. Garcia$^{1}$, M. Aronovich$^{1,2}$, A. P. Peconick$^{1}$, R. A. N. Pereira$^{2,5}$, and M. N. Pereira$^{1,5}$. $^{1}$University of Lavras, Lavras, Brazil, $^{2}$Minas Gerais Ag Research Enterprise (Epmag), Lavras, Brazil, $^{3}$Phileo Lesaffre Animal Care, Lille, France, $^{4}$Rio de Janeiro State Agricultural Research Enterprise (Pesagro), Rio de Janeiro, Brazil, $^{5}$Better Nature Research Center, Ijaci, Brazil.

This experiment evaluated if strain type would induce differential response of lactating cows to Saccharomyces cerevisiae supplementation during Brazilian summer (THI > 68 for 86.4% of time). Thirty Holsteins (169 ± 87 DM) were milked 3x/d and individually fed on TMR for a 2-week covariate period and blocked by parity (1 vs. >1) and milk yield before assignment to a treatment for 56 d. Treatments were: strains SC 47 or NCYC 996 (Phileo Lesaffre) or Control (CTL). Pre-planned contrasts were C1) CTL vs. SC 47 + NCYC 996 and C2) SC 47 vs. NCYC 996. Yeast products (10 g/cow/d) were mixed to a cup of ground corn and top dressed to the TMR in the morning. Viable and non-viable cells/g were: 1.00 × 1010 and 1.03 × 1010 for SC 47 and 1.76 × 1010 and 0.93 × 1010 for NCYC 996, respectively. Yeast products did not affect milk yield (35.8 kg/d, $P > 0.69$), solids (1.048 protein, 0.953 fat, and 1.572 lactose) kg/d, $P > 0.37$), and DMI (19.4 kg/d, $P > 0.62$). Linear SCC scores were 4.94 for CTL (384,000 cells/mL), 4.17 for SC 47 (225,000 cells/mL), and 4.37 for NCYC 996 (258,000 cells/mL) ($P < 0.01$ for C1). Immune response was evaluated on d -1, 7, and 15 relative to vaccination with an inactivated rabies vaccine on d 35. Total leukocytes and interleukin-4 (IL-4) increased in all treatments in response to vaccination. Compared...
with SC 47, NCYC 996 tended to increase IL-4 on d –1 (P = 0.10 for C2) and reduced it on d 15 (P = 0.03 for C2). Gamma-interferon was not affected by treatments (P ≥ 0.19). Total-tract apparent digestibility of nutrients and the daily urinary allantoin excretion did not differ (P ≥ 0.20). Yeast increased ingestion time per day and per DMI and meal duration (P ≤ 0.02 for C1). Plasma niacin concentration on d 14 and 56 did not differ (P ≥ 0.62). Rectal temperature at 7 p.m. tended to be lower on NCYC 996 than SC 47 (39.4 vs. 39.1°C, P = 0.06 for C2), but respiratory frequency and sweating rate did not change (P ≥ 0.32). Plasma concentration at 0, 2, 4, and 12 h post-feeding did not differ (P ≥ 0.22). Live yeast supplementation reduced milk SCC and affected immunity, but had no impact on animal performance, diet digestibility, and plasma niacin concentration and thermo-regulation.

Key Words: heat stress, immune function, yeast

T251  Evaluation of acidified milk for feeding dairy calves in tropical climates. M. G. Coelho, F. L. M. Silva, M. D. Silva, A. P. Silva, A. C. Silva, J. Hartmann, and C. M. M. Bittar*, ESALQ, Universidade de Sao Paulo, Piracicaba, Sao Paulo Brazil.

The acidification of milk is used as a way of maintaining low bacteriological load. In the tropical climate, the high temperatures can make the use of this technique difficult, increasing diarrhea occurrence and reducing animal’s performance. During the summer (max. 29°C; min. 17.4°C), 44 male and female Holstein newborn calves were used in a randomized blocks experimental design and fed different liquid feed: commercial milk replacer (20.7% CP; 15.5% fat; 12.5% solids); whole milk; and acidified milk. Milk was acidified to a pH of 4.2 with lactic acid, 12–24 h before feeding, and kept in room temperature. Animals were individually housed with free access to water and concentrate. Calves were fed 6L/d of liquid diet (2 meals) until weaning at 56 d. Acidified milk resulted in performance and blood metabolites similar to that observed for whole milk, with higher weight gain and lower fecal scores as compared with milk replacer. Acidify whole milk to pH 4.2 is a good alternative to store the milk in high environmental temperatures for feeding dairy calves.

Key Words: lactic acid, liquid diet, pH

T252  Supplementation to late lactation dairy cows during summer with dead yeast culture. J. D. L. Dias¹, R. B. Silva¹,², L. E. C. Graças¹, K. Ferreira¹, L. C. Resende¹, R. C. Araujo³, R. A. N. Pereira⁴,⁵, and M. N. Pereira⁴,⁵,¹ University of Lavras, Lavras, Brazil, ²Better Nature Research Center, Itajai, Brazil, ³Grasp Industria e Comercio, Curitiba, Brazil, ⁴Minas Gerais State Ag Research Enterprise (Epmag), Lavras, Brazil.

The supplementation of dairy cows with yeast probiotics may increase diet digestibility, plasma niacin concentration, heat dissipation, and lactation performance. We evaluated the response of Holsteins in late lactation (234 ± 91 DIM) to dead yeast culture (YC. Fifteen g/d, Factor SC, Grasp. Saccharomyces cerevisiae) during Brazilian summer (THI >68 for 92.2% of time). Thirty-two cows were individually fed a standard TMR for 14 d and treatments Control (CTL) or YC for 35 d, in a covariate adjusted randomized block design. Response was evaluated in wk 5 or as repeated measures over time. Cows were milked 3x/d and treatments were orally dosed to each cow after each milking. Plasma niacin was 1.50 and 1.66 µg/mL on CTL and YC, respectively (P = 0.02). The YC reduced rectal temperature, respiratory rate, and body surface temperature (P ≤ 0.05) and tended to increase the sweating rate (P = 0.13) evaluated twice per week at 0730, 1500, and 2200 h. The proportion of cows with rectal temperature ≥39.2°C on CTL and YC was, respectively: 8 and 0% at 0730 h, 52 and 25% at 1500 h, and 35 and 26% at 2200 h (P ≤ 0.05). GENMOD of SAS). Plasma glucose in wk 3 and 5 were increased by YC (P = 0.01). The total-tract apparent digestibility of nutrients, plasma urea-N concentration, ruminal VFA profile, urinary allantoin excretion, ingestion and chewing behavior, and the proportion of daily intake in the morning, afternoon, and night were not affected by treatment (P ≥ 0.16). Cows on CTL rejected feed particles >19 mm in the morning (P = 0.02), in the afternoon were less selective against long feed particles (P = 0.05) and in favor of particles <8 mm (P = 0.03), and did not sort against short particles at night (P = 0.05). Milk yield was 30.5 kg/d for CTL and 30.2 for YC (P = 0.58). The YC reduced DMI (19.4 vs. 20.3 kg/d. P = 0.04) and increased the milk to DMI ratio (1.64 vs. 1.50. P = 0.05). Milk solids yield and concentration, linear SCC score, and MUN did not differ (P ≥ 0.21). The YC increased plasma niacin concentration, heat dissipation, and feed efficiency of late lactation dairy cows.

Key Words: heat stress, niacin, yeast

Table 1 (abstract T251).

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th></th>
<th></th>
<th>SEM</th>
<th>T</th>
<th>A</th>
<th>T × A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk replacer</td>
<td>Whole milk</td>
<td>Acidified milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentrate, g/d</td>
<td>245.8</td>
<td>395.2</td>
<td>344.2</td>
<td>63.04</td>
<td>0.095</td>
<td>&lt;0.0001</td>
<td>0.512</td>
</tr>
<tr>
<td>Liquid diet, L/d</td>
<td>5,424.8b</td>
<td>5,634.6a</td>
<td>5,624.5a</td>
<td>42.49</td>
<td>0.002</td>
<td>&lt;0.0001</td>
<td>0.01</td>
</tr>
<tr>
<td>Fecal score</td>
<td>2.48c</td>
<td>1.79a</td>
<td>1.96b</td>
<td>0.069</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.122</td>
</tr>
<tr>
<td>Weight at birth, kg</td>
<td>33.2</td>
<td>32.1</td>
<td>32.0</td>
<td>1.81</td>
<td>0.412</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Weight at weaning, kg</td>
<td>38.5b</td>
<td>48.4a</td>
<td>48.0a</td>
<td>1.67</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight gain, kg/d</td>
<td>0.234b</td>
<td>0.631a</td>
<td>0.640a</td>
<td>0.0304</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hematocrit,%</td>
<td>19.25b</td>
<td>22.88a</td>
<td>23.59a</td>
<td>0.586</td>
<td>&lt;0.0001</td>
<td>0.023</td>
<td>0.224</td>
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<tr>
<td>Glucose, mg/dL</td>
<td>113.2b</td>
<td>127.6a</td>
<td>119.2b</td>
<td>2.91</td>
<td>0.001</td>
<td>0.0115</td>
<td>0.083</td>
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<tr>
<td>Lactate, mg/dL</td>
<td>10.5</td>
<td>11.1</td>
<td>11.6</td>
<td>0.62</td>
<td>0.4829</td>
<td>&lt;0.0001</td>
<td>0.0256</td>
</tr>
<tr>
<td>Protein, g/dL</td>
<td>6.09b</td>
<td>6.62a</td>
<td>6.63a</td>
<td>0.122</td>
<td>0.0017</td>
<td>&lt;0.0001</td>
<td>0.335</td>
</tr>
<tr>
<td>BHB, mmol/L</td>
<td>0.112b</td>
<td>0.157a</td>
<td>0.155a</td>
<td>0.0074</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
<td>0.1647</td>
</tr>
</tbody>
</table>

*Means with different letters within a row indicate statistical difference (P < 0.05).
²T = treatment effect; A = age effect; T × A: Interaction treatment × age effect.
Effects of continuous i.m. infusions of acetic acid (AA) or sodium acetate (NA) infused into the rumen (RU) or into the abomasum (AB) were evaluated on feeding behavior, dry matter intake (DMI), and metabolic response of cows. Six rumen-cannulated multiparous Holstein cows (11.8 ± 4 d in milk) were utilized in a 6 × 6 Latin square design experiment balanced for carryover effects with a 2 × 3 factorial arrangement of treatments. Treatments were AA and NA, with sodium chloride (NC) as a control, infused at a rate of 0.75 mol/h (0.5 L/h) into the RU or AB for the first 8 h following feeding with a rest day between infusion days. Treatment sequences were assigned to cows randomly. Feeding behavior was recorded by a computerized data acquisition system and blood was sampled at 0, 4, and 8 h relative to the start of infusion. Data were analyzed by ANOVA; the model included the fixed effects of treatment and period and the random effect of cow; treatments were compared with preplanned contrasts. We hypothesized that AA was more hypophagic than NA, and that infusion into the AB was more hypophagic than into the RU. Treatments decreased DMI 30% (4.3 kg/8h vs. 6.1 kg/8h) and NA-AB (4.0 kg/8h) decreased DMI compared with other treatments and NC. Plasma NEFA concentration was increased (P < 0.01) compared with NC. AA-RU decreased plasma glucose concentration over time (interaction, P = 0.02). Sodium acetate did not affect DMI during the rest day (P > 0.05). All treatments increased plasma acetate and β-hydroxybutyrate concentrations over time (interaction, P < 0.001) and decreased plasma insulin concentration (P = 0.01) compared with NC. AA-AB decreased plasma glucose concentration over time (interaction, P = 0.01) compared with other treatments and NC. Plasma NEFA concentration was increased over time with higher concentrations for AA-AB and AA-RU compared with other treatments and NC (interaction, P = 0.02). Sodium acetate did not decrease DMI compared with control or the other treatments but the reasons for the different effects are not known.

Key Words: fresh cow, feed intake, acetic acid

T254  Original XPC and NutriTek increase volatile fatty acid production in an in vitro rumen model on forage samples from various global regions. L. B. Gualdrón-Duarte* and M. S. Allen, Michigan State University, East Lansing, MI.

Supplementation of grazing cows with a blend of essential oils and capsaicin or monensin. R. B. Silva1,2, W. R. Silva1, R. C. Cunha1, B. B. C. Junqueira1, M. A. S. Lara1, J. F. Santos1, R. C. Araujo4, R. A. N. Pereira2,4, and M. N. Pereira*1,2, 1University of Lavras, Lavras, Brazil, 2Better Nature Research Center, Ijaci, Brazil, 3Cooperativa Castrolanda, Castro, Brazil, 4Grasp Industria e Comercio, Curitiba, Brazil, 5Minas Gerais Ag Research Enterprise (Epamig), Lavras, Brazil.

Monensin and essential oils can be beneficial to lactating cows on grazing systems adopting high grain feeding. We evaluated the effect of a microencapsulated blend of capsaicin, carvacrol, cinnamaldehyde, and eugenol (CEO. Four g/d) relative to monensin (MON. 300 mg/d) or control (CTL) on lactation performance, pasture intake, total-tract apparent digestibility, rumen microbial yield, and rumination of grazing Holsteins. After a 14-d standardization period (Covariate), 60 cows were evaluated on feeding behavior, dry matter intake (DMI), and metabolic response of cows. Six rumen-cannulated multiparous Holstein cows (11.8 ± 4 d in milk) were utilized in a 6 × 6 Latin square design experiment balanced for carryover effects with a 2 × 3 factorial arrangement of treatments. Treatments were AA and NA, with sodium chloride (NC) as a control, infused at a rate of 0.75 mol/h (0.5 L/h) into the RU or AB for the first 8 h following feeding with a rest day between infusion days. Treatment sequences were assigned to cows randomly. Feeding behavior was recorded by a computerized data acquisition system and blood was sampled at 0, 4, and 8 h relative to the start of infusion. Data were analyzed by ANOVA; the model included the fixed effects of treatment and period and the random effect of cow; treatments were compared with preplanned contrasts. We hypothesized that AA was more hypophagic than NA, and that infusion into the AB was more hypophagic than into the RU. Treatments decreased DMI 30% (4.3 kg/8h vs. 6.1 kg/8h, P < 0.01) compared with NC. AA-RU decreased plasma glucose concentration over time (interaction, P < 0.01); NA-RU did not reduce DMI (7.0 kg/8h), while AA-RU (2.6 kg/8h), AA-AB (3.7 kg/8h) and NA-AB (4.0 kg/8h) decreased DMI compared with NC. Treatments did not affect DMI during the rest day (P > 0.05). All treatments increased plasma acetate and β-hydroxybutyrate concentrations over time (interaction, P < 0.001) and decreased plasma insulin concentration (P = 0.01) compared with NC. AA-AB decreased plasma glucose concentration over time (interaction, P = 0.01) compared with other treatments and NC. Plasma NEFA concentration was increased over time with higher concentrations for AA-AB and AA-RU compared with other treatments and NC (interaction, P = 0.02). Sodium acetate did not decrease DMI compared with control or the other treatments but the reasons for the different effects are not known.

Key Words: fresh cow, feed intake, acetic acid

T254  Original XPC and NutriTek increase volatile fatty acid production in an in vitro rumen model on forage samples from various global regions. L. B. Gualdrón-Duarte* and M. S. Allen, Michigan State University, East Lansing, MI.

Original XPC and NutriTek increase volatile fatty acid production in an in vitro rumen model on forage samples from various global regions. L. B. Gualdrón-Duarte* and M. S. Allen, Michigan State University, East Lansing, MI.

The objective of this study was to evaluate the effect of 2 Saccharomyces cerevisiae fermentation products - Diamond V Original XPC (XPC) and NutriTek (NutriTek) - on in vitro VFA production on forage samples from various global regions. Twelve different forage samples were collected from 4 regions: Europe [haylage (H EU) and grass silage (GS EU)]; Russia [corn silage (CS RU), grass haylage (GH RU), and alfalfa haylage (AH RU)]; China [corn silage (CS CN), sweet corn stalk silage (SCS CN), and sheep grass (SG CN)]; and US [Iowa corn silage (CS1 US), New York corn silage (CS2 US), alfalfa haylage (AH US), and alfalfa hay (A US)]. Serum bottles (100 mL) containing 0.3 g forage and treatment (Control, XPC, or NutriTek) were inoculated with buffered rumen fluid and incubated for up to 24 h. Seven experiments were performed to generate 10 replicates per treatment, diet, and time point. Data were analyzed using the GLM model of JMP and significance was defined as P ≤ 0.05. Percent increases in acetate (A), propionate (P), butyrate (B), and total VFA (T) concentrations are reported in Table 1. Significant treatment effects were observed in all forage samples tested within 24 h with few exceptions indicated with asterisk (*). In conclusion, XPC enhanced in vitro VFA production over Control on majority of forage samples tested. NutriTek further enhanced VFA production over XPC on the same range of forage samples.

Key Words: XPC, NutriTek, forage

Table 1 (abstract T254). Percent increases of VFA by XPC and NutriTek

<table>
<thead>
<tr>
<th>Forage</th>
<th>XPC over Control</th>
<th>NutriTek over XPC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>P</td>
</tr>
<tr>
<td>H EU</td>
<td>5.8</td>
<td>10.4</td>
</tr>
<tr>
<td>GS EU</td>
<td>11.6</td>
<td>17.0</td>
</tr>
<tr>
<td>GH RU</td>
<td>12.5</td>
<td>13.2</td>
</tr>
<tr>
<td>AH RU</td>
<td>5.7*</td>
<td>8.1*</td>
</tr>
<tr>
<td>CS CN</td>
<td>6.9*</td>
<td>9.4</td>
</tr>
<tr>
<td>SCS CN</td>
<td>8.3</td>
<td>9.0</td>
</tr>
<tr>
<td>SG CN</td>
<td>11.2</td>
<td>16.0</td>
</tr>
<tr>
<td>CS1 US</td>
<td>3.6*</td>
<td>33.8</td>
</tr>
<tr>
<td>CS2 US</td>
<td>7.8</td>
<td>30.5</td>
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<tr>
<td>AH US</td>
<td>6.0</td>
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</tr>
<tr>
<td>A US</td>
<td>7.6</td>
<td>11.1</td>
</tr>
</tbody>
</table>
(183 ± 95 DIM) were blocked by parity and milk yield and assigned to a treatment for 28 d with response evaluated on wk 4. Pre-planned contrasts were: C1) CTL vs. CEO and C2) CEO vs. MON. Capsules with treatments were given orally 1×/d. Cows were milked 2×/d and locked-fed 5.54 kg/d of corn silage DM (7.9% CP, 55.1% NDF) and 2.70 kg of concentrate DM (16.3% CP, 14.7% NDF) at 0230, 1130, 1430, and 1700 h. Paddocks of ryegrass-white clover (17.6% CP, 49.9% NDF) were grazed for 324 ± 53.7 min/d and pre- and post-grazing heights of 12.2 cm and 10.1 cm, respectively. Cows were orally dosed from d 14 to 28 with Cr2O3 to estimate fecal production. Pasture was harvested with a rumen fistulated cow allowed to graze for 50 min after ruminal evacuation. The in situ content of iNDF in feeds (244 h) estimated pasture intake and total-tract digestibility. The allantoin to creatinine ratio of urine spot samples estimated rumen microbial yield. Rumination was recorded automatically with neck collars (SCR, Israel). Treatments had no effect on pasture intake (7.22 kg/d), milk yield (36.6 kg/d), solids not fat (SNF) yield and concentration, BW (679 kg), BCS (2.9), and rumination activity (546 min/d) (P ≥ 0.28 for C1 and C2). The CEO reduced the concentration of urea-N in milk (≤ 0.03 for C1 and C2) and plasma P activity (546 min/d) (P ≤ 0.03 for C1 and C2) and the urinary allantoin to creatinine ratio tended to be reduced by MON (P = 0.10 for C2). Lactation performance and pasture intake were not affected by MON and CEO.

Key Words: capsaicin, essential oils, monensin

T257 Supplementation of lactating cows with a blend of essential oils or monensin: Thermoregulation and blood variables. R. B. Silva1,2, W. R. Silva1, C. D. S. Oliveira1, A. C. C. Lacreta Junior1, L. H. L. Chaflun1, R. C. Araujo3, R. A. N. Pereira4, and M. N. Pereira1,2. 1University of Lavras, Lavras, Brazil, 2Better Nature Research Center, Lavras, Brazil, 3Grasp Industrria e Comercio, Curitiba, Brazil, 4Minas Gerais State Ag Research Enterprise (Epamig), Lavras, Brazil.

Capsaicin can promote peripheral blood flow and essential oils can have digestive and systemic action in mammals. We evaluated the effect of a blend of capsaicin, carvacrol, cinnamaldehyde, and eugenol (CEO, 176 mg/kg of TMR DM) relative to monensin (MON. Fifteen mg/kg of TMR DM) or control (CTL) on body homeothermy and systemic variables of dairy cows. Thirty individually fed Holsteins (189 ± 83 DIM) received a standard TMR for 14 d and a treatment for 56 d, in a covariate adjusted randomized block design with repeated measures over time. Pre-planned contrasts were: C1) CTL vs. CEO and C2) CEO vs. MON. Treatments had no effect on the concentrations of plasma D-lactate, BHBA, aspartate, and gamma-glutamyl transferases at 12-h post-feeding on d 25 and 53 and glucose on d 14, 25, 39, and 53 at 0, 4, and 12 h post-feeding (P ≥ 0.30 for C1 and C2). The CEO increased PO2, the % oxygen saturation of hemoglobin, and the % oxygen of total volume in jugular blood on d 24 and 52 (P ≤ 0.03 for C1 and C2). On d 26 and 58, the diameter of the caudal artery evaluated by doppler-image ultrasonography tended to be larger on CEO than CTL (P = 0.08 for C1) and artery area was increased (P = 0.04 for C1). Blood flow and pulsatility did not differ among treatments (P ≥ 0.30 for C1 and C2). Rectal temperature (RT), skin surface temperature (ST), and respiratory frequency were measured at 3–4 d intervals (16 measures) at 8, 16, and 22 h. Cows on CEO tended to have lower RT at 16 h (P = 0.12 for C1), but did not differ at 8 h and 22 h (P = 0.22 for C1 and C2). The proportion of cows with rectal temperature ≥39.2°C at 16 h was 18.7% for CEO and 30% for CTL and MON (P = 0.02), and at 8 h 10.4% and 19%, respectively (P = 0.02), with no treatment effect at 8 h (6%, P = 0.37) (GENMOD of SAS). The ST of CEO cows at 8 h was higher than CTL (P = 0.01 for C1) and tended to be higher than MON (P = 0.07 for C2). Respiratory frequency did not differ (P ≥ 0.43 for C1 and C2). The CEO increased the sweating rate (P = 0.05 for C1 and C2) and the evaporative heat loss (P = 0.01 for C1 and P = 0.12 for C2) measured once per week. The CEO increased heat dissipation and oxygen concentration in blood of dairy cows, showing to have systemic action.

Key Words: capsaicin, essential oils, monensin

T256 Supplementation of lactating cows with a blend of essential oils and capsaicin or monensin: Performance and digestion. R. B. Silva1,2, W. R. Silva1, C. D. S. Oliveira1, R. C. Araujo3, R. A. N. Pereira1,2, and M. N. Pereira1,2. 1University of Lavras, Lavras, Brazil, 2Better Nature Research Center, Lavras, Brazil, 3Grasp Industrria e Comercio, Curitiba, Brazil, 4Minas Gerais State Ag Research Enterprise (Epamig), Lavras, Brazil.

Monensin and essential oils can manipulate ruminal fermentation profile. We evaluated the effect of a microencapsulated blend of capsaicin, carvacrol, cinnamaldehyde, and eugenol (CEO, 200 mg/kg of TMR DM) relative to monensin (MON. Fifteen mg/kg of TMR DM) or control (CTL) on lactation performance, intake pattern, rumen fermentation profile, and diet digestibility of dairy cows. Thirty individually fed Holsteins (189 ± 83 DIM) received a standard diet for 14 d and a treatment for 56 d, in a covariate adjusted randomized block design with repeated measures over time. Pre-planned contrasts were C1) CTL vs. CEO and C2) CEO vs. MON. The CEO increased milk protein secretion compared with MON (+ 51 g/d, P = 0.04) and tended to increased relative to CTL (+ 38 g/d, P = 0.12). The CEO also tended to increase milk yield relative to CTL (33.0 vs. 34.4 kg/d, P = 0.11) and ECM (+ 1.3 kg/d, P = 0.15) and fat yield (+ 60 g/d, P = 0.13) relative to MON. Cows on CEO had greater covariate adjusted BW and daily gain (P < 0.01) and tended to have higher BCS (P = 0.13) than CTL. The DMI (21.0 kg/d), milk/DMI (1.59), ECM/DMI (1.51), the total-tract apparent digestibility of OM and NDF, and the digestible OM intake (DOMI) were similar among treatments (P ≥ 0.25). Urinary allantoin excretion was reduced by MON (P ≤ 0.05 for C2). The CEO increased the proportion of acetate in ruminal VFA (P = 0.04 for C1 and P = 0.02 for C2) and reduced butyrate (P = 0.05 for C1 and P = 0.03 for C2) and methyl-butyrate (P = 0.13 for C1 and P = 0.01 for C2), and MON tended to increase propionate (P = 0.12 for C2). The A/P ratio tended to be increased by CEO (P = 0.12 for C1 and P = 0.07 for C2). Cows on MON had lower proportion of the daily intake at night and selected in favor of small feed particles in the afternoon (P < 0.01 for C2) and CEO induced more refusal of long feed particles in the morning (P = 0.02 for C1). Rumination/DMI tended to be reduced by CEO (P = 0.06 for C1 and C2) and total chewing/DMI was reduced compared with MON (P = 0.05 for C2). The CEO had no effect on diet digestibility and feed efficiency, but increased acetate in rumen fluid, weight gain, and lactation performance.

Key Words: capsaicin, essential oils, monensin

T258 Production effects of phytonutrients alone or in combination with yeast culture in lactating dairy cows. J. Oh*1, M. Harper1, A. Melgar1, E. Wall2, and A. Hristov1, 1The Pennsylvania State University, University Park, PA, 2Pancosma, Geneva, Switzerland.

As rumen modifiers with distinct modes of action, yeast and phytonutrients may have additive effects on ruminal fermentation and animal productivity. To test this concept, an 8-wk, randomized complete block design study with 36 Holstein cows (average days in milk, 117 ± 37.5...
The aim of this study was to evaluate the effect of an exogenous amylase on postprandial digestion of starch in cattle. Four rumen-fistulated heifers were assigned to a 2 x 2 Latin square with 2 experimental periods lasting 23 d each with 10 d of diet adaption followed by 13 d of abomasal infusion, additive effects of the 2 dietary supplements were not observed.

Key Words: phytomunrient, yeast culture, dairy cow

**T259** Effect of an abomasal amylase administration on postprandial starch digestion in heifers. K. Hansen1, E. Westreicher-Kristen1, A. Tröscher*2, R. Blank1, U. Dickhöfer3, and A. Susenbeth1, 1Universität Hohenheim, Stuttgart, Germany, 2BASF SE, Ludwigshafen, Germany, 3Universität Hohenheim, Stuttgart, Germany.

The aim of this study was to evaluate the effect of an exogenous amylase on postprandial digestion of starch in cattle. Four rumen-fistulated heifers were assigned to a 2 x 2 Latin square with 2 experimental periods lasting 23 d each with 10 d of diet adaption followed by 13 d of abomasal infusion (10 h/d) and sample collection. During the first 3 d of each infusion phase, isotonic saline solution was infused (1 L/h) for measurement of basal values in feces, followed by daily infusions of 880 g corn starch (suspension of 1 kg/10 L water) with or without amylase solution (2%; 50 mL/h). The heifers were fed 5.5 kg/d of a starch-free diet consisting of 64.9% grass hay, 33.0% dried beet pulp, 0.9% urea and 1.2% of a mineral and vitamin premix (DM basis) in 2 equal meals. Titanium dioxide (10 g/d) was ruminally administered in 2 dosages for estimation of fecal excretion. Four fecal grab samples per day were obtained during collection phase, pooled by animal and analyzed for DM, N, purified bases and volatile fatty acids (VFA). Microbial N was estimated from purified bases assuming a purine N: microbial N ratio of 0.116. Abomasal infusion of starch increased fecal excretion of VFA and microbial N (P < 0.05), which indicates an enhanced carbohydrate fermentation in the hindgut and therefore limited digestion of starch in the small intestine. Lactate excretion was increased at the second day of starch infusion (P < 0.05) but returned to basal values within 2 d, which was probably due to changes in composition of microbtkia in the hindgut (e.g., increase of lactate-utilizing bacteria). This agrees with increasing proportion of butyrate in total excreted VFA (P < 0.05) as an end product of lactate utilization. The increase of lactate excretion at the second day of infusion was lower with amylase administration (P < 0.05), which indicates a possible positive effect of amylase supplementation to animals not adapted to starch digestion.

**Key Words:** postprandial digestion, exogenous enzyme, hindgut fermentation

**T260** Effect of a *Saccharomyces cerevisiae*-based direct-fed microbial product and an enzyme extract from *Aspergillus oryzae* and *Aspergillus niger* on productivity and enteric gas emission in lactating dairy cows. J. Oh*1, M. Harper1, A. Melgar1, D. P. Carpenter2, and A. Hristov1, 1The Pennsylvania State University, University Park, PA, 2PMI Nutritional Additive, Arden Hills, MN.

Dietary supplementation of live yeast and fungal enzymes may have beneficial effects on productivity and rumen fermentation in ruminant animals. The objective of this experiment was to investigate the effects of a *Saccharomyces cerevisiae*-based direct-fed microbial product (DFM) and an enzyme extract from *Aspergillus oryzae* and *Aspergillus niger* (ENZ) on feed intake, milk production and composition, and enteric gas emission in lactating dairy cows. Eighteen Holstein cows (115 ± 42.0 kg in milk; 609 ± 77.9 kg body weight) were used in a 3 x 3 Latin square design experiment with 3, 28-d periods. Treatments were: (1) control (no additive), (2) 28 g/cow/d DFM, and (3) 10 g/cow/d ENZ. Treatments were top-dressed at the time of feeding in the morning. The basal diet consisted of (DM basis): 44.5% corn silage, 10.5% alfalfa haylage, 5.0% grass hay, and 40% concentrates and contained 16.5% CP and 32.0% NDF. Feed intake and milk production were monitored daily and gas emission was measured during the last week of each experimental period using the GreenFeed System. Dry matter intake was not affected (P = 0.53) by treatments (average 25.3 kg/d; SEM = 0.97). Compared with control, DFM increased (P = 0.03) milk yield (39.7 vs. 41.9 kg/d, respectively). Feed efficiency was not affected by treatments (average 1.61 kg/kg; SEM = 0.04). Concentrations of milk fat, true protein, and lactose (average 3.50, 3.03, and 4.84%, respectively) and energy-corrected milk yield (average 38.1 kg/d) were not different (P ≥ 0.38) among treatments. Milk urea nitrogen was also not affected (P = 0.39) by treatment. Treatments had no effect (P ≥ 0.17) on enteric methane (average 344 g/d, SEM = 16.2) or carbon dioxide emission and methane yield (average 13.8 g/kg DMI; SEM = 0.56) or intensity (9.5 g/kg ECM; SEM = 0.49). In this experiment, the *Saccharomyces cerevisiae*-based microbial product increased milk production without affecting enteric methane emission. The fungal enzyme product had no effect on productivity or gas excretion.

**Key Words:** direct-fed microbial, fungal enzyme, methane
calves (32 male and 32 female) were studied from birth until wk 11 of age. All calves received colostrum and transition milk until d 3 of age and from d 4 onwards calves were fed MR in amounts of either 6 L/d (Res; n = 32) or ad libitum (Adl; n = 32) for 8 wk. In both feeding groups half of the calves were fed MR with 0.24% B or same MR with no B supplement. From wk 9 to wk 10 MR was linearly reduced in all calves to 2 kg/d. Hay, water, and concentrate (CON) were offered ad libitum. Feed intake was measured daily and body weight (BW) weekly. Blood samples for analyzing IGF-I, IGF binding proteins (IGFBP), insulin, and glucose were taken on d 1, 2, 4, and 7, then weekly or biweekly (IGFBP) until wk 11 of age. Liver samples were taken on d 50 and at the end of the study (d 80 of age) to measure gene expression of the somatotropic axis. Data were analyzed by the Mixed Model of SAS with MR feeding, B supplementation, time, and sex as fixed effects. MR consumption and BW were greater, but CON intake was lower in Adl than in Res groups (P < 0.001). Plasma concentrations of IGF-I, IGFBP-3, insulin, and glucose were greater (P < 0.01) and plasma concentration of IGFBP-2 was lower (P < 0.05) in Adl than in Res. B supplementation depressed (P < 0.05) plasma IGF-I from wk 1–4 and in wk 9. On d 50, mRNA abundance of the growth hormone receptor and IGF-I was greater (P < 0.02) and of IGFBP-2 mRNA was lower in Adl than in Res. At the end of the study, IGFBP-2 mRNA was greater in Adl than in Res and increased by B. Ad libitum MR feeding stimulated the systemic and hepatic somatotropic axis, and mirrored the greater growth rate during the intensive MR feeding. Butyrate supplementation did not stimulate growth performance but partly depressed the IGF system.

**Key Words:** ad libitum milk replacer feeding, butyrate, somatotropic axis

**T262**

**Effect of sodium acetate supplementation on in vitro production of volatile fatty acids and gases.** L. M. Judd* and R. A. Kohn, University of Maryland, College Park, MD.

Whereas volatile fatty acid (VFA) production may be affected by product concentrations, this experiment assessed the effect of sodium acetate (NaOAc, 50 mM) addition on VFA and gas profile during in vitro fermentation. Rumen fluid samples (n = 16) with 1% timothy hay were incubated with or without 50 mM NaOAc addition. Tubes were equilibrated with a 50/50 gas mixture of CO2 and N2, and incubated at 39°C while shaking with 20-mL syringes attached to collect gases. VFA and gas production were measured at 0, 4, 16, 24, and 48 h. Means for treatments with acetate addition vs. control were analyzed using the Student’s t-test and are reported when different 

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Acetate addition decreased acetate production at each interval: 0–4 h (−4 vs. 14 mM; β = 0.02); 4–16 h (11 vs. 26 mM; SE ± 3.7); 16–24 h (15 vs. 31 mM; SE ± 4.9); and 24–48 h (22 vs. 36 mM; SE ± 4.2). Acetate addition decreased propionate production from 0 to 4 h (4 vs. 6 mM; SE ± 0.5). Ratio of produced acetate:propionate (A:P) decreased from acetate addition for all intervals: 0–4 h (−0.8 vs. 2.4; SE ± 0.96); 4–16 h (1.1 vs. 2.3; SE ± 0.41); 16–24 h (1.1 vs. 2.3; SE ± 0.34); and 24–48 h (1.3 vs. 2.2; SE ± 0.23). The A:P ratio being close to 1 may be a result of acetate production being inhibited by NaOAc addition. NaOAc addition did not affect butyrate production, but decreased ratio of produced acetate:butyrate (A:B) for each interval: 0–4 h (−2.9 vs. 5.5; SE ± 2.6), 4–16 h (2.0 and 5.0; SE ± 0.89), 16–24 h (2.3 vs. 5.1; SE ± 0.74) and 24–48 h (3.0 and 5.2; SE ± 0.54). Acetate addition decreased total VFA production: 0–4 h (3 mM vs. 23 mM; SE ± 3.8) and 4–16 h (28 vs. 45 mM; SE ± 4.8). Addition of NaOAc to rumen fluid decreased subsequent production of acetate more than other VFA, and decreased gas production.

**Key Words:** volatile fatty acids, enteric gases, in vitro fermentation

**T263**

**Fava bean (Vicia faba L. major) inclusion in dairy cow diets: Effect on enteric methane production and milk performance.** C. Cherif1, F. Hassanat1, S. Claveau1, J. Girard2, R. Gervais2, and C. Benchaar3, 1Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada. 2Agrinova, Alma, QC, Canada. 3Département des Sciences Animales, Université Laval, Québec, QC, Canada.

The objective of this study was to determine the effect of including fava bean in dairy cow diets on intake, milk production and enteric CH4 emissions. Nine lactating cows (DIM = 121 ± 28; milk yield = 41.2 ± 5.6 kg) were used in a replicated 3 × 3 Latin square (38-d period; 14-d adaptation). Cows were fed (ad libitum) a TMR composed (on DM basis) of 58.5% forages (20% corn silage; 35% alfalfa silage; 3.5% timothy hay) and 41.5% concentrates. In the concentrate portion, soybean meal and ground corn grain (Control) were completely and partially replaced, respectively, with either rolled (RFB) or ground (GFB) fava bean (17% DM). Diets were isonitrogenous (16% CP) and isoenergetic (NEi = 1.50 Mcal/kg). Intake, digestibility and milk performance were determined over 6 consecutive days while CH4 was measured using respiration chambers (5 consecutive days). Data were analyzed using the MIXED procedure (SAS) and differences between treatments and the control were declared significant (P ≤ 0.05) using Dunnett’s comparison test. Intake (25.7 kg/d) and digestibility (68.8%) of DM were not affected (P > 0.50) by the inclusion of GFB or RFB in the diet. Compared with the control, yield of ECM was not affected when cows were fed RFB (39.2 and 38.5 kg/d, respectively; P = 0.34), but declined when cows were fed GFB (37.7 kg/d; P = 0.02). Enteric CH4 production averaged 539 g/d and was not affected by dietary treatments (P = 0.74). Methane emissions expressed as g/kg DMI, % of gross energy intake or g/kg ECM were not affected by dietary treatments and averaged 20.9 g/kg (P = 0.14), 6.3% (P = 0.14) and 14.0 g/kg (P = 0.46), respectively. Results from this study show that replacing soybean meal and ground corn by 17% of RFB or GFB on isonitrogenous and isoenergetic basis had no effect on enteric CH4 emission. Milk production (ECM) was not changed by feeding RFB but decreased when cows were fed GFB, compared with the control diet. In conclusion, if provided rolled rather than ground in dairy cow diets, fava bean has no adverse effects on enteric CH4 emission and milk performance.

**Key Words:** fava bean, enteric methane, milk production

**T264**

**Effect of phytogenic feed supplements added to starter grain on weight gain and rumen development in Holstein calves.** H. A. Rossow1, K. Mitchell1, A. Johnson2*, and B. Miller2, 1University of California Davis, Tulare, CA, 2Biomin America Inc, San Antonio, TX.

The goal of pre wean calf operations is to maximize rumen development and weight gain. Feed supplements that increase starter intake should also encourage rumen development and increase weight gain. The objective of this study was to compare rumen development and body weight gain in pre wean calves given 2 different starter supplements, phytogenic blend A (A) or phytogenic blend B (B) (Biomin, San Anto-
The objective of this study was to determine the effect of replacing one hundred twenty-four Holstein calves were randomly assigned to 1 of 3 treatments, Control, A or B, at 1 d of age. Control (nothing added), A or B were added to individual feed buckets at each feeding at the rate of 0.25 g/kg starter at AM and PM feedings. Both starter intake and milk intakes were assessed daily. Calves were weighed at enrollment and at weaning, and blood samples were collected from a subset of 38 calves and analyzed for glucose (Glu, mg/dL) and β-hydroxybutyrate levels (BHB, mmol/L) with Precision Extra blood meters (Abbott Diabetes Care Inc., Alameda, CA) to assess rumen development. Weekly average DMI, milk intake, Glu and BHB were analyzed using the Mixed procedure of SAS (v. 9.4) with repeated measures by calf; fixed effects treatments and random effect week. Weekly average DMI (P < 0.01), BHB (P < 0.01) and Glu (P < 0.01) were different by week but not by treatment. However, weekly milk intake was less for group B (P < 0.05). Total DMI, initial bodyweight, final body weight and gain were analyzed using the Mixed procedure of SAS with repeated measures by calf, fixed effects, sex, birthdate, and BW at 142 and 224 days. There were no differences in initial bodyweight and effects of Hutch, birthdate, or sex among treatments. Product B group was numerically greater in total DMI, gain, ADG and had faster rumen development indicated by overall higher BHBA values but differences were not significant among treatments. Therefore, supplementation with B decreased milk intake but calves maintained similar starter DMI, gain and ADG.

Key Words: phytonic supplement, calf starter

T265 Fava bean (Vicia faba L. major) inclusion in dairy cow diets: Effects on nitrogen utilization. C. Cheriti1, F. Hassanat1, S. Claveau2, J. Girard2, R. Gervais3, and C. Benchaar1, 1Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada, 2Agrinova, Alma, QC, Canada, 3Département des Sciences Animales, Université Laval, Québec, Québec, Canada.

The objective of this study was to determine the effect of replacing on isonitrogenous (16% CP) and isoenergetic (NEL = 1.50 Mcal/kg) basis soybean meal and corn grain with fava bean (17%, DM basis) in dairy cow diets on N utilization. For this purpose, 9 lactating cows (DIM = 121 ± 28; milk yield = 41.2 ± 5.6 kg) were used in a replicated 3 × 3 Latin square (38-d period; 14-d adaptation). Treatments were: control diet (CTL), rolled fava bean diet (RFB), and ground fava bean diet (GFB). Intake of N, N excretion (fecal and urinary), milk N secretion, ruminal NH3 concentration was determined over 2 consecutive days. Data were analyzed using the MIXED procedure of SAS (v. 9.4) with repeated measures by calf; fixed effects, treatments and the control were declared significant (P ≤ 0.05) using Dunnett’s comparison test. Nitrogen intake averaged 659 g/d and was not affected by the inclusion of RFB or GFB in the diet. Ruminal NH3 concentration was higher for cows fed RFB and GFB diets (11.8 and 11.3 mM, respectively) than for cows fed CTL diet (9.8 mM). In contrast, MUN was unaffected by dietary treatments (11.6 mg/dL). Excretion of N in feces and urine was similar among dietary treatments (222 and 224 g/d, on average, respectively). Milk N efficiency (milk N secretion as a proportion of N intake) was similar between cows fed the CTL diet (29.6%) and those fed the RFB diet (29.0%), but decreased when cows were fed the GFB diet (28.5%). Feeding cows the RFB diet or the CTL diet had no effect on retained N (g/d or as a proportion of N intake). However, retained N was numerically higher for cows fed the GFB diet compared with cows fed the CTL diet (31.9 vs. 19.3 g/d and 4.85 vs. 2.86%, respectively). Results from this study suggest that similar efficiency of N utilization can be achieved when RFB replaces soybean meal and corn grain in dairy cow diets on isonitrogenous and isoenergetic basis. However, milk N efficiency can be depressed if GFB is used in dairy cow diets. In conclusion, at 17% dietary inclusion, fava bean should be fed rolled rather than ground to avoid negative effects of N utilization in dairy cows.

Key Words: fava bean, N utilization, dairy cow

| Performance of calves fed different liquid diets (LD) associated with environmental enrichment (EE) |
|-------------------------------------------------|----------------|----------------|----------------|----------------|----------------|
| BW, kg                                          | Control        | EE             | Control         | EE             | Control        | EE             |
| 59.4±1.54                                       | 59.8±1.18      | 56.1±1.66      | 57.5±1.54       | 0.125           | 0.578          | 0.972          | <0.0001        | 0.06           |
| BW gain, g/d                                    | 712±33.5       | 787±38.7       | 683±35.6        | 695±38.8       | 0.121           | 0.252          | 0.417          | <0.0008        | 0.19           |
| Starter intake, g/d                             | 280±35.8       | 203±42.5       | 327±38.5        | 254±42.6       | 0.236           | 0.082          | 0.971          | <0.0001        | 0.07           |
| Heart girth, cm                                 | 91.3±1.91      | 93.7±2.39      | 91.4±2.05       | 92.8±2.26      | 0.851           | 0.393          | 0.828          | <0.0001        | 0.28           |
| Hip width, cm                                   | 24.5±0.37      | 24.4±0.44      | 23.8±0.39       | 24.1±0.44      | 0.218           | 0.746          | 0.623          | <0.0001        | 0.53           |
| Withers height, cm                              | 84.5±0.77      | 85.7±0.92      | 83.4±0.83       | 84.2±0.93      | 0.141           | 0.270          | 0.823          | <0.0001        | 0.61           |

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Calves fed whole milk associated with environmental enrichment were heavier than calves fed milk replacer from wk 5 until wk 8 (P < 0.06).

**Key Words:** nipple, stationary brush, welfare

**T267**  
**Title:** Metabolic profile, feeding behavior and production responses of lactating dairy cows supplemented with a combination of osmotic feed ingredients (I.C.E.) and submitted to high temperature and humidity environment.  
**Authors:** J. Franck1,2, F. Terra1, A. Barbosa1,2, M. N. Corrêa1,2, F. A. B. Del Pino1,2, D. B. Araújo1, and E. Schmitt1,2.  
**Institution:** Federal University of Pelotas, Capão do Leão, Brazil; NUPPEC, Capão do Leão, Brazil; Cargill Animal Nutrition, Campinas, Brazil.

The Internal Cooling Elements (I.C.E.) is a commercial feed technology designed and manufactured by Cargill, containing a combination of key ingredients, such as amino acids, minerals and other osmotics, that have been used as a nutritional alternative to reduce body temperature and sustain dairy cows performance during heat stress. We hypothesized that I.C.E. supplementation for lactating dairy cows submitted to high temperature and humidity environment would overcome the effects of heat stress and improve performance. Sixteen lactating, multiparous Holstein cows (65 ± 15 d in milk) were balanced for previous milk production, body condition score, parity, somatic cell count, and randomly assigned to either receive (GICE; n = 8) or not (CG; n = 8) I.C.E., 60 g/cow daily (as-fed basis) for 6 weeks. Cows were kept in a free-stall barn with the same management, feeding and cooling system during experimental period. Diets were iso-energetic for both treatment groups. Temperature and humidity logger was used to record environmental data and temperature-humidity index. All animals received a collar with a sensor to monitor and register rumination, activity and idleness time (C-Tech HealthyCow, Chipsinside, Santa Maria, Brazil). Blood samples were collected and recorded every 3 d after first milking. Average THI during the role experiment period was 71.6 (59.6 min.; 83.6 max.). Higher serum albumin (2.6 ± 0.43 vs. 2.4 ± 0.47 mg/dL, P = 0.03) and lower urea nitrogen (27.1 ± 5.4 vs. 28.0 ± 5.7 mg/dL, P = 0.02) were observed for GICE, suggesting changes in protein metabolism. No difference on NEFA concentration between the treatment groups. The GICE presented higher rumination time (P < 0.01) according the day’s hour (7am: 33.2 vs. 29.0 min, 11 a.m.: 30.2 vs. 23.25 min, 1pm: 31.3 vs. 26.2 min, 19pm: 29.9 vs. 22.6 min, 22pm: 31.0 vs. 24.3 min). The milk production also was higher in GICE (27.08 ± 3.2 vs. 24.92 ± 3.2 kg / d; P = 0.05). These results indicate that I.C.E. supplementation may mitigate the negative effects of heat stress in lactating dairy cows, increasing milk production.

**Key Words:** transition cow, OmniGen-AF, health

**T269**  
**Title:** C16:0 supplementation alters markers of adipose tissue lipolysis and inflammation in early lactation dairy cows.  
**Authors:** J. de Souza*, C. Strieder-Barboza, G. A. Contreras, and A. L. Lock, Michigan State University, East Lansing, MI.

We evaluated the effects of feeding a C16:0 supplement (85% C16:0) on markers of adipose tissue (AT) lipolysis and inflammation in early lactation dairy cows. Sixteen multiparous cows were used in a randomized complete block design experiment and assigned to either a control diet containing no supplemental fat (CON) or a C16:0 supplemented diet (PA; 1.5% diet DM) that was fed from calving to 24 DIM. Subcutaneous AT were collected at d −14 and d 10 of the study. Gene expression was assessed by qPCR and flow cytometry was used to determine AT immune cell trafficking and phenotype. The statistical model included the random effect of block, the fixed effect of treatment, and variables measured at d −14 as covariate. Compared with CON, PA increased the expression of genes related to lipolysis including LIPE (P = 0.03), ABHD5 (P < 0.01), and FABP4 (P = 0.05). We measured by Western blot the activity of hormone-sensitive lipase (HSL) and observed that compared with CON, PA increased the ratio of pHSL/HSL (P = 0.06) indicating enhanced lipolysis. There was no effect of treatment on the expression of genes related to lipogenesis including FASN, SCD1, and ELOVL6 (all P > 0.46), but compared with CON, PA reduced the expression of ADIPOQ (P = 0.05). Compared with CON, PA increased the expression of genes related to inflammation including TNF (P < 0.01), SIRPA (P = 0.05), and IL6 (P = 0.07), but did not affect CCL22 (P = 0.54) or IL10 (P = 0.84). Compared with CON, PA increased the expression of CCL2 (P < 0.01) that encodes the macrophage chemoattracting protein-1 and increased the expression of CD44 (P = 0.02), a macrophage receptor. The increase in lipolysis gene expression (LIPE) was correlated with BW loss (r = 0.44, P = 0.05), milk yield (r = 0.50, P = 0.01), and ECM (r = 0.58, P < 0.01). Flow cytometry revealed that compared with CON, PA increased AT macrophage trafficking as...
reflected in the increased number of CD172a+ (P = 0.05) and CD14+ (P = 0.09) cells. Our results demonstrate that feeding C16:0 during early lactation increased lipolysis to support the yield of milk and ECM and may increase inflammation in adipose tissue.

**Key Words:** gene, lipid metabolism, palmitic acid

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**T270**  
**Long-term effects of C16:0 supplementation on production responses of lactating dairy cows.** J. de Souza*, A. L. Lock, Michigan State University, East Lansing, MI.

We evaluated the long-term effects of feeding a C16:0 supplement (85% C16:0) on production responses of primiparous and multiparous mid-lactation dairy cows. Forty Holstein cows (144 ± 44 DIM; 18 primiparous and 22 multiparous) were blocked by parity, milk production, and BCS, and assigned to either a control diet containing no supplemental fat (CON) or a C16:0 supplemented diet (PA; 1.5% diet DM) for 10 wks. PA replaced soyhulls and diets contained (% DM) 21% forage NDF, 17% CP, and 27% starch. After the treatment period, all cows received the CON diet for 2 wks to evaluate carryover effects. The statistical model included the random effect of block and cow within parity, and the fixed effect of treatment, parity, time, and their interactions. During the 10-wk treatment period, compared with CON, PA consistently increased DMI (31.0 vs. 29.5 kg/d; P < 0.01), milk yield (49.4 vs. 45.6 kg/d; P < 0.01), milk fat content (3.35 vs. 3.15%; P < 0.01), milk fat yield (1.56 vs. 1.41 kg/d; P = 0.03), yield of 16-carbon milk FA (582 vs. 545 g/d; P < 0.01), ECM (47.1 vs. 43.4 kg/d; P < 0.01), feed efficiency (3.5% ECM/DMI; 1.52 vs. 1.47; P < 0.05) and tended to increase milk protein yield (1.40 vs. 1.31 kg/d; P = 0.06). There was no effect of treatment on the yield of de novo milk FA (318 vs. 342 g/d; P = 0.37), yield of preformed milk FA (555 vs. 549 g/d; P = 0.84), BW (698 vs. 689 kg; P = 0.71), or BCS (3.26 vs. 3.31; P = 0.65). Treatment by parity interactions were observed for ECM (interaction, P = 0.08), and milk fat yield (interaction, P = 0.05) due to PA increasing these variables to a greater extent in multiparous than primiparous cows. In contrast, compared with CON, PA increased BW change in primiparous, but not in multiparous cows (interaction, P = 0.09). During the carryover period, compared with CON, PA tended to increase ECM (42.2 vs. 40.4 kg/d; P = 0.07), and milk fat yield (1.33 vs. 1.28 kg/d; P = 0.07). In conclusion, production responses of dairy cows to C16:0 supplementation were consistent throughout the 10-wk treatment period, and had carryover effects on the yield of milk fat and ECM. Overall, C16:0 increased DMI, milk yield, milk fat yield, ECM, and feed efficiency in mid-lactation dairy cows.

**Key Words:** milk fat, palmitic acid, parity

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**T271**  
**Altering the ratio of dietary C16:0 and cis-9 C18:1 modifies the fatty acid profile of plasma lipid fractions and adipose tissue.** J. de Souza*, C. Strieder-Barboza, H. Eerdun, G. A. Contreiras, and A. L. Lock, Michigan State University, East Lansing, MI.

We evaluated the effects of altering the dietary ratio of C16:0 and cis-9 C18:1 on the fatty acid (FA) profile of plasma lipid fractions and adipose tissue (AT), and the association of dietary FA ratio with body weight (BW) change of lactating dairy cows. Cows (n = 24; 124 ± 30 DIM) were blocked by milk yield and assigned to FA treatments fed for 35 d. The FA treatments supplemented at 1.5% diet DM were (1) 80:10 (80% C16:0 + 10% cis-9 C18:1); (2) 73:17 (73% C16:0 + 17% cis-9 C18:1); (3) 66:24 (66% C16:0 + 24% cis-9 C18:1); and (4) 60:30 (60% C16:0 + 30% cis-9 C18:1). Subcutaneous AT samples from the tail region and blood samples from tail vein were collected on the last d of the study. The FA profile of AT and plasma phospholipids (PL), cholesterol esters (CE), triglycerides (TG), and free FA (FFA) determined. The statistical model included the random effect of cow and the fixed effect of treatment. Increasing cis-9 C18:1 in FA treatments increased the concentration of C16:0 (linear, P = 0.10), C18:0 (linear, P = 0.01), cis-9 C18:1 (linear, P = 0.01), trans-10 C18:1 (linear, P = 0.01), and cis-9, trans-11 C18:2 (linear, P = 0.01) in plasma TG. Increasing cis-9 C18:1 in FA treatments reduced C18:0 (linear, P = 0.01), and increased cis-9 C18:1 (cubic, P = 0.04) in plasma FFA. There were no effects of treatments on C16:0 and C18:0 concentrations in PL and CE (P > 0.25); however, increasing cis-9 C18:1 in FA treatments increased cis-9 C18:1 concentration in plasma PL and CE (both linear, P = 0.10). In AT, increasing cis-9 C18:1 in FA treatments increased the concentration of C18:0 (linear, P = 0.01) and cis-9 C18:1 (linear, P = 0.01), but reduced C16:0 (quadratic, P = 0.03). There were positive correlations between the concentration of cis-9 C18:1 in AT and BW change (r = 0.66, P < 0.01) and the concentration of cis-9 C18:1 in AT and plasma insulin (r = 0.44, P = 0.03). There were no correlations between BW change and AT concentration of C16:0 (r = −0.17, P = 0.44) or C18:0 (r = −0.04, P = 0.84). Our results demonstrate that altering the dietary ratio of C16:0 and cis-9 C18:1 changes the FA profile of AT and plasma lipid fractions. Changes in BW are positively associated with cis-9 C18:1 in AT and plasma insulin.

**Key Words:** adipose tissue, fatty acids, plasma lipid

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**T272**  
**Altering source of chelated trace minerals improves milk fat in commercial dairy.** H. Tucker* and M. Vazquez-Anon, Novus International, St. Charles, MO.

Little research is available directly comparing chelated mineral sources in a commercial dairy setting. The objective of this study was to determine effects of altering source of chelated trace minerals on lactation and reproductive performance in a commercial dairy herd. Six pens (3/treatment; 2,400 cows total; 6 mo duration) received the same isominal diets, differing only in supplemental Cu, Mn, and Zn source. Supplemental Cu, Mn, and Zn were provided as metal methionine hydroxy analog chelate (MMHAC) or metal chelates of methionine and lysine (MMKC). Analyzed dietary mineral content (average ± SD) was 21.1 ± 1.9, 74.0 ± 8.1, and 78.0 ± 5.2 ppm Cu, Mn and Zn for MMHAC; 20.4 ± 2.2, 77.4 ± 12.3, and 79.6 ± 7.6 ppm Cu, Mn, and Zn for MMKC. Monthly DHIA milk yield, fat, solids nonfat % (8.77 versus 8.80 P = 0.02), fat % (3.45 versus 3.51 ± 0.20; P < 0.01), and solids nonfat % (8.77 versus 8.80 ± 0.10; P = 0.04). Failure to see differences in reproductive measures between treatments suggests little differences in the bioavailability of the evaluated chelated mineral sources. Observed increases in milk fat suggest additional value for MMHAC because of methionine hydroxy analog. Methionine hydroxy analog has previously been associated with increased milk fat. Together this data suggests that differing source of chelated trace mineral results in changes in lactation performance,
particularly milk fat, over time that may significantly affect dairy farm revenue.

**Key Words:** chelated minerals, dairy, performance


Nearly 70% of energy and 60–85% of protein requirements of the dairy cow are met from microbial fermentation, indicating a critical need for maximizing rumen function and describing rumen microbiota. However, it is still not known how diet and microbes interact to enhance milk yield in dairy cows. The purpose of this study is to compare the ruminal bacterial composition in the high and low yielding dairy cows within and between 2 dairy herds. Eighty 5 Holstein dairy cows in mid-lactation (80–180 d in milk) were selected from 2 farms: Farm 12 (M305 = 12,300 kg; n = 47; 24 primiparous cows, 23 multiparous cows) and Farm 9 (M305 = 9,700 kg; n = 38; 19 primiparous cows, 19 multiparous cows). Each study cow was sampled once using the stomach tube method and processed for 16S rRNA ampiclon sequencing using the Ion Torrent (PGM) platform. Differences in bacterial communities between farms were greater (Adonis: R² = 0.16; P < 0.05) than within farms. Five bacterial lineages, namely Prevotella (48–51%), Bacteroidales (10–12%), unclassified bacteria (5–8%), Succinivibrionaceae (1.4–6.6%) and unclassified Prevotellaceae (3.8–4.7%) were observed to differentiate the community clustering patterns between the farms. A notable finding is the greater (P < 0.05) contribution of Succinivibrionaceae in Farm 12 compared with Farm 9. Furthermore, in Farm 12, this bacterial population was higher (P < 0.05) in the higher yielding cows compared with the lower yielding cows in both primiparous and multiparous groups. Prevotella, S24–7, and Succinivibrionaceae were found in greater abundance on Farm 12 and were positively correlated with milk yield. Differences in rumen bacterial populations observed between the 2 farms can be attributed to dietary composition, particularly differences in forage type and proportion in diets. A combination of corn silage and alfalfa silage may have contributed to the increased proportion of Proteobacteria. It was concluded that Farm 12 had a greater proportion of specialist bacteria that have the potential to support enhanced rumen function.

**Key Words:** dairy cows, ruminal microbiota, dairy herds

T274  Evaluation of solubles syrup from microbially enhanced soy protein production as a supplement for growing dairy heifers. C. R. Schossw and J. L. Anderson, Dairy and Food Science Department, South Dakota State University, Brookings, SD.

The objective of this research was to conduct a preliminary study to determine if solubles syrup from a microbially-enhanced soy protein (MSP) production process has potential as a supplement for growing dairy heifers. A 6-wk randomized complete block design study was conducted using 14 Holstein and 4 Brown Swiss heifers [310 ± 16.4 d of age; body weight (BW) 337.6 ± 33 kg] to evaluate effects of diet on growth performance. Treatments were: 1) a control total mixed ration (TMR) with corn, soybean meal and distillers dried grains with solubles as concentrate ingredients (CON) and 2) a TMR with 6.5% MSP solubles syrup (DM basis) in partial replacement of soybean meal and corn (SYP). Both diets contained 34% corn silage, 35% grass hay, and 2.6% mineral mix and were isonitrogenous and isocaloric. Heifers were individually fed TMR for ad libitum intakes using a Calan gate feeding system. Frame sizes, BW, and body condition score (BCS) were measured on 2 d during wk 0, 2, 4, and 6 of the feeding period. Rumen fluid was sampled via esophageal tube 2 d during wk 0 and 6 and 1 d during wk 2 and 4 at 4 h post-feeding. Data were analyzed with MIXED procedures with repeated measures in SAS 9.4. There were no interactions of treatment by wk for any of the parameters evaluated. Treatments had similar (P > 0.05) DMI (9.9, and 9.7 kg/d for CON and SYP, respectively; SEM = 0.49), body weight (366.2, and 365.3 kg; SEM = 2.73), ADG (1.00 and 0.97 kg/d; SEM = 0.09), gain:feed (0.11, and 0.13; SEM = 0.02) and BCS (3.10, and 3.10; SEM = 0.30). Frame growth measures including hip height (134.9, and 131.3 cm; SEM = 1.92), withers height (131.0, and 131.0 cm; SEM = 0.40), heart girth (156.7, and 160.7 cm; SEM = 3.21), body length (124.5 and 129.2 cm; SEM = 2.64) and hip width (41.8 and 41.7; SEM = 0.38) and their rates of change were the same between treatments. Rumen pH (6.9, and 6.9; SEM = 0.072) was not affected by treatment. Results demonstrated that partially replacing soybean meal and ground corn with MSP solubles syrup maintained heifer growth performance with a similar gain:feed.

**Key Words:** microbially enhanced soy protein solubles, dairy heifer, growth performance

T275  Dairy heifer growth performance when fed hydroponically grown barley sprouts. R. D. Lawrence and J. L. Anderson, Dairy and Food Science Department, South Dakota State University, Brookings, SD.

Our objective was to determine the effects of feeding hydroponically grown barley sprouts (HydroGreen Inc., Renner, SD) to dairy heifers on growth and rumen fermentation. A 12-wk randomized complete block design study was conducted using 20 Holstein and 4 Brown Swiss heifers [215.1 ± 25 d of age; body weight (BW) 229.7 ± 39 kg]. Treatments were (1) control (CON) diet which was a total mixed ration (TMR) with grass hay, corn silage, and ground corn and soybean meal as major concentrate ingredients; and (2) a TMR with 14% (DM basis) hydroponic barley sprouts (HYD) replacing a portion of the concentrate mix. Diets were fed for ad libitum intakes and formulated to be isonitrogenous and isocaloric (DM basis), although the CON was 66% DM and HYD was 44% DM. Intakes were measured using the Calan gates. Frame sizes, BW, and body condition scores (BCS) were measured on 2 d during wk 0, 2, 4, 6, 8, 10, and 12. Rumen fluid was collected 4 h post feeding via esophageal tube every 4 wk. Data were analyzed in MIXED procedures of SAS 9.4 with repeated measures. Heifer DMI was greater (P < 0.01) for HYD (7.5, and 8.0 kg/d for CON and HYD, respectively; SEM = 0.06), Body weight (289.7, and 282.4 kg; SEM = 2.02) and gain:feed (0.16, and 0.13; SEM = 0.01) were greater (P < 0.01) for the CON treatment. Withers height (121.7 and 121.9 cm; SEM = 0.54) and hip width (37.3 and 37.1 cm; SEM = 0.57) and BCS (3.11, and 3.10; SEM = 0.02) were similar (P > 0.05), but heart girth was greater (P < 0.01) for the CON heifers (146.0 cm, and 145.0 cm; SEM = 0.62). Rumen ammonia-N (21.0, and 24.5 mg/dL; SEM = 2.15), pH (6.75, and 6.70; SEM = 0.06), total volatile fatty acids (95.0, and 98.0 mM; SEM = 2.32) and acetate to propionate ratio (3.4, and 3.3; SEM = 0.13) were similar. Results indicated that replacing ground corn and some soybean meal with hydroponic fresh barley sprouts maintained rumen fermentation and heifer body frame growth with slightly decreased gain:feed. The decreased gain:feed was most likely because of the overall high moisture content in the HYD TMR.

**Key Words:** hydroponic feed, dairy heifer, growth performance
The cow’s daily pattern of feed intake creates differences in nutrient consumption across the day. Feeding a high-fiber diet during the high-intake period of the day is a potential strategy to stabilize rumen fermentation and nutrient absorption. Rumen samples from a previously published experiment (Rottman et al., J. Dairy Sci. 98:7), which fed multiple diets differing in neutral detergent fiber and starch across the day, were used to determine the effects on the daily patterns of selected microbial populations. Briefly, diets included a control (33.3% NDF), a low fiber diet (LF; 29.6% NDF), and a high-fiber diet (HF; 34.8% NDF). Nine cannulated Holstein cows were fed 1 of 3 diet combinations in a 3 × 3 Latin square design: (1) 100% daily offering of the control diet at 0900 h (CON); (2) 70% daily offering of HF at 0900 h and 30% daily offering of LF at 2200 h (HL); or (3) 30% daily offering of LF at 0900 h and 70% daily offering of HF at 1300 h (LH). Microbial DNA was extracted from rumen digesta representing every 3 h across the day. The relative abundances of bacteria, fungi, ciliate protozoa, Fibrobacter succinogenes, Ruminococcus albus, Butyryrivibrio fibrisolvens, Selenomonas ruminantium, Butyryrivibrio hungatei, Prevotella bryantii, Megaphaera elsdenii, and Streptococcus bovis was measured using quantitative PCR. Data were analyzed using the mixed procedure of SAS with a repeated statement and tested the effects of treatment, time, and their interaction. The time of day affected the relative abundance of all microbial groups (P < 0.05) and nearly all were modified by feeding strategy. Notably, HL and LH treatments caused dramatic increases in the relative abundances of S. bovis (4-fold), S. ruminantium (2-fold), and B. hungatei (~3-fold) at 0900 h, that was not observed in the control (P < 0.05). Total bacteria abundance varied by treatment, and the lowest daily variation occurred in HL. Results suggest that the daily pattern of certain microbes is modified by altering the amounts of fiber and starch fed throughout the day, and feeding a high-NDF diet during peak intake may stabilize bacterial abundance.

Key Words: daily rhythm, rumen microbes, feed intake

### T277

**Acute high-grain challenge triggers a hepatic inflammatory response and alteration of lipid metabolism in Holstein but not Jersey cows.** T. Xu1,2, F. C. Cardoso2, E. Trevisi3, X. Shen1, and J. J. Loor2, 1College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China, 2Department of Animal Sciences, University of Illinois, Urbana, IL, 3Università Cattolica del Sacro Cuore, Piacenza, Italy.

Long-term feeding of high-grain diets to dairy cows often results in systemic inflammation characterized by alterations in acute-phase proteins and other biomarkers, both in plasma and immune-responsive tissues like the liver. The systemic changes that underlie an acute high-grain feeding challenge remain unclear. The current study involved 6 Holstein and 6 Jersey cows in a replicated 3 × 3 Latin square. Periods (10 d) were divided into 4 stages (S): S1, d 1 to 3, served as baseline with TMR ad libitum; S2, d 4, served as restricted feeding, with cows offered 50% of the TMR fed on S1; S3, d 5, a grain challenge was performed, in which cows were fed a TMR ad libitum without (CON) or with an additional 20% pellet wheat-barley (1:1; HIG) top-dressed onto the TMR based on DMI obtained in S1; S4, d 6 to 10, served as recovery during which cows were allowed ad libitum access to the TMR. Among 28 biomarkers detected in plasma after 12 h of feeding on d 5, the concentrations of fatty acids and bilirubin increased in HIG Holstein but not in Jersey cows. In Holsteins, feeding HIG also increased total protein and albumin and decreased ceruloplasmin, myeloperoxidase and alkaline phosphatase concentrations. At the molecular level, however, hepatic genes associated with inflammation (IL1B, IL6, TNF, TLR4, MYD88, and NFKB1) were upregulated in Holsteins fed HIG compared with CON. Despite such response, expression of the acute-phase proteins SAA and HP in both Holsteins and Jerseys fed HIG compared with CON was markedly downregulated. In Holsteins fed HIG versus CON, the marked downregulation of SCD, ELOVL6, and MTTP together with upregulated CPT1A, ACOX1 and APOA5 indicated alterations in lipid metabolism during grain challenge. Genes related to TCA cycle and ketogenesis (PDK4, HMGC52 and ACAT1) also were upregulated in Jerseys fed HIG. Data indicate that Jerseys tolerated better the high-grain challenge. An acute high-grain challenge in Holsteins induced marked alterations in lipid metabolism in the liver at least in part due to a localized inflammatory response. It remains to be determined if acute high-grain challenges can elicit long-term liver tissue damage, which could negatively affect the welfare of the cow.

**Key Words:** high-grain diet, liver response

### T278

**Assessing potentially digestible NDF and energy content of canola meal from twelve Canadian crushing plants over four production years.** E. M. Paula1, J. L. P. Daniel2, H. H. A. Costa3, and A. Faciola1, 1University of Nevada, Reno, NV, 2Universidade Estadual de Maringa, Maringa, PR, Brazil, 3Universidade Estadual Vale do Acauá, Sobral, Ceara, Brazil.

The objective of this study was to assess NDF digestibility and energy content of canola meals (CM) produced in Canada over a 4-year period. Canola meal samples were collected from 12 Canadian crushing plants over 4 years (total = 48) and analyzed for chemical composition, poten-
tially digestible NDF (pdNDF), total digestible nutrients at maintenance (TDN1x), and NEL simulating a cow consuming 3× maintenance (NEL3x). To estimate TDN1x and NEL3x, pdNDF was calculated as: (1) pdNDFOBS = (NDF − NDICP − iNDF), using observed CM iNDF values after 288-h in situ ruminal incubations; (2) pdNDFNCPS = (NDF − NDICP − ADL) × \{1 − [ADL/(NDF − NDICP)]0.667\}, according to NRC 2001; (3) pdNDFCNCPS = (NDF − NDICP − iNDF), according to the CNCPS that calculates iNDF as acid detergent lignin (ADL) × 2.4. Concentrations of NDF, NDICP, and ADL in all equations were given in % of DM. Truly digestible NDF was estimated multiplying the observed and predicted pdNDF by 0.75. Then TDN1x and NEL3x were calculated assuming a diet with 74% of TDN1x according to NRC 2001 equations. Regressions of predicted (NRC or CNCPS) vs. observed values were performed using Proc Reg of SAS (Table 1). pdNDFOBS, pdNDFNCPS, and pdNDFCNCPS averaged 15.8, 2.4%, and 2.4% of DM, respectively. The TDN1x average were 73, 67, and 64%, respectively. The NEL3x average were 1.88, 1.73, and 1.63 Mcal/kg, and ranged from 1.73 to 2.08; 1.51 to 1.94; and 1.4 to 1.87 Mcal/kg DM for NEL3NCPS, NEL3NCPCS, and NEL3NCPCS, respectively. Our results indicate that NEL3x from CM diets may be underestimated in current nutritional models due to underestimations in CM NDF digestibility. More accurate information on CM NDF digestibility may improve energy content estimation, thus improving diet formulation accuracy.

Key Words: acid detergent lignin, indigestible NDF, in situ

T279 Implications of rumen inoculation on the ruminal bacterial populations in dairy cows with diet-induced milk fat depression. D. Pitta1, N. Indugo1, B. Vecchiarelli1, D. Rico1, and K. Harvatine2,1
University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, PA; 2Penn State University, University Park, PA; 3Centre de Recherche en Sciences Animales de Deschambault, Deschambault, QC, Canada.

Ten Holstein cows were used to investigate the changes in ruminal bacterial populations in response to induction and recovery from diet-induced milk fat depression (MFD). Further, the impact on the ruminal bacterial composition of the cows with MFD inoculated with rumen contents from non-milk-fat-depressed donor cows was evaluated. MFD was induced during the first 10 d of each period by feeding a low-fiber and high-polysaturated fatty acid (FA) diet (induction diet; 26.1% neutral detergent fiber, 5.8% FA (1.9% C18:2)], resulting in a 30% decrease in milk fat yield. Induction was followed by a recovery phase where all cows were switched to a high-forage, low-polysaturated FA diet (31.8% NDF, 4.2% total FA (1.2% C18:2)) and were allocated to (1) control (no inoculation) or (2) ruminal inoculation with donor cow digesta (8 kg/d for 6 d). Ruminal samples were collected at the end of induction (d10) and through recovery (d13, d16 and d28) of each period, separated to solid and liquid fractions, extracted for DNA, PCR-amplified for V1-V2 hypervariable region of the 16S rRNA gene and sequenced using Ion Torrent (PGM) platform for bacterial diversity. The 16S pyrosequence reads were analyzed using the QIIME (v 1.8.0) and all statistical analysis were performed using SAS (V 9.3) and R (V 3.3.1). In both fractions, d10 bacterial communities were observed to be different (P < 0.05) from d13, d16 and d28. MFD (d10) was typified by a reduction (P < 0.05) in Bacteroidetes and Fibrobacteres and an increase (P < 0.05) in Firmicutes and Actinobacteria across both fractions. Both Bacteroidetes and Fibrobacteres recovered as early as 3 d into the recovery period their sensitivity to induction diet. Inoculation of rumen contents from non-MFD donor cows to MFD cows revealed that transfaunation procedure did not result in wholesale shifts in the ruminal microbiota, however, influenced a few genera from Bacteroidetes, Fibrobacteria and Spirochaetes in the liquid fraction. These microbial changes diminished after d16 indicating that the impact of inoculation on the rumen microbiota is a transient effect. It can be concluded that diet-induced MFD is accompanied by microbial dysbiosis in the rumen.

Key Words: milk fat depression, ruminal inoculation, ruminal bacteria

T280 Effects of varying prepartum DCAD and calcium concentrations on pre- and postpartum body weight, intake, milk yield and milk composition. A. L. Diehl1, J. K. Bernard1, S. Tao1, T. N. Smith1, D. J. Kirk2, D. J. McClean2, and J. D. Chapman2,
1University of Georgia, Tifton, GA, 2Phibro Animal Health, Corp., Teaneck, NJ.

Eighty-two multiparous Holstein cows were enrolled 28 d before calving through 63 DIM between January and October, 2016 and assigned to 1 of 4 dietary treatments in a randomized block design experiment with a 2 × 2 factorial arrangement of treatments. Dietary treatments provided 2 dietary cation-anion concentrations (DCAD): −22 mEq/100 g DM (NEG) or −3 mEq/100 g DM (NEU); and 2 dietary Ca concentrations: 1.0% or 1.5% of DM. Cows were individually fed and DM recorded daily. After calving, cows were milked 3 times daily, yield recorded for each milking and samples collected once weekly for analysis of components. Body weight and BCS were recorded 21 d before calving, and 0, 21, and 63 DIM. No interactions were observed between DCAD and Ca for any variables measured. No significant differences (P > 0.10) were observed in BW or BCS due to DCAD or Ca. Prepartum intake was 15.1, 13.0, 17.3, and 14.8 kg/d for 1.0%-NEG, 1.5%-NEG, 1.0%-NEU, and 1.5%-NEU, respectively. Prepartum DM was lower (P = 0.0096) for NEG compared with NEU (16.0 and 14.2 kg/d, respectively) and lower (P = 0.0038) for 1.5 compared with 1.0% Ca (14.0 and 16.2 kg/d, respectively). Postpartum daily DMI was not different (P > 0.10) among treatments and averaged 24.0, 23.0, 22.5, and 23.0 kg/d for 1.0%-NEG, 1.5%-NEG, 1.0%-NEU, and 1.5%-NEU, respectively. Daily milk yield was not different among treatments (P > 0.10) but an interaction (P = 0.0125) was observed for DCAD and DIM (P = 0.0125) due to higher milk yield after 45 DIM for NEG compared with NEU. No differences (P > 0.10) were observed in milk component percentage or yield among treatments. In this trial, feeding −22 mEq/100 g DM compared with −3 mEq/100 g DM, or 1.5% Ca compared with 1.0% Ca reduced MFD prepartum. Feeding −22 mEq/100 g DM prepartum supported increased milk yield after 45 to 55 DIM.

Key Words: DCAD, calcium, dry matter intake

T281 Comparing selected corn grains using in vitro starch digestion or gas production. D. R. Mertens1, N. Schlau2, and D. Taysom2,1Mertens Innovation & Research LLC, Belleville, WI; 2Dairyland Laboratories Inc., Arcadia, WI.

In vitro (IV) starch digestion (IVSD) is used to evaluate starch sources for dairy rations. The study objective was to determine the efficacy of IVSD and IV gas production (IVGP) in estimating fractional rates of starch digestion (kds). Three high-moisture corn samples with high, medium or low IVSD and 2 dry corn samples with high and low IVSD were selected from 20 random commercial corn grains. Samples were oven-dried and ground using a Wiley mill (4-mm screen). Blended ruminal fluid from 3 steers fed a 30% starch diet was used to measure IVSD at 3, 8, and 24 h and IVGP from 0 to 24 h using an Ankom RF system. Duplicate samples were measured using the same inoculum and media in 3 IV runs. Across all samples and times, IVGP was highly cor-

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related with grams of digested starch: IVGP (mL/g) = 388 x g digested starch; R² = 0.975, SE = 8.0; P < 0.0001. As a reference, MIR_kd for starch was calculated using logarithmic transformation (log-trans) and a constant lag with IVSD(8h). Reference MIR_kd were different among grains and IV runs. Both kds and lag can be calculated using log-trans with IVSD(3,8h) giving the relationship: MIR_kd(8h) = 0.014 + 0.8693 x kds(3,8h); R² = 0.991, SE = 0.0039, P < 0.001. Average lag(3, 8h) was 2.02 h and did not vary among grains, but varied between IV runs (P < 0.009). Solver (MS Excel add-in) was used to estimate lag, rate of gas production (kgp), and kds for IVSD(3,8,24h): MIR_kd(8h) = −0.0197 + 1.0348 x kds(3–24h); R² = 0.969, SE = 0.0071, P < 0.001. As with MIR_kd(8h), kds(3–24h) varied among runs (P < 0.001). Variation between IV runs was less for IVGP compared with IVSD. When separate intercepts were allowed for each run, the prediction was: MIR_kd(8h) = IVrun + 0.9805 x kgp; R² = 0.861, SE = 0.0155, P < 0.001. High correlations between kds calculated from IVSD at 3, 8 and 24h and the small variation among grains in IVSD at 3 and 24 h suggest these times add little when estimating kds. The high correlation between IVGP and g of digested starch during fermentation, and the relationship between kds and kgp suggest that IVGP will be useful in defining the kinetics of carbohydrate digestion in grain.

Key Words: Ankom RF, starch kinetics, corn

T282  Steam explosion of corn stover: Saccharification, fermentation, and microbial colonization in rumen of dairy cows. G. Li14, S. Zhao*1,4, N. Zheng12, and J. Wang13. 1,4Ministry of Agriculture-Key Laboratory of Quality & Safety Control for Milk and Dairy Products, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. 2Ministry of Agriculture-Laboratory of Quality and Safety Risk Assessment for Dairy Products, Beijing, China. 3Ministry of Agriculture-Milk and Dairy Product Inspection Center, Beijing, China. 4State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

Corn stover has great potential utility as a ruminant feed because it contains large proportions of cellulose and hemicellulose. The purpose of this study was to characterize the saccharification, fermentation, and microbial colonization of steam-exploded corn stover. The steam explosion parameters for corn stover were optimized using a Box–Behnken design, based on 3 factors: steam pressure (1.0, 1.6, or 2.2 MPa), processing time (30, 115, or 200 s), and moisture content (10%, 30%, or 50%). The chemical components and morphological structure of steam-exploded corn stover were analyzed. The in sacco incubation of steam-exploded corn stover was assessed with nylon bags in the rumen of 4 dairy cows, in which steam-exploded corn stover was incubated for 48 h, analyzed for degradation, and examined with scanning electron microscopy (SEM) to observe the microbes present. The software Design-Expert was used for optimum levels statistics. Date were analyzed using the one-way ANOVA procedure of SAS. The optimal steam explosion conditions were 1.51 MPa, 180 s, and 10% moisture, insofar as they maximized the gas production rate during rumen fermentation in vitro. Steam explosion significantly reduced the content of neutral detergent fiber (NDF) and hemicellulose in the corn stover by 14.9% and 54.3%, respectively, and increased the production of total reducing sugars and xylose. SEM showed that the steam-exploded corn stover became more disordered and looser, suggesting that it is more accessible to rumen microbes. Fourier transform infrared spectroscopy indicated that the removal of hemicellulose and the abundance of cellulose increased after steam explosion. After rumen incubation for 4, 24, 36, or 48 h, the steam-exploded corn stover had higher cellulose and hemicellulose degradation (P < 0.05) than the raw corn stover. SEM showed that steam explosion markedly increased the bacterial colonization and biofilm formation on the surface of the corn stover. In conclusion, steam explosion facilitates the attachment of microbes to corn stover and its degradation in the rumens of dairy cows.

Key Words: corn stover, steam explosion, rumen fermentation

T283  Effects of clay on ruminal degradability of alfalfa hay, grass hay, wet brewer’s grains, ground corn, corn silage, and soybean meal. M. E. Weatherly*, S. A. Sulzberger1, A. Pineda1, Y. Khidoyatov2, M. R. Murphy3, and F. C. Cardoso4. 1University of Illinois, Department of Animal Sciences, Urbana, IL. 2United Minerals Group, Kyiv, Ukraine.

Our objective was to determine the ruminal degradability of feedstuffs in response to 3 concentrations of dietary clay in lactating dairy cows. Treatments were: no clay (EcoMix, UMG, Ukraine) (CON), 1% (1%), or 2% (2%) of dietary DM as EcoMix. Samples (8 g) of dried and ground alfalfa hay (AH), grass hay (GH), wet brewe’r’s grains (WBG), ground corn (GC), corn silage (CS), or soybean meal (SBM) were placed into (3 replicates per feed) polyester bags and incubated for 0, 2, 4, 8, 12, 48, 72, or 96 h in 3 rumen-cannulated cows. Recovered bags were analyzed for DM, NDF, ADF, starch, and CP. Soluble (S), digestible (D), and indigestible (I) fractions of each nutrient, fractional rate of digestion (Kd), and effective degradability (ED) were estimated for each feedstuff; treatment, and cow combination. Statistical analysis was performed using the MIXED procedure of SAS. Quadratic effects occurred for S and Kd for GH DM. Soluble DM was 0.14, 0.17, and 0.12% DM for CON, 1%, and 2% (P = 0.03). Dry matter Kd for GH was 0.026, 0.015, and 0.022 h⁻¹ for CON, 1%, and 2% (P = 0.02). Linear effects occurred for D and ED for WBG DM. Digestible DM was 0.59, 0.66 and 0.76% DM for CON, 1%, and 2% (P = 0.04). Dry matter ED for WBG was 0.44, 0.41, and 0.31 for CON, 1%, and 2% (P = 0.02). Quadratic effects occurred for S, I, and ED for SBM DM. Soluble DM was 0.26, 0.34 and 0.15% for CON, 1%, and 2% (P = 0.04). Indigestible DM was 0.09, 0.02 and 0.11% for CON, 1%, and 2% (P = 0.02). Dry matter ED for SBM was 0.48, 0.57, and 0.39 for CON, 1%, and 2% (P = 0.002). Linear effects occurred for digestible SBM ADF and soluble and digestible SBM starch. The ADF digestible fraction for SBM decreased linearly for CON, 1%, and 2% with 0.63, 0.47, 0.33% (P = 0.03). Starch soluble fraction for SBM decreased linearly for CON, 1%, and 2% with 0.23, 0.23, 0.18% (P = 0.04). Starch digestible fraction for SBM increased linearly for CON, 1%, and 2% with 0.77, 0.77, 0.82% (P = 0.04). In conclusion, WBG DM decreased linearly with clay supplementation; whereas, that of SBM DM was maximized with clay at 1% of dietary DM.

Key Words: degradability, clay

T284  Effective fiber for lactating dairy cows: A physically adjusted NDF (paNDF) system. R. R. White1, M. B. Hall2, J. L. Firkins3, and P. J. Kononoff4. 1Department of Animal and Poultry Science, Virginia Tech, Blacksburg, VA. 2U.S. Dairy Forage Research Center, Madison, WI. 3Department of Animal Sciences, The Ohio State University, Columbus, OH. 4Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE.

Multiplying dietary NDF by particle size (PS) has been used as an estimate of physically effective fiber (peNDF). Our objectives were to (1) to compare the use of peNDF as dietary NDF × PS vs. use of individual NDF and PS descriptors in a physically adjusted NDF (paNDF) system
when used with other factors to predict dry matter (DM) intake (DMI), rumination time, and ruminal pH in lactating dairy cows, and (2) leverage equations derived in a meta-analysis into an ensemble modeling system for predicting dietary physical and chemical characteristics required to maintain desired rumen conditions. Each response variable tested had 8 models in a 2 (peNDF, paNDF) × 2 (diet, diet+ruminal factors) × 2 (DM, as fcd (AF) basis) factorial arrangement. PS descriptors were determined with the Penn State Particle Separator. Treatment means (n = 241) from 60 publications were used to derive models by backward elimination, weighted, mixed effect regression. Models containing peNDF terms had similar or lower prediction accuracy and precision than did models without peNDF terms. peNDF models of ruminal pH did not differ substantially from paNDF models. All variables from the meta-analyses were P < 0.05, and variables that entered the ensemble models included mean PS (MPS), AF or DM proportions retained on 19- and 8-mm sieves of the PSPS, DMI, diet concentrations of forage, forage NDF, crude protein, starch, NDF, and the interaction terms of starch × MPS, ADF/NDF, and rumination time/DMI. The peNDF system predicted that the minimum proportion of material (DM basis) retained on the 8-mm sieve should increase with decreasing forage NDF or dietary NDF. To maintain ruminal pH, an ensemble of peNDF models predicted that the minimum proportion of DM retained on the 8 mm sieve should increase with decreasing forage NDF or NDF; the minimum proportion of DM on the 8 mm sieve should increase as dietary starch increased, but also depends on the proportion of DM on the 19 mm sieve. Results of this study agree with and quantify known interrelationships between the chemical and physical forms of diets that affect ruminal pH in dairy cows, and offer the potential to generate feeding recommendations.

Key Words: ensemble models, particle size, effective fiber

T285 Effects of virginiamycin supplementation on milk yield and its composition in high-producing dairy cows. P. M. Souza1, J. K. Poncheki1, L. Barbosa2, D. P. D. Lanna1, and R. Almeida*1, 1Universidade Federal do Paraná, Curitiba, PR, Brazil, 2Phibro Animal Health, Campinas, SP, Brazil, 3Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, SP, Brazil.

The objective of this study was to evaluate the effects of antimicrobial virginiamycin on milk production and composition in high-producing dairy cows. In a commercial herd in Castro, Paraná State, Southern Brazil, 180 multiparous Holstein cows, bST-treated and milked 3x/day, were blocked by parity, milk yield and DIM. Within each block, cows were randomly allocated to 2 groups; control (CT) and treatment (VM), with VM group being supplemented with 300 mg/cow/day of virginiamycin (V-Max, Phibro Animal Health), and CT group receiving the same amount of corn meal. In the 5-d covariable period, cows had on average 2.9 ± 1.0 lactations, 111 ± 59 DIM, and 51.2 ± 6.4 kg/d milk yield. The experimental period had 6 weeks, and 3 milk collection periods were performed, totaling 45 milk samples for each cow. The corn silage and ryegrass haylage based-diet had on average 17.8% CP, 37.8% NDF, 17.7% ADF, 5.0% fat, and 7.4% ash. Milk composition (fat, protein, casein, lactose and total solids contents), as well as MUN and SCC were determined. Data were analyzed using the MIXED procedure of SAS with a model containing the effects of block, cow within treatment, period (wk 3 and 6), day, and treatment. Milk yield and its composition in the covariable period were included in the model as covariate. Virginiamycin supplemented cows produce more milk (50.34 vs. 49.31 ± 0.34 kg/d; P = 0.03), with higher milk protein (2.98 vs. 2.93 ± 0.01%; P < 0.01) and milk casein (2.32 vs. 2.27 ± 0.01%; P < 0.01) contents than control cows. Virginiamycin treated cows also show higher milk fat (1.704 vs. 1.640 ± 0.020 kg/d; P = 0.02), milk protein (1.471 vs. 1.424 ± 0.012 kg/d; P < 0.01), milk casein (1.143 vs. 1.106 ± 0.009 kg/d; P < 0.01), milk lactose (2.341 vs. 2.288 ± 0.017 kg/d; P = 0.03), and milk total solids (5.897 vs. 5.753 ± 0.051 kg/d; P < 0.05) amounts. No differences (P > 0.05) were found for milk fat, lactose, and total solids contents, as well as MUN and SCC linear score between controls and VM-treated cows. This work demonstrated virginiamycin improves milk production and milk composition, and it could become an important additive for lactating dairy cows.

Key Words: antimicrobial, milk composition

T286 Evaluation of chemical composition and in vitro protein and fiber digestibility of corn dried distillers grains with solubles originating from seven sources. E. Dufour1, J. Judy1, K. Herrick2, and P. Kononoff2, 1University of Nebraska-Lincoln, Lincoln, NE, 2Poet Nutrition LLC, Sioux Falls, SD.

The chemical composition and nutrient digestibility of corn dried distillers grains with solubles (DDGS) may vary by source. The objectives of this study were to characterize chemical composition and evaluate differences in digestibility of DDGS originating from 7 different dry milling facilities. Data were analyzed as a completely randomized design assuming the experimental unit was production site, which was replicated by collecting 4 independent samples over an approximate 1-mo period. Significant (P < 0.05) differences between sources were observed in crude protein (ranging from 29.6 to 31.2 + 0.32%), NDF (ranging from 29.4 to 32.7 + 0.52%), ash-free NDF on an organic matter basis (ranging from 28.98 to 32.25 + 0.53%), and crude fat (ranging from 5.53 to 7.52 + 0.27%). Rumen-undegradable protein (RUP) and its intestinally digestible fraction (dRUP) were determined according to the methods described by Ross et al. (2013). Additionally, total-tract NDF digestibility (TTNDFD) was tested using the methods described by Combs (2013) which included fermentations at 24, 30, 48, and 240 h. Significant differences (P < 0.01) in RUP and dRUP were observed across sources ranging from 65.3 to 89.1 + 2.77% of CP and 56.4 to 77.5 + 0.24% of CP for RUP and dRUP respectively. As determined by 240 h fermentations the proportion of potentially digestible NDF also differed (P < 0.01) by source and ranged from 83.0 to 86.8 + 0.40% of NDF. Greater differences (P < 0.05) were observed between sources in TTNDFD which ranged from 60.0 to 70.5 + 1.59% of NDF across sources with rate of potentially digestible NDF ranging from 4.79 to 6.82 + 0.48%/h. Results indicate that both nutrient composition and digestibility vary across production sites of DDGS. Fiber and protein digestibilities should be analyzed to ensure appropriate feeding and inclusion of DDGS in high-producing dairy diets for optimal performance.

Key Words: DDGS, intestinal digestion, rumen NDF digestion

T287 Effects of DHA and ARA on performance, nutrient metabolism, and activation of the immune system in Holstein heifers. C. F. Vargas-Rodriguez, K. E. Olagaran*, R. Rusk, L. K. Mamedova, J. L. McGill, and B. J. Bradford, Kansas State University, Manhattan, KS.

Polysaturated FA have modulatory effects in a wide range of biological functions. The objectives for this study were (1) to determine if docosahexaenoic (DHA) or arachidonic acid (ARA) affect pre-weaning glucose and lipid metabolism, and (2) to determine if altered supply of these n-3 and n-6 polysaturated fatty acids (PUFA) in milk replacer modulates the immune response to vaccination. Thirty newborn Holstein heifers were blocked by date of birth and, within block, randomly assigned to
5 treatments: control (choice white grease), low dose ARA (1.6 g/d), high dose ARA (4.8 g/d), low dose DHA (1.6 g/d) and high dose DHA (4.8 g/d). Treatments provided approximately 1% added lipid (DM basis), with choice white grease added to ARA and DHA treatments. Treatments were blended into a milk replacer that was 28% CP and 20% fat (DM basis) and 1.89 L was fed 3 times/d until weaning at 63 d. Multivalent vaccines were administered on approximately d 49 and 63. Data were analyzed with mixed models including the fixed effects of treatment, time, and their interaction, and the random effect of block and heifer within block. DMI, ADG and feed efficiency were not affected by treatments, but ARA decreased hip height growth and increased the incidence of abnormal fecal consistency ($P < 0.05$). Glucose and insulin concentrations were greatest for control and least for the low PUFA doses ($P < 0.05$). Vaccination response was assessed by plasma TNF-α and haptoglobin concentrations 24-h post-vaccination, BRSV and BVD1/2 antibody titers at 2 wk, and proliferation of CD4+ T helper cells and CD8+ cytotoxic T cells at 6 to 10 d; none of these responses different by treatment ($P > 0.10$). The supplementation of either PUFA modulated glucose metabolism, ARA had subtle negative impacts on digestive health and skeletal growth, but neither PUFA source significantly altered the immune response to vaccination in pre-weaning calves.

**Key Words:** inflammation, pre-ruminant, polyunsaturated fatty acid

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**T288** Maternal ethyl-cellulose rumen-protected methionine supplementation alters blood biomarkers and immune function in neonatal Holstein calves. A. S. Alharthi¹, F. Batistel¹, C. Parys², A. Helmbrecht², M. A. Ballou³, E. Trevisi⁴, and J. J. Loor¹, ¹University of Illinois at Urbana-Champaign, Urbana, IL, ²Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany, ³Department of Animal Sciences, Texas Tech University, Lubbock, TX, ⁴Istituto di Zootecnica, Facoltà di Scienze Agrarie Alimentari ed Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy.

The objective of this study was to investigate the effect of ethyl-cellulose rumen-protected methionine supplementation during late-pregnancy of dams on blood markers and neutrophil function of calves. We used 16 Holstein heifer calves born to cows receiving during the last 4 weeks of pregnancy a control diet (CON; 1.47 Mcal/kg dry matter and 15.3% crude protein) or CON plus ethyl cellulose rumen-protected methionine (MET; Mepron®, Evonik Industries AG, Germany). After birth, calves (n = 4 in each group) were randomly allocated considering dam treatment andcolostrum as follows: calves from CON cows and colostrum from CON cows; calves from CON cows and colostrum from MET cows; calves from MET cows and colostrum from MET cows; and calves from MET cows and colostrum from CON cows. Blood was collected from the jugular vein at birth (before receiving colostrum, 0 d), 24 h after first colostrum and at 7, 21, 42 and 50 d of age. Birth body weight did not differ among calves. At birth calves from dams fed MET had a tendency for greater creatinine ($P = 0.10$; 248 vs 204 μmol/L ± 17), NEFA ($P = 0.15$; 1.22 vs 0.99 mmol/L ± 0.11), and albumin ($P = 0.13$; 30.6 vs 29.2 g/L ± 0.43). Over time, whole blood neutrophil phagocytosis in response to an in vitro challenge increased (MET × day, $P = 0.02$) to a greater extent in MET calves. Overall, MET calves had greater (0 to 52 d) neutrophil oxidative burst ($P = 0.09$; 61 vs 53% ± 3). Furthermore, these calves had lower concentration of nitric oxide ($P = 0.03$; 146.9 vs 166.8 μmol/L ± 5.9) and lower ($P = 0.05$; 147.2 vs 165.0 μmol/L ± 5.9) plasma ferric reducing ability (an antioxidant capacity assay). Regardless of cow diet, all heifers receiving colostrum from MET cows had overall greater ($P = 0.05$; 7.1 vs 6.4 mmol/L ± 0.25) blood glucose and lower ($P = 0.03$; 147 vs 167 μmol/L ± 5) nitric oxide concentration. Our results highlight the potential role of MET supply during late-gestation on oxidative stress and immune function during early life. The link between these factors and growth performance remain to be elucidated.

**Key Words:** fetal programming, nutrition, nutrigenomics
During the anestrus season, the use of progesterone (P4) priming followed by human chorionic gonadotropin (hCG) 24 h later, has shown to stimulate follicular growth and estrus behavior in goats. Hitherto, the possible effect of such estrus induction protocol has not been carried out in goats with different breeds. The study was performed during de anestrus season (May, 26° N), and considered the Alpine (n = 15; 37.4 ± 8.5 kg) and Criollo (n = 15; 39.7 ± 4.3 kg) goats; none of the goats presented corpus luteum at the onset of the treatment. Both groups received 20 mg of P4 (0.4 mL -1 i.m., Progesteron, Zoetis, Mexico) plus 100 IU of hCG (0.1 mL -1 i.m., Chorulon, Intervet, Mexico) 24 h later. Estrus behavior was evaluated twice a day through 10 min during 5 d with the use of 3 aproned sexually active males treated with testosterone; males were in contact with females only during the estrus detection session. Ovulation was quantified through transrectal ultrasonography scanning (Aloka 500, 7.5 MHz) every 12 h up to 120 h after hCG administration. Ovulation was considered to occur when a preovulatory follicle (= 6 mm) present in the previous day disappeared in the subsequent ultrasonographic examination. While both estrus response and ovulation were analyzed with a Fisher exact test, the estrus interval and the time to ovulation were analyzed with an independent t-test; ovulation rate between breeds was compared with the Kruskal-Wallis test. No differences (P > 0.05) in estrus response were detected between groups; goats from both breeds showed estrus behavior and ovulation (100%). No differences (P > 0.05) regarding the interval period to standing estrus (60 ± 8 and 54 ± 6 h), time to ovulation (92 ± 11 and 91 ± 10 h) and ovulation rate (1.5 ± 0.5 and 1.4 ± 0.5) were detected after hCG administration between the Alpine and Criollo does, respectively. Although the number of replicates within breed is limited, results of our study established no differences between goats upon estrus response and synchronization of ovulation. This confirms the results of Alvarado-Espino et al. (Anim Reprod Sci. 2016) that 100 IU hCG is the appropriated dosage to obtain 100% oestrus in goats.

Key Words: follicular growth, estrus, gonadotropin

**Small Ruminant II**

**T289** Estrus and ovarian response in Alpine and Criollo goats primed with progesterone plus human chorionic gonadotropin in anestrous period. A. S. Alvarado-Espino1, C. A. Meza-Herrera2, E. Carrillo1, R. Rivas-Muñoz2, O. Ángel-García1, S. Moreno-Avalos1, M. A. De Santiago-Miramontes1, and F. G. Véliz1,1 Universidad Autónoma Agraria Antonio Narro, Torreón, Coahuila, México, 2Universidad Autónoma Chapingo, Bermejillo, Durango, México, 3Instituto Tecnológico de Torreón, Torreón, Coahuila, México.

The objective of this study was to evaluate the reproductive performance of adult bucks with different body condition induced exogenously to sexually activity. This study was carried out in the north of Mexico (26°N, 104°W) using 12 confined adult male goats, divided into 2 groups (n = 6 each) fed for 9 weeks (February–April 2016) on a low diet (LT group) containing 7.7% CP and 2.2 Mcal EM/kg and high (HT group), which contributed 11.9% CP and 2.5 Mcal EM/kg. To HT group was added 400 g/d commercial concentrate (18% CP) from the 5th to 9th week. Both groups received 25 mg of testosterone, via IM each third day for 3 weeks (13th April to May 1st). Body condition score (scale 1–4), body weight (kg) intensity of sexual odor (scale 1–3), scrotal circumference (cm) and seminal quality parameters: ejaculate volume (ml), sperm concentration (millions/mL), motility (scale 1–5) and viability (%) were evaluated weekly, these data were analyzed with an ANOVA test, in SAS 2002, through an experimental design of random blocks. On April 30 and May 1, a sexual behavior test was performed. Each male was evaluated individually quantifying sexual behaviors (search and consummation). These data were compared using a Chi-squared test. There were differences between groups (P < 0.05) in: body weight (58.5 ± 0.6 vs. 61.9 ± 0.6), body condition score (1.4 ± 0.03 vs. 2.4 ± 0.03), scrotal circumference (27.6 ± 0.7 vs. 30.1 ± 0.7), volume of ejaculate (0.53 ± 0.07 vs. 0.91 ± 0.07), sperm concentration (3322.7 ± 256.47 vs. 3787.2 ± 256.47), motility (1.7 ± 0.19 vs. 2.7 ± 0.19), viability (48.2 ±
4.7 vs. 68.5 ± 4.7). Nevertheless, the intensity of sexual odor was not different (0.18 ± 0.03 vs. 0.30 ± 0.03). Sexual behavior of search was lower (P < 0.001) in the LT group than in the HT group (42.3% vs. 57.6%), as well as the consummation behavior (P = 0.006; 27.7% vs. 72.2%). We conclude that a good body condition score at the end of the sexual resting period in bucks, increased body weight, scrotal circumference, sperm quality and the sexual behavior which can be an important factor to induce female goats to ovulate through the male effect.

**Key Words:** male goat, food level, sexual response

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**T292**  
**Effects of forage to concentrate ratio in dairy ewes in early lactation: 2. Milk fatty acid profile and cheese-yielding traits.**  
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As the second part of the study reported by Elhadi et al. (Abstract M331), the effects of forage:concentrate ratio (F:C, %) were studied in 72 dairy ewes (Manchega, MN, n = 36; Lacaune, LC, n = 36) in early lactation. Treatments were: high- (HF, 70:30), medium- (MF, 55:45) and low-forage (LF, 40:60). Ewes were fed indoors, in pens of 6, the HF diet during 4 wk and then the experimental diets (wk 5 to 8). Milk was sampled individually on wk 7 for composition (NIR system; Foss, Nordersted, DE) and coagulation traits (Optigraph; Ysebaert, Frepillon, FR). Tank milk samples were collected by group and fat extracted (2000 g, 15 min, 4°C) and converted to FAME by base catalyzed transesterification for FA analysis by gas-chromatography. Milk composition varied by breed, being richer for main components in MN than in LC (P < 0.001), with no dietary effects on fat, protein and solids contents (P = 0.95 to 0.052), except for firmness at min 45 and coagulation time, rate of curd aggregation and laboratory cheese-yield index 2.50. Nevertheless diet × breed interactions were detected in most milk components and FA profile data (P = 0.01 to 0.001). Regarding the extreme diets (HF vs. LF), no effects were observed in the FA saturation profile in MN ewes, whereas SFA decreased (~2%, P < 0.001) and MUFA (4%, P = 0.004) and PUFA (12%, P < 0.001) increased in LC ewes. Furthermore, comparing HF vs. LF, the C 16 increased in the LC (2%; P = 0.031), whereas C 16 and C 16 decreased (~2% and ~5%, respectively; P < 0.05) and C < 16 increased, in the MN (6%; P = 0.010). Changes in atherogenicity index were negligible. No effect of concentrate was detected on rumen coagulation time, rate of curd aggregation and laboratory cheese-yield in both breeds (P = 0.95 to 0.052), except for firmness at min 45 and rate of curd aggregation, that increased (23% and 47%, respectively; P < 0.05) in the HF vs. MF comparison in MN ewes. In conclusion, slight changes in FA profile, and no benefits in cheese traits, were detected when the ratio forage:concentrate fell below the level required to satisfy the nutrition requirements of medium and high yielding dairy ewes.

**Key Words:** fatty acid, cheese, curd

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**T293**  
**Out of breeding season sexual biostimulations of Dorper rams improve sexual behavior but not the male effect.**  
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The aim was to evaluate if the contact of sexually inactive rams with estrogenized females induces the male sexual behavior during the reproductive resting season (May) and if these treated rams are, in turn, able to stimulate the reproductive response of anovulatory nulliparous ewes in the northern Mexico (26°N). Dorper rams (n = 9) were divided into 3 groups (n = 3 each) with homogeneous live weight and body condition score and exposed during 6 d to: 1) 2 untreated ewes during 24 h (CG); 2) 2 estrogenized ewes (2 mg i.m. of estradiol cypionate every 3 d for 9 d) during 12 h daily (G12); 3) 2 estrogenized ewes for 24 h, replaced by other 2 ewes every 24 h (G24). Once rams were exposed to the “female effect”, 60 nulliparous ewes (diagnosed anovulatory by transrectal ultrasonography) were divided into 3 groups (n = 20 each) and were exposed to the previously treated rams. During the first 2 d, both the appetite sexual behavior (ASB; i.e., anogenital sniffing, body sniffing, vocalization, kicking and flehmen) and the consummatory sexual behavior (CSB; attempt mount and complete mount) were evaluated 1 h daily. Estrus were monitored twice daily for 5 d to detect estrus signs, and ovulation rate was determined by transrectal ultrasonography. The ASB and CSB frequencies were compared using a Fisher’s test; estrus and ovulatory activities were compared using javascript:il chil2(Systat 12). Groups CG, G12 and G24 showed ASB values of 29, 35 and 35%, respectively (P > 0.05). Values for CSB in the CG were 17% (P > 0.05), observed the highest in G12 and G24 (38 and 44%; P < 0.05). No differences (P > 0.05) were detected regarding the percentage of females showing estrus, with 85, 71 and 93% in the CG, G12 and G24 groups. In the same way, the percentage of ovulations did not differ (P > 0.05) among groups (84, 71 and 86%, for the CG, G12 and G24). This study showed that the sexual biostimulation of Dorper rams with estrogenized females during 12 or 24 h generated a high consummatory sexual behavior. When these rams exposed to the “female effect” were put in contact with anovulatory nulliparous ewes, the presence of the treated males did not promote any difference regarding the observed reproductive outcomes, considering estrus response and ovulatory activity.

**Key Words:** biostimulation, behavior, ewes

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**T294**  
**Milk fatty acid profile of dairy ewes fed contrasting sources of energy supplementation.**  
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Polyunsaturated fatty acids are biohydrogenated in the rumen producing diverse intermediates according to diet. Thus, milk fatty acid (FA) composition could be affected by diet manipulation with special attention on those FA with positive effects on human health. The aim of the study was to evaluate the effect of 2 contrasting sources of energy supplementation (soybean hulls and corn grain) on milk FA profile of dairy ewes fed fresh ryegrass. Ten mature dairy ewes (30 DIM; 65.8 ± 8.67 kg) were assigned to 2 treatments in a complete randomized design: SH (soybean hulls) and CG (corn grain). Daily ration was offered at 4% BW in a 50:50 forage to concentrate ratio and balanced with soybean meal to meet 16% CP. Milk samples of 50 mL were collected at each milking (0800 and 1600 h) during 7 consecutive days, after a diet adaptation period of 14 d. Samples were pooled per animal and a 50 mL subsample was taken for analysis. Fat extraction was assessed for further FA methylation and gas
chromatography analysis. Data were analyzed in a complete randomized design using the MIXED procedure (sheep was included as a random effect) and means compared by Tukey test ($P < 0.05$; SAS Institute, 2008). No differences were observed for SCFA, MCFA, C16:0, C16:1, C18:0, C18:1c9, C18:1t10, C18:1t11, C18:2c9t11 and C18:2t10c12 ($P > 0.05$), although the supplement had a significant effect on linoleic and linolenic acid, being 3.38 vs. 1.86 g/100g FA and 0.71 vs. 0.42 g/100 g FA in SH and CG, respectively ($P < 0.05$). Despite having similar milk FA profiles, PUFA concentration was higher in SH than CG (6.77 vs. 4.49 g/100 g FA; $P < 0.05$). The C20:5 did not differ between treatments ($P > 0.05$) whereas C22:6 tended to be higher in SH than CG (0.043 vs. 0.031 g/100 g FA; $P < 0.1$). The SH showed higher n-3 (0.89 vs. 0.58 g/100g FA) and n-6 (3.43 vs. 1.94 g/100g FA) concentrations ($P < 0.05$). Regarding the delta-9-desaturase and atherogenicity indexes, no effect was obtained ($P > 0.05$). The results indicate that SH constitute a promising supplementation strategy for dairy ewes to obtain higher PUFA and n-3 levels in milk.

Key Words: milk fatty acids, energy supplementation, sheep
Dairy cattle have changed considerably in the past 100 years. They now are generally taller and thinner with larger udders that produce considerably more milk. Genomic selection and the ability to modify specific genes will enable us to make changes even faster in the future. The discipline of animal breeding is generally concerned with genetic change; however, all disciplines must adapt to the animals that are produced. Moreover, dairy cattle genetics are related across countries. Thus, it is imperative that we consider the future of the dairy cow from a multidisciplinary and international approach. Although the presentations in this symposium are led mostly by geneticists, this symposium is for all dairy scientists, especially for graduate students in the production division. You are the ones who will discover how to best feed and manage the cows of the future!

Key Words: genomics, selection, dairy production

Dairy-based foods will increase in importance in human diets because of dairy’s role in meeting protein needs sustainably. Dairy enterprises will relocate to regions that have adequate rainfall or water resources and suitable climates. Technologies that will be used have emerged conceptually at basic scientific levels. Dairy enterprises will use laterally-integrated systems comprising physically-separated facilities for pre-weaned heifers, replacement heifers, early dry cows, transition cows, milk cows and dairy beef. Each unit will be managed as if it were a superorganism. Scale of dairy enterprises will increase and manual labor will decrease through automation, robotics and sensors. Resources will be harvested from manure and reused. Perennial crops, including perennial maize and high-starch energy grasses, will replace annual maize as major feed sources. Dairy enterprises will be subjected to more regulations and will put greater emphasis on sustainable agro-ecological systems. Milk output will be contracted with processors and manufacturers, and milk will be separated automatically into pools at farms according to processing characteristics. Dairy cows worldwide will be gene-based rather than breed-based and will comprise genes that have been edited, synthesized or transferred. Cows will be smaller and healthier and selected for their environmental region. Milk yield will exceed 25,000 kg per cow per year in North America. Genetic introductions into herds in Australia, genetic improvement, selection index

Selective breeding has been practiced since domestication, but early breeders commonly selected on appearance (e.g., coat color) rather than quantitative phenotypes (e.g., milk yield). A breeding index converts information about several traits into 1 number used for selection and also to predict an animal’s own performance. Calculation of selection indices is straightforward when phenotype and pedigree data are available. Prediction of economic values 3 to 10 years in the future, when the offspring of matings planned using the index will be lactating, is more challenging. The first USDA selection index included only milk and fat yield, while the latest version of the lifetime net merit (LNM) index includes 13 traits, with some traits actually composites of other traits. Selection indices are revised to reflect improved knowledge of biology, new sources of data, and changing economic conditions. Single-trait selection often suffers from antagonistic correlations with traits not in the selection objective. Multiple-trait selection avoids those problems at the cost of less-than-maximal progress for individual traits. How many and which traits to include is not simple to determine because traits are not independent. Many countries use indices that reflect the needs of different producers in different environments. While the emphasis placed on trait groups differs, most indices include yield, fertility, health, and type traits. Addition of milk composition, feed intake, and other traits is possible but are more costly to collect, and many are not yet directly rewarded in the marketplace, such as with incentives from milk processing plants. As the number of traits grows there is increasing interest in custom selection indices for closely matching genotypes to the environments in which they will perform. Traditional selection required recording lots of cows across many farms, but genomic selection favors collecting more detailed information from cooperating farms. A similar strategy may be useful in less developed countries. Recording important new traits on a small fraction of cows can quickly benefit the whole population through genomics. Gene editing may be used to increase the frequency of high-value Mendelian traits, such as polled.

Key Words: dairy cow, genetic improvement, selection index

Genomic selection has opened up opportunities for developing new breeding values that rely on phenotypes that use dedicated reference populations of genotyped cows. There are also opportunities to advance phenotype collection through automation and identifying predictor traits that can be measured cost-effectively. One model is to identify the best phenotypes to measure in research herds and then increase observations (perhaps using predictors) in genotyped commercial herds. Further advances in the accuracy of genomic prediction can be gained from the use of sequence data, in addition to gene expression studies, which can lead to improved persistence of genomic breeding values across generations. In Australia integrating data collection with a research and implementation platform is the platform for delivering new methodologies and breeding values. For example, we have recently delivered the Feed Saved breeding values to industry and are soon to deliver genomic
breeding values for Heat Tolerance. Identifying traits to include in the national objective will be the focus of future breeding value research, such as expanding the number of health traits breeding values available. However, industry, market and social drivers may see the emergence of new breeding values, such as cow level methane emissions, gestation length or niche milk products. To date, selection objectives have been similar globally, but it is possible that they may diverge into the future. Selection index methodology is still needed to ensure that the weights on each trait in the index are appropriate, although the weights are subtly altered to respond to respective industry and consumer requirements. So far nationwide indices remain standard practice, but this may change in the future, especially as tools to deliver information back to farmers become more sophisticated. Already bull selection tools and personalised genetic trends are available, but the capture of economic and farm data will see the emergence of even more tools. Increasing the rate of genetic gain in the genomics era remains a challenge in Australia, so industry engagement is paramount.

Key Words: genomic selection, novel traits, selection index

Building a better cow. How can we be sure she is adaptable? D. P. Berry*, Teagasc Moorepark, Fermoy, Co. Cork, Ireland.

Intuition suggests that if a trait is under genetic control, then selection for improved performance will increase the frequency of the genetic variant conferring that advantage (as well as co-inherited genetic variants). If selection persists then eventually all of the individuals carrying the unfavorable variant may be culled thus resulting in only the one variant of the mutation in the population and thus logically an exhaustion of genetic variability. Because sustainable genetic gain, or the ability to adapt to a changing environment (in light of changing economic and social policy as well as changes in weather and climate), is predicated on the presence of genetic variability, this expected loss in genetic variation, suggest that the response to selection or ability to adapt genetically will reduce and eventually halt. Although genetic variance is expected to reduce in the initial generations of selection, empirical evidence, in general, does not support the thesis of an eventual exhaustion of genetic variability; arguably the most recognized evidence originates from the Illinois corn lines selected for high and low content of oil in the kernel. The quantity of exploitable genetic variation in a population is dictated by evolutionary forces such as selection, migration, mutation and genetic drift. The extent of variability introduced by mutations is actually high and is thought to represent 0.1% of the environmental variance; this is equivalent to approximately 0.3% for a trait with a coefficient of genetic variability of 10%. Moreover, developments in genomic tools and approaches, both through whole genome selection and genome editing has the potential to reduce the demise of standing genetic variation and even (strategically) introduce genetic variability. For example, genome editing has recently been used to edit the CD163 gene in pigs to increase resistance to the recently isolated arterivirus porcine reproductive and respiratory syndrome (PRRS). Reality, however, does not always reflect potential, and pressures to capture market share may unduly place greater emphasis on short-term genetic gain to the determinant of long-term gain.

Key Words: genetic, evolution, dairy
Animal Health III

277 Management practices for male calves on Canadian dairy farms. D. L. Renaud*, T. F. Duffield, S. J. LeBlanc, D. B. Haley, and D. F. Kelton, Department of Population Medicine, University of Guelph, Guelph, ON, Canada.

Morbidity, mortality, and antimicrobial use and resistance are major concerns in the rearing of male dairy calves, so information to support disease prevention is important. The objective of this cross-sectional study was to describe management practices associated with the care of male calves during their first days of life on Canadian dairy farms. A survey completed by dairy producers across Canada from March 1 to April 30, 2015. The survey had 192 questions covering producer background, farm characteristics, biosecurity practices, disease prevalence, calf health, animal welfare, lameness, milking hygiene, reproduction and internet/social media use. A total of 1,025 surveys were completed online, by telephone, or by mail, representing 9% of all dairy farms in Canada. Five percent of respondents (n = 49) answered that they had euthanized at least one male calf at birth in the previous year and blunt force trauma was used commonly in these cases. The majority of respondents always fed colostrum to male calves, however 9% (n = 80) did not always feed colostrum. Nearly 40% (n = 418) of respondents reported always dipping the navels of male calves, 12% (n = 123) vaccinated male calves and 17% (n = 180) did not provide the same quantity of feed to male calves as heifer calves. The care of male calves differed greatly depending on geographical region of the respondents. However, some regional effects may be confounded by economic conditions and the logistics of marketing male dairy calves in different parts of the country. Herd size was another important variable, with larger herd size being associated with using an appropriate method of euthanasia at birth, but with larger farms also being less likely to always feed colostrum to their male calves, or feed them the same as female calves. Familiarity with the Code of Practice for the Care and Handling of Dairy Cattle by respondents was associated with better care of male calves on dairy farms. The results of this survey suggest that there is variable treatment of male dairy calves on Canadian dairy farms and that there are opportunities to improve health management of male calves on the farms of origin.

Key Words: male calf, management, mortality


The objective of this study was to determine neonatal management risk factors on dairy farms associated with mortality on veal farms. From April to October 2016, 52 dairy farms that supply male calves to 2 veal operations were visited. During the visit, a questionnaire was administered covering all areas of calf management, calves 1–10 d of age were examined using a standardized health scoring system and blood was collected to test for passive transfer of immunoglobulins. The veal calf mortality risk for each dairy farm was calculated based on the number of calves shipped and the number that died during 2016. To adjust for differences in calf mortality at the 2 veal operations, a facility-adjusted mortality rate was calculated for each dairy farm, and the farms were classified as high or low mortality farms. Using the high and low mortality outcome, a logistic regression model was used to assess farm risk factors. Suppliers to veal farm 1 had a mean mortality risk of 9% in 2016 and 39% of calves (n = 27) examined on the dairy farms supplying veal farm 1 had an identifiable health abnormality. In contrast, suppliers to veal farm 2 had a mean mortality risk of 3% in 2016 and 29% of calves (n = 33) examined on the dairy farms supplying veal farm 2 had an identifiable abnormality. The proportion of calves with failure of passive transfer on all dairy farms was very low, with only 13% of calves (n = 24) tested having a serum IgG < 10mg/ml. Feeding stored (refrigerated or frozen) colostrum (P = 0.03) compared with fresh colostrum, bedding male calves on wood shavings (P = 0.03) or chopped straw (P = 0.04) compared with long straw on the source dairy farm, and the herd veterinarian not frequently asking about the health and performance of the calves (P = < 0.01) were all positively associated with the dairy farm being classified as a high mortality farm. Checking the calving pen less frequently during the day (P = 0.02) compared more frequently during the day was negatively associated with being classified as a high mortality dairy farm. The results of this study suggest that male calf management practices on dairy farms have an impact on mortality during the growing period on veal farms.

Key Words: male calf, management, mortality

279 Aluminized reflective covers: Effect on calf behavior, health, and performance during summer. D. Manriquez*1, H. Valenzuela2, S. Paudyal1, A. Velasquez1, J. Velez2, and P. Pinedo1, 1Colorado State University, Fort Collins, CO, 2Aurora Organic Dairy, Boulder, CO.

Our objective was to evaluate the effect of commercial aluminized reflective covers (ARC) for polyethylene hutches on behavior, health, and performance of pre-weaned Holstein calves during summer in Northern Colorado. Hutch interior THI and inner wall temperature were also assessed. Heifers (n = 195) enrolled at 1d of life and housed in individual hutches with (n = 97) or without (n = 98) ARC were monitored until 60d of life. Behavior, health, rectal temperature, respiratory rate, and hutch wall temperature were measured twice per week between 12:00 and 2:00 p.m. Calves were weighed at birth and at 60d. Ambient and hutch temperature and humidity were continuously recorded using automatic loggers (4 covered and 4 control empty hutches). Fecal, nasal, eye, ear, and hydration abnormalities were assessed using a health score system. Days were categorized according to max ambient THI as low (<72 units) and high. Behavior and health scores were analyzed using logistic regression analysis. Temperature, THI, rectal temperature and respiratory rate were evaluated as repeated measures. Use of ARC and ambient THI were associated with calf behavior (P = 0.02 and P < 0.0001): The odds of remaining inside the hutch were 1.3 times greater for calves in control hutches vs. those in covered hutches. When THI >72 the odds of remaining inside of the hutch were 2 times greater. Health scores were not associated with use of ARC. Rectal temperature and respiratory rate did not differ among calves in covered and control hutches (39.2 ± 0.02 vs. 39.2 ± 0.02°C and 61 ± 1.2 vs. 58 ± 1.2 breaths/min). ADG did not differ between covered and control hutches (0.54 ± 0.02 vs. 0.53 ± 0.02 kg/day, respectively). The average ambient THI was 66.8 (min 43.2, max 81.1 units). THI was greater in covered (64.8 ± 0.05) compared with control (64.1 ± 0.05) hutchs (P < 0.001). Inner wall temperature was lower in covered vs. control hutches by 1°C (P < 0.001). Even though ARC effectively reduced wall temperature, this effect was not sufficient to have a positive impact on calf health and performance under these study settings. Higher THI in covered hutches...
may explain differences in behavior, where control calves preferred to stay inside the hutches on high THI periods.

**Key Words:** reflective, cover, calf

### 280 Associations of management practices and calf health on dairy farms using automated milk feeders in southern Ontario.

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Data on management practices with automated milk feeders (AMF) are needed to identify determinants of calf health and welfare. Seventeen dairy farms with AMF in Ontario (Canada) were visited 4 times, seasonally, over 1 year. All calves (n = 1488) in pens (n = 35) with AMF were health scored to identify number of calves with diarrhea (CD) and bovine respiratory disease (BRD). Data on calf, feeder, and pen management practices were analyzed using mixed-effects negative binomial regression models. Overall calf-level prevalence of CD and BRD were 23% and 17%, respectively. Median (IQR) within-pen prevalence of CD and BRD was 17% (7–37) and 11% (0–28), respectively. Median age (IQR) for diarrheic calves was 25 d old (12–42), while for calves with BRD was 43 d old (21–60). Predictors associated with reduced within-pen prevalence of CD were the feeding of probiotics (risk ratio [RR] = 0.5, P = 0.01), cleaning feeder hoses daily compared with weekly (RR = 0.53, P < 0.03), and individual-housing (vs. group-housing) before introduction to the AMF pen (RR = 0.6, P = 0.07). In contrast, use of maternity pens other than for calves tended to be a risk factor (RR = 1.6, P = 0.07). Predictors associated with increased within-pen prevalence of BRD were sharing air with cattle 5–8 mo old (RR = 3.4, P = 0.01) or 1+ year (RR = 2.0, P = 0.07), and introduction to the AMF pen after 9 d old compared with < 3 d (RR = 2.1, P = 0.04) or 3 to 6 d (RR 2.0, P = 0.02). Increased total solids in milk replacer (RR = 0.93, P = 0.04) and dry top bedding over a wet bedding pack no deeper than 5 cm (RR = 0.6, P = 0.04) were protective. To conclude, isolation from older animals, and frequent cleaning of the feeder and pen may help reduce disease in group-housed calves fed with an AMF.

**Key Words:** male calf, health screening

### 282 Behavior activity detected via 3D acceleration before diarrhea events in neonatal dairy calves. J. F. Castillo*1,2, F. Rosa2, J. J. Loor3, J. S. Osorio2, and F. C. Cardoso3, 1Escuela Agrícola Panamericana El Zamorano, El Zamorano, Honduras, 2South Dakota State University, Brookings, SD, 3University of Illinois, Champaign-Urbana, IL.

Diarrhea is the single most common source of morbidity and mortality in preweaning dairy calves with significant economic losses for the dairy industry. Therefore, automated in-line systems that can identify calves at risk to develop diarrhea can have a substantial impact in diminishing the negative impacts of this disease. Therefore, the aim of this experiment was to assess various behaviors (e.g., standing and lying time) via the use of 3D accelerometers (Onset; Pocasset, MA) mounted in the rear left leg of neonatal dairy calves during a diarrhea event. Twenty-eight Holstein neonatal calves were housed in individual hutches from birth to 7 wk of age at the University of Illinois Dairy Cattle Research Unit. Milk replacer, starter, fecal score (FS), and rectal temperature was recorded daily. BW and withers height was measured weekly. Data loggers of 3D acceleration were set to record every 60-s during the trial. The overall fecal score (FS) for all calves reached a maximal point at 10 d (FS = 2.3 ± 0.10) or 2 wk of age, therefore, all the accelerometer data were analyzed within the first 15 d of life. Calves were classified by a retrospective analysis of FS as healthy (non-Scour; FS<i>2; n = 18) or scour (Scour; FS ≥ 2; n = 10). As expected FS was greater (P < 0.01) in the scour group (1.25 ± 1.60) from birth to 7 wk of age. Rectal temperature tended (P = 0.12) to be greater in the scour group in comparison to the non-Scour. Starter intake tended (P = 0.15) to be greater (0.76 vs 0.91 kg/d) in non-Scour calves. Although neither BW (P = 0.28) nor withers height (P = 0.27) were affected by scour, ADG tended (P = 0.10) to be greater (0.54 vs 0.65 kg/d) in the non-Scour group. Overall standing and lying time were not affected (P = 0.72) by diarrhea. Standing bouts tended (P = 0.15) to be greater in Scour calves, while a trend (P = 0.08) for greater left side lying duration was observed in non-Scour calves. Interestingly, the latter effects occurred mainly during the days (3–7 d) leading to maximal scour at 10 d of age. This suggests that these behaviors could be potentially used to identify calves at risk to develop diarrhea.

**Key Words:** accelerometer, behavior prediction, calf scour
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Real time determination of immunoglobulins levels in colostrum by using on-line computerized a herd management system.  
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Colostrum is the first mammary glands secretion at parturition. In ruminants, the neonates are born agammaglobulinemic because there is no Ig transfer through the placenta. Thus, passive immunization through the colostrum helps the newborn to contend pathogens and survive the first weeks of life. The gold standard of Ig and/or Ig isotypes: IgG1 & IgG2, IgM and IgA is their determination by an immunoassay such as ELISA. However, these assays require laboratory equipment, are time consuming and therefore not applicable under farm conditions. On site, rapid assays such as Colostrometer or Brix refractometer are used. To date, many dairy farms are equipped with on-line computerized data acquisition systems. The aim of the study was to calibrate data collected on-line for evaluating the level of immunoglobulins in the colostrum. The study included 4 dairy farms with parlors equipped with an on-line computerized AfiMilk Herd Management system including AfiLab milk spectrometer (AfiMilk, Israel). Samples were tested by Colostrometer and by Brix calibrated for Ig. Total IgG was determined by ELISA BIO k 165/2 kit (Bio-X Diagnostics S.A., 5580 Rochefort, Belgium).

The accuracy of the on-line AfiLab system compared to traditional ELISA methodology was evaluated by calculating the correlation coefficient (r) for A and B. The correlation coefficient for A and B was 0.88 for A and 0.53 for B. Assuming practice of dichotomic IgG cutoff at 50 mg/mL, the sensitivity was 88.2 and specificity 87.9%. In conclusion, it was possible to create a “bank” of ‘good’ colostrum by compiling the data obtained by the AfiLab analyzers, which is now implemented into the AfiMilk system.

Key Words: online analysis, colostrum, immunoglobulin G

284  
Validation of commercial luminometry swabs for enumeration of total bacteria and coliform counts in colostrum feeding equipment.  
D. L. Renaud, T. F. Duffield, D. B. Haley, S. J. LeBlanc, and D. F. Kelton, Department of Population Medicine, University of Guelph, Guelph, ON, Canada.

Colostrum feeding is an integral component of neonatal calf care with many effects on calf health and productivity, yet failure of passive transfer remains common on many dairy farms. A sufficient quantity and quality colostrum must be fed quickly to the newborn calf. Colostrum with a total bacteria count (TBC) > 100,000 cfu/mL may impair IgG absorption and contribute to disease. The objective of this study was to validate Hygiena AquaSnap (AS) and MicroSnap (MS) swabs for detection of elevated bacterial counts in colostrum-feeding equipment. AS and MS swabs offer a potential rapid calf-side alternative to traditional bacterial culture. The reagents in the swabs produce a light-generating reaction when in contact with bacterial adenosine triphosphate, which is quantified in relative light units (RLU) with a luminometer. From April to October 2016, 18 esophageal tube feeders, 49 nipple bottles and 6 pails from 52 dairy farms in Ontario were evaluated for cleanliness. Sterile physiological saline (15 mL) was poured into each piece of equipment, mixed for 2 min to ensure total surface coverage and poured into a sterile collection container through the feeding end. All wash fluid was split into equal aliquots, with one being evaluated by the by conventional culture and the other evaluated using both the AS and MS swabs. Non-parametric receiver operator curves were generated using STATA 14 for each of AS and MS, comparing the RLU to bacterial counts. The area under the curve (AUC) comparing the AS swab to TBC (cut point > 100,000 cfu/mL) was 0.89 (95% Confidence Interval (CI): 0.8–1) and using a cut point of 631 RLU correctly classified 84% of samples with a sensitivity of 88% and a specificity of 77%. The AUC comparing the MS swab to total coliform count (cut point > 100,000 cfu/mL) was 0.85 (95% CI: 0.7–1) and using a cut point of 44 RLU correctly classified 89% of samples with a sensitivity of 83% and a specificity of 90%. The results suggest that the AS and MS swabs can be used as an alternative to traditional lab bacterial counts to evaluate cleanliness of colostrum-feeding equipment.

Key Words: calf, colostrum, contamination
**286 Comparison of Johne’s disease prevalence on organic and conventional dairy farms in Pennsylvania.** M.-E. Fecteau*, T. L. Fyock, H. W. Aceto, H. J. Karremen, and R. W. Sweeney, Department of Clinical Studies-New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

Johne’s disease (JD), caused by Mycobacterium avium ssp. paratuberculosis (MAP), affects approximately 70% of all US dairies. To determine if JD prevalence on PA organic dairy farms is different than that of conventional dairy farms; to identify differences in management practices between organic and conventional farms; and to identify risk factors associated with a higher prevalence of JD. Fifty PA dairy farms (26 certified organic, 24 conventional) participating in DHIA testing were included. Individual milk samples were tested for MAP antibodies via ELISA. Information regarding management practices was gathered during a farm visit. Univariable statistical comparisons were made by use of logistic and linear regression. Multivariable analysis was employed to look for risk factors and associations. A total of 2,739 cows were included in the study. Organic herds had a median of 39 lactating cows (range, 20–211 cows); while conventional herds had a median of 58 lactating cows (range, 20–114 cows) (P = 0.02). Average daily milk production was significantly higher in the conventional group (mean, 32.3 kg/d) versus the organic group (mean, 22.3 kg/d) (P = 0.05). Prevalence between herd types was not statistically different with 13/26 (50%) positive organic herds versus 14/24 (58%) positive conventional herds (P = 0.16). Among positive herds, the proportion of JD+ cows was higher (though not statistically different) for organic herds (2.3%) versus conventional herds (1.6%) (P = 0.24). Risk factors associated with JD+ on organic farms included: lack of routine vaccination, sharing of pasture and water source between adult cows and replacement heifers, and use of nurse cows. Risk factors associated with JD+ on conventional farms included: purchasing of animals, sharing of pasture and water source between adult cows and replacement heifers, feeding of whole milk to calves, and use of group maternity pens. The prevalence of JD on PA organic farms is no different than that of conventional farms matched by size. Although differences in management practices were identified between herd types, these differences did not have a significant effect on JD prevalence.

**Key Words:** Johne’s disease, organic dairy farm, conventional dairy farm

**287 Dry cow treatment, antimicrobial residues in colostrum, and resistance in new born calves.** A. G. J. Velthuis*1, M. A. Gonggrijp1, A. E. Heuvelink1, C. Kappert1, D. Mevius1, and T. Lam1,2, 1GD Animal Health, Deventer, the Netherlands, 2Utrecht University, Department Farm Animal Health, Utrecht, the Netherlands.

This study aimed to quantify the prevalence and level of antibiotic residues (AR) of dry-cow therapies in colostrum fed to calves and in their feces and to evaluate the association between these residues and extended-spectrum β-lactamase- and AmpC-producing Escherichia coli in calf feces. On 10 dairy farms, colostrum samples were taken from the 1st to the 5th bucket (milking) that was fed to the new born calves. The colostrum originated from 87 cows: 20 cows dried off with 500 mg cloxacillin, 38 with 600 mg cloxacillin and 29 dried off with no antibiotics. Fecal samples were taken from then calves on 1, 7 and 14 d of age. The colostrum samples and the 7 fecal samples were evaluated for the presence and level of AR using a microbiological screening method and subsequently by LC-MS. All samples were screened for E. coli with non-wild-type susceptibility for cefotaxime (MIC >0.25 mg/L) and isolates were confirmed phenotypically as ESBL/AmpC-producing by the combination disc-diffusion test using cefotaxime and ceftazidime with and without clavulanic acid and cefoxitin. In 60% (CI 47–73%) of the colostrum samples of cows dried off with cloxacillin, cloxacillin residues were detected. The median concentration in the 1st milking was 148µg/kg and in a pooled sample of the 2–5th milking 67µg/kg. AR levels did not differ between cows treated with 500 or 600 mg cloxacillin. No AR were found in the fecal calf samples. Two out of 173 colostrum samples (2%, CI: 0–8%) tested positive for ESBL/AmpC E.coli, both were pooled samples from the 2–5th milking from cows treated with cloxacillin. ESBL/AmpC-E.coli were isolated from 12% (CI 6–20%) d 1 fecal samples, from 38% (CI 28–49%) d 7 samples, and from 35% (CI 24–47%) d 14 samples. No significant association was found between the dry cow treatment with cloxacillin or the presence of antimicrobial residues in the colostrum and the presence or amount of ESBL/AmpC E.coli in calf fecal samples. This in line with the fact that cloxacillin is not selecting for ESBL/AmpC-producing E. coli.

**Key Words:** antibiotic residue, colostrum, resistance

**288 Lameness on Canadian dairy farms: Measured and farmer-perceived prevalence, and associations with management practices.** S. L. Croyle*, C. Bauman, S. J. LeBlanc, and D. F. Kelton, University of Guelph, Guelph, ON, Canada.

The objectives of this study were to (1) estimate herd-level lameness prevalence (HLLP) on Canadian dairy farms, (2) compare the detected HLLP to the perceived lameness estimated by farmers, and (3) assess the associations between hoof-health management practice (HHMP) and HLLP. A cross-sectional study (National Dairy Study (NDS)) was conducted in the summer of 2015. The NDS consisted of a questionnaire and a follow-up farm visit. The questionnaire had an 11% response rate (n = 1,157) and contained farmer HLLP estimates, herd demographics, HHMP (e.g., use of a footbath). On-farm, HLLP was assessed using locomotion score (LS) for cows in freestall/pack farms, or in-stall lameness score (SLS) for cows in tie-stalls. The 14 assessors achieved a group inter-rater reliability Fleiss’s kappa score of 0.63 (substantial agreement), and a rater-expert Byrt’s Kappa of 0.73 (substantial agreement), ranging from 0.62 to 0.78. Lameness assessments were performed on a representative sample of milking cows on 374 farms across Canada. HLLP was determined by 1) the proportion of cows with LS ≥ 3 on a 5 point scale, where 1 = normal, 3 = mild, and 5 = severely lame or 2) the proportion of cows with SLS of ≥ 2 out of 4 behavioral indicators of lameness, where < 2 indicated a non-lame cow, and ≥ 2 indicated a lame cow. The mean HLLP was 29.2%, which was 2.8 times greater, on average, than the prevalence estimated (10.3%) by the farmers. In multivariable models, the use of deeper bedding was associated with lower HLLP. Pack barns were associated with lower HLLP (8.3%) when compared with freestall (20.1%), which was lower than tie stall (29.6%) (P < 0.05). Using a professional trimmer was associated with lower HLLP when compared with farmer/vet (15.7% vs 19.5%, P < 0.05). Providing the milking herd pasture access at least part of the year was associated with lower HLLP compared with no pasture access (14.4% vs 21.2%, P < 0.05). Results from this study highlight the need to educate farmers on detecting lameness, and provide insight into HHMP that may reduce the HLLP across Canada.

**Key Words:** lameness detection, farmer estimate, bedding depth
Dairy Foods Symposium:
Biofilm Formation on Dairy Separation Membranes

289 Exopolysaccharides produced by lactic starter cultures
impact biofilm formation on separation membranes. N. Garcia-Fernandez2, S. Anand2, and A. Hassan*1, 1Daisy Brand, Garland, TX, 2South Dakota State University, Brookings, SD.

Exopolysaccharide (EPS)-producing lactic cultures (LC) have been used to improve body and texture of fermented dairy products for decades. Research at South Dakota State University indicated that reduced fat Cheddar cheese made with EPS-producing cultures had similar textural and melting properties to its full fat counterpart. Furthermore, whey from such cheeses contained EPS that enhanced the functional properties of whey protein concentrate. We hypothesized that the EPS-producing bacteria surviving whey pasteurization would attach to the separation membrane surface and form biofilm. We also hypothesized that the extent of biofilm formation would depend on the type of EPS. To test our hypotheses, we used EPS-producing thermophilic and mesophilic cultures and their EPS-negative mutants to form biofilm on the retentate side of whey reverse osmosis (RO) membranes. To simulate the composition of concentrated whey expected at the membrane surface, we used 10% solution of 35% protein whey protein concentrate as the growth medium. The role of EPS in microbial attachment in the absence of growth (incubation at 4°C) and biofilm formation was investigated. The relationship between biofilm formation and cell surface characteristics was also determined. Results showed that EPS produced by LC may enhance or interfere with bacterial cell attachment and biofilm formation depending on their molecular characteristics. The growth medium did not affect the tendency of the test strains to form biofilm. Bacterial cell surface charge did not seem to impact attachment or biofilm formation on RO membranes. Generally, high cell surface hydrophobicity was associated with greater biofilm formation. In addition to its role in biofilm formation by the producing strains, EPS from LC could also impact biofilm produced by cocultures. A study with slime-producing spore-forming bacteria surviving whey pasteurization would attach to the separation membrane surface and form biofilm. We also hypothesized that the extent of biofilm formation would depend on the type of EPS. To test our hypotheses, we used EPS-producing thermophilic and mesophilic cultures and their EPS-negative mutants to form biofilm on the retentate side of whey reverse osmosis (RO) membranes. To simulate the composition of concentrated whey expected at the membrane surface, we used 10% solution of 35% protein whey protein concentrate as the growth medium. The role of EPS in microbial attachment in the absence of growth (incubation at 4°C) and biofilm formation was investigated. The relationship between biofilm formation and cell surface characteristics was also determined. Results showed that EPS produced by LC may enhance or interfere with bacterial cell attachment and biofilm formation depending on their molecular characteristics. The growth medium did not affect the tendency of the test strains to form biofilm. Bacterial cell surface charge did not seem to impact attachment or biofilm formation on RO membranes. Generally, high cell surface hydrophobicity was associated with greater biofilm formation. In addition to its role in biofilm formation by the producing strains, EPS from LC could also impact biofilm produced by cocultures. A study with slime-producing spore-forming bacteria supported data from the lactic acid bacteria experiments and showed that the hydrophobicity of the extra polymeric substances, whether it is EPS or polyamino acids, plays an important role in biofilm formation on dairy separation membranes.

Key Words: exopolysaccharide-producing lactic cultures, biofilm, membrane separation

290 The role of biofilms in the quality of dairy products in whey processing plants. S. Flint*, S. N. M. Zain, and R. Bennett, Massey Institute of Food Science and Technology, Massey University, Palmerston North, New Zealand.

Whey originates from microbial fermentation processes such as cheese and casein manufacture and is loaded with microorganisms from those operations. Thermalization is used to reduce bacterial numbers in whey before processing, however, bacteria that survive this are able grow within the manufacturing plant and contaminate the product. In hot processing plants, the growth of thermostolerant bacteria such as Streptococcus thermophilus can reach high levels on manufacturing plant surfaces blocking ultrafiltration modules. The current cold UF systems operating at 10°C solved that problem, however we are still facing microbial quality issues. Scrapings from ultrafiltration membranes reveal a variety of bacteria – many of which cannot survive thermalization and are hypothesized to enter via the water used for dialysis or cleaning. Control of water quality is therefore important. One problem that persists

in today’s whey manufacturing plants is the growth of spore-forming bacteria that survive thermalization. Bacillus licheniformis is a common contaminant which we assume originates from raw milk but propagates as biofilm on manufacturing plant surfaces. Although many strains cannot ferment lactose, they grow readily on stainless steel surfaces in the presence of whey. Their growth is limited to zones close to their optimum growth temperature of 37°C before whey concentration and dialysis as growth is influenced by ions and protein concentration. They produce spores and protease, potential problems in whey products. The challenge is to manipulate conditions to prevent biofilm growth of B. licheniformis in manufacturing plant.

Key Words: Lactobacillus plantarum, Klebsiella, cation

291 Controlling microbial biofilms. P. S. Stewart*, Montana State University, Bozeman, MT.

This presentation will discuss fundamental physical, chemical, and biological concepts important to understanding control of detrimental biofilms such as those that can foul and contaminate food processing equipment. Three phenomena that are important in the action of antimicrobial agents against a biofilm will be examined: diffusion, hydrodynamics, and physiology. The penetration of a biocide into a biofilm is governed by the balance of reaction and diffusion. Oxidizing agents in particular are subject to retarded or incomplete penetration due to their inactivation within the biofilm. Oxygen and nutrient concentration gradients within biofilms lead to stratified patterns of anabolic activity. For example, microelectrode technology demonstrates the presence of anoxic niches in biofilms exposed to aerated medium. Staining techniques reveal that the same biofilm can harbor, in distinct spatial niches, growing and dormant cells. Variation in the physiological activity is accompanied by alterations in susceptibility to antimicrobials. Time-lapse imaging of biofilms subjected to antimicrobial treatments reveals that in many cases these treatments do not remove the biofilm. In instances where removal is observed it is clear that forces applied by the flowing fluid are an important component of the removal process. The biofilm defense against biocides and antibiotic is multifactorial and so requires integrated and interdisciplinary science.

Key Words: biofilm, biocide, fouling


Reverse osmosis (RO) treatment systems have features which make them attractive for bacteria to settle, colonize and form biofilms. Often the water is warm, at least seasonally, and provides a continuous source of dissolved, assimilable nutrients; the combination of which provide high growth conditions for bacteria. Also, the RO element itself has a high proportion of surface area for the bacteria to attach. Interestingly, the entire RO system does not equally become inhabited by bacteria. It is found that the feed side of the system is more prone to colonization and biofilm formation than the reject side of the system. Comparing the environment (e.g., velocity, flux, solute concentration) of each side of the RO system provide some parameters to explore in an effort to
identify mechanisms for reducing biofilm formation in RO systems. Fundamental research highlighting some of the more influential parameters will be presented.

Key Words: reverse osmosis, biofouling, research

293 The role of quorum sensing in biofilm formation by bacteria in the dairy processing environment. M. Griffiths*, University of Guelph, Guelph, ON, Canada.

Biofilms are known to be a source of contamination of dairy products with bacteria such as pseudomonads and Bacillus spp. This source of contamination will become increasingly more important as the size of milk processing plants increase and the time that production lines operate becomes longer. Biofilm formation is also an important reason why bacteria persist in processing plants. Biofilm formation is controlled by quorum sensing and the chemicals responsible for this cell-to-cell communication vary between gram-positive and gram-negative bacteria. Much research has been conducted to determine if interruption of bacterial communication can be used to prevent biofilm formation and, hence, improve the shelf-life and safety of dairy products. This presentation will discuss the mechanisms of quorum sensing, its importance to the formation of biofilms in the dairy processing plant and the potential for targeting quorum sensing to control environmental contamination.

Key Words: biofilm, quorum sensing, environmental contamination
Mid-infrared analysis of Cheddar cheese. B. Margolies* and D. Barbano, Cornell University, Ithaca, NY.

Our objective was to develop a rapid method for measuring fat, protein, solids, and salt content of Cheddar cheese using a mid-infrared (MIR) transmittance analysis. Currently, quality assurance is done using near-infrared (NIR). For MIR analysis, Cheddar cheese (about 9 g) was blended with a sodium metasilicate solution (about 85 g). Cheese was blended to a uniform particle size (about 3 to 4 mm). The blended cheese (4°C) was added to a sodium metasilicate solution at 60 to 65°C in a stainless steel blender jar, 3 drops of a silicone-based antifoam were added, and the mixture was blended for 15 s at low speed followed by 45 s at high speed. The blended sample was poured into a 60-mL snap-lid plastic vial and placed in a 40°C water bath before analysis using a MIR milk analyzer. An infrared spectra and conductivity reading were collected for each sample. Measurement of fat and protein were done using traditional wavelengths used for milk analysis. Salt measurement was a combination of infrared traditional wavelengths and conductivity. Total solids was determined by a summation of fat, protein, and salt. Reference values for cheese solids were determined directly by forced air oven drying and salt was determined by a silver nitrated titration (Volhard method). The same solution of cheese/sodium metasilicate analyzed on the MIR was analyzed using Mojonnier ether extraction and Kjeldahl total nitrogen to obtain reference values for fat and protein content. Calibration slope and intercept adjustment for each component were done using linear regression. Standard error of predictions (SEP) for fat, protein, solids, and salt were generally less than 0.20. Typical SEP values for NIR for cheese fat, moisture, and protein are >0.3. MIR analysis of cheese may offer a more accurate alternative to NIR testing for routine quality control testing in a cheese factory, while reducing the amount of reference chemistry testing required to achieve a good calibration relative to that of NIR.

Key Words: mid-infrared, near-infrared, Cheddar cheese

Cholesterol, fatty acid profile, and mineral content of commercial cheeses predicted by near-infrared transmittance spectroscopy. CL Manuelian*, S. S. Currò, M. Penasa, and M. De Marchi, University of Padova, Legnaro, Padova, Italy.

Cheese supplies bioactive peptides, fatty acids (FA), minerals, and vitamins essential for human health. Common laboratory analyses of these components are expensive and time consuming. Near-infrared spectroscopy is a rapid, objective, non-destructive, and cheap method to determine several composition traits. However, heterogeneity of cheese, and low concentration of FA and minerals make their prediction difficult. This study aimed to develop prediction models for cholesterol, FA profile, and mineral content of commercial European cheeses using near infrared transmittance (NIT) spectroscopy. A total of 145 ground cheese samples from different dairy species and ripening time (fresh to 24 mo) were scanned with a NIT spectrophotometer every 2 nm from 850 to 1,050 nm wavelength. Sample spectra were matched with absolute content of cholesterol, FA, and mineral reference data to develop prediction models. Modified partial least squares regressions were validated through external validation after dividing the data in calibration (75%) and external validation (25%) sets. Cheese moisture, fat, protein, total solids, and cholesterol averaged 43.24 ± 0.97%, 27.24 ± 0.47%, 24.87 ± 0.54%, 56.76 ± 0.97%, and 0.07 ± 0.001%, respectively. Cholesterol content was inadequately predicted, exhibiting a coefficient of determination of external validation (R²ExV) of 0.50 and a residual prediction deviation of external validation (RPDExV) of 1.36. Satisfactory models were developed for saturated, unsaturated, monounsaturated, and polyunsaturated FA, and myristic, palmitic, oleic, and some minor FA (R²ExV from 0.87 to 0.97, RPDExV from 2.74 to 4.73). Promising predictions were obtained for Ca, Na, P, S, Mg, Zn, and Cu (R²ExV from −0.94 to 0.83; RPDExV from −3.73 to 2.35). Results of the present study are a prelude to the at-line utilization of prediction models for the most abundant cheese FA and minerals.

Key Words: buffalo, cow, trace mineral

Is fatty acid composition of retail cheeses influenced by the scale of production? E. Vargas-Bello-Pérez*, C. Geldsetzer-Mendoza*, M. S. Morales*, P. Toro-Mujica1, M. A. Fellenberg1, R. A. Ibáñez1, and P. Gómez-Cortés1, 1Departamento de Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile, 2Departamento de Fomento de la Producción Animal, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile, 2Instituto de Investigación en Ciencias de la Alimentación, Universidad Autónoma de Madrid, Nicolás Cabrera 9, Madrid, Spain.

The objective of the present study was to assess if the scale of production of dairy plants has an effect on the fatty acid (FA) composition of retail cheeses. Cheese samples (n = 60) were obtained from local retail stores during summer season (Santiago, Chile). Retail samples consisted of Gouda (n = 18), Chanco (n = 11) and Mantecoso (n = 31) cheeses. Cheeses were manufactured from 8 different district regions from Chile: Coquimbo, Valparaíso, O’Higgins, Bio-Bio, Araucanía, Los Lagos, Los Ríos and Metropolitana. Samples were classified based on the scale of dairy plant production: small-scale (<3500 L milk/d; n = 18) and large-scale (>3500 L milk/d; n = 42). Samples were analyzed for FA composition by gas chromatography-flame ionization detector (GC-FID) and consequent principal component analysis (PCA). In average, cheeses (g/100g total FAME) resulted in 73 of saturated FA, 23 of monounsaturated FA and 3 of polyunsaturated FA. PCA of the FA data yielded 2 significant principal components (PC), which accounted for 74% of the total variance in the data set. PC1 was related to saturated FA (C8:0, C10:0, C15:0, C16:0 and C17:0) and monounsaturated FA (C20:2 and C22:6n3). Moreover, Mantecoso cheese samples were clearly discriminated from the rest along PC1. In contrast, PC2 differentiated Chanco and Gouda cheeses by polyunsaturated FA (C20:2 and C22:6n3). Moreover, Mantecoso cheeses obtained from large-scale production plants were related to increased levels of saturated FA, whereas those from Chanco and Gouda cheeses from small-scale dairy plants were associated with increased contents of monounsaturated and polyunsaturated FA. Our data partly showed that the FA composition of retail cheeses is influenced by the scale of production; however, further research considering FA composition of cheese milk as well as on-farm management practices will be required to further understand the origin of the observed differences in this study. This study was sponsored by a research grant from Pontificia Universidad Católica de Chile (Proyecto Puente P1608).

Key Words: milk, principal component analysis, lipids

Impact of green tea polyphenols on functionality and sensory acceptability of buffalo milk Cheddar cheese. M. A. Murtaza*, I. Hafiz*, and M. Anees-ur-Rehman*, 1Institute of Food
Green tea is a rich source of polyphenols, predominantly flavonoids having antioxidant properties. The objective of the study was to assess the impact of green tea extract addition on composition, functionality and sensory acceptability of buffalo milk Cheddar cheese. The cheddar cheese was manufactured from buffalo milk standardized at 4% fat content. The tea extract was added as 0.1, 0.2 and 0.3% in milk. The cheese samples along with a control were prepared in triplicates and ripened at 6–8°C for 4 mo. The cheese was analyzed for basic composition, phenolic content, texture profile, color and sensory perception during storage. The addition of extract did not influence the protein, fat and minerals content. The moisture in cheese was reduced significantly with the increase in extract concentration. The mean phenol retention coefficient was found 0.70 and non-significant increase was found with respect to extract concentration. The extract addition also affected the cheese color with slight decrease in lightness (L* value) and increase in redness (a* value) and yellowness (b* value). Regarding texture profile, cheese hardness increased while springiness and cohesiveness decreased significantly with the increased concentration of extract. On sensory evaluation (9-point hedonic scale), as the concentration of extract increased, the scores awarded for flavor, color and texture of cheese decreased but product was greatly acceptable (scores >6) up to the extract level of 0.2%. The influence on color and flavor was due to the color and flavor of the extract however, the alteration in texture shows the interaction of extract with casein matrix and its retention in final product. Hence, it was concluded that green tea extract increases the antiradical activity of cheese and extract up to 0.2% of milk can adequately be carried through Cheddar cheese to get its nutritional and health impacts.

Key Words: buffalo milk, Cheddar cheese, green tea polyphenols

298 Effect of pH modification on chymosin-induced coagulation of concentrated casein micelles suspensions. Z. Zhao*1 and M. Corredig1,2, 1University of Guelph, Guelph, ON, Canada, 2Gay Lea Foods, Guelph, ON, Canada.

The objective of this research was to investigate the influence of pH-modification and concentration on the chymosin-induced gelation of milk. Milk was adjusted to pH 6.0 and 5.5 and then concentrated to 3× using osmotic stress. The concentrated were compared with those prepared with milk at native pH. The gelation behavior was monitored using rheology and light scattering, in situ. Partial acidification accelerated the coagulation of casein micelles and the gelation time decreased from 55 ± 2.5 min to 9.0 ± 2.0 min and 6.0 ± 0.5 min at pH 6.0 and 5.5, respectively. Concentration (3×) increased the gel strength with the highest elastic value 977 ± 81 Pa was found at pH 6.0. These results confirmed previous literature data. It was demonstrated that in untreated milk, the gelation time was not affected by casein concentration, while in the case of the pre-acidified samples, the gelation times increased after concentration. This was attributed to a change in the composition of the serum phase, including an increase in soluble proteins. It was also found that more caseins were released for the pre-acidified samples during the concentration process. When the concentrated acidified casein micelles were added to untreated milk, the gels showed higher elastic modulus (around 372 ± 30 Pa) compared with control (146 ± 10Pa). This effect was not due to free calcium concentration, as when the mixtures were equilibrated against milk, no gelation was observed. The lack of gelation could not be solely attributed to changes in diffusible calcium phosphate, but to a partial solubilisation of the casein micelles. The results demonstrated that both soluble caseins and diffusible calcium phosphate play an important role in the coagulation of casein micelles induced by chymosin.

Key Words: micelle, acidification, rennet

299 Effects of different commercial proteolytic enzymes used in the production of enzyme-modified cheese on the cheese ripening parameters. G. Goveч1, P. Salum2, D. Bas3, P. Kendircи4, and Z. Erbay*5, 1Department of Food Engineering, Institute of Natural and Applied Sciences, Adana Science and Technology University, Adana, Turkey, 2Department of Food Engineering, Institute of Natural and Applied Sciences, Cukurova University, Adana, Turkey, 3Department of Food Engineering, Faculty of Engineering, Cankırı Karatekin University, Cankırı, Turkey, 4Department of Gastronomy and Culinary Arts, Faculty of Tourism, Katip Çelebi University, İzmir, Turkey, 5Department of Food Engineering, Faculty of Engineering and Natural Sciences, Adana Science and Technology University, Adana, Turkey.

Cheese is the most remarkable dairy product due its variability, high market coverage and flavor. An important ratio of worldwide cheese production is used as an ingredient for the production of other foods. The main reason for using the cheese as ingredient is its flavor. Unique flavor of cheese is developed during the ripening period. The ripening is a high-cost process and standardization of the product is not easy. It is possible to develop and intensify cheese flavor in a short time period under controlled conditions by the aid of enzymatic reactions. The product obtained with this method is called enzyme modified cheese (EMC). In the production of EMC, proteolytic and lipolytic enzymes are used. However, the enzyme type and incubation time differ according to the targeted cheese flavor and these parameters should be determined with experimental studies. In this study, the effects of proteolytic enzymes on the cheese ripening parameters were determined. Fresh white cheese was used as raw material and 5 different commercial enzymes including endopeptidases (Neutrase and Promod 215MDP) and exopeptidases (Flavorzyme, Flavorpro 937MDP and Flavorpro Umami 852MDP) were tested at 4 different incubation times (12, 24, 36 and 48 h). The soluble nitrogen fractions (nitrogen soluble in water, trichloroacetic acid, phosphotungstic acid and total free amino acid contents) were analyzed and ripening indices (ripening extension, ripening depth and free amino acid indices) were calculated. Results showed that all ripening parameters changed significantly during incubation period (P < 0.05). The ripening extension index varied in the range of 46.2–77.9%, while the ripening depth index and the free amino acid index values were calculated in the range of 25.9–67.4% and 8.0–34.4%, respectively. Exopeptidases showed higher proteolysis rates. The highest rate for ripening was obtained by Flavorpro Umami 852MDP, followed by Flavorzyme.

Key Words: enzyme-modified cheese, ripening, proteolysis
The objective of this study was to determine the effect of soybean meal (SB) or canola meal (CM) with or without inclusion of micro-encapsulated sodium butyrate (MSB) in calf starter mixtures on gastrointestinal tract (GIT) development. Twenty-eight Holstein-Friesian bull calves (8.7 ± 0.8 d of age, 43.0 ± 4.4 kg at the start of the study) were blocked by date of birth and initial BW and fed 1 of the 4 pelleted starters containing (1) SB; (2) SB+MSB; (3) CM and (4) CM+MSB. Crude protein (CP) content of the starters was (%DM): 1) 21.9; 2) 21.7; 3) 20.7 and 4) 20.3. The CM constituted 35.2%, SB 24.2% and MSB 0.3% of the respective starters DM. Calves were fed milk replacer (MR, 21.7% CP) at 0.85 kg/d for 35 d and then 0.43 kg/d for following 7 d. Calves were weaned at 51.7 ± 0.8 d of age and were killed at 72.1 ± 0.9 d of age. The GIT was dissected for morphometry measurements and tissue samples were used for histological assessment and brush border enzyme activity determination. Data were analyzed as a 2 × 2 factorial design. Pre-weaning starter intake and performance of calves during the summer months in northern New York. K. Hultquist*, C. Ballard, and C. Havekes, William H. Miner Agricultural Research Institute, Chazy, NY.

The objective of this study was to evaluate the addition of fat to milk replacer as a supplemental energy source to reduce the negative effects of heat stress on growth and performance of dairy calves. Sixty calves (27 heifers and 33 bulls) housed in individual hutches were enrolled in a randomized block design from June 7 to Oct. 7, 2016 with THI ranging from 33 to 81. Calves were blocked by age and sex and randomly assigned to treatment: 1) milk replacer with no added fat (CON), 2) milk replacer with added fat when daily temperature exceeded 26°C (FTEMP), and 3) milk replacer with added fat for all study days (FALL). Calves were fed the same amount of milk replacer (26% crude protein, 18% fat, and 13% solids) twice daily following a step-up-step-down feeding strategy from 2 to 57 d of age. Fat was added at 1.2% of total reconstituted milk replacer for FTEMP and FALL increasing total solids to 14.2%. Calves had ad libitum access to a pelleted starter and water. Body weight, hip height, hip width, serum glucose, and serum nonesterified fatty acids were measured weekly. Intakes and health (body temperature, respiration, skin tent, eye recession, cough, nasal discharge, and fecal) were evaluated daily. Intake, growth, and feed efficiency data were averaged by week and analyzed using the GLIMMIX procedure of SAS. Health data were analyzed using logistic regression. The effect of feeding treatments was assessed using preplanned contrasts, comparing CON vs fat supplementation (FS = FTEMP + FALL) and FTEMP vs FALL. Average daily gain was 0.06 kg/d greater for FS from 2 to 43 d of age before weaning started. However, overall average daily gain (2 to 57 d) was not significant among treatments. Hip height and hip width also did not differ among treatments. Dry matter intake was increased for FS resulting in greater overall feed efficiency for CON-fed calves. Serum nonesterified fatty acids were greater for FS than CON. Calves fed FS had higher respiratory rates with FALL being greater than FTEMP. All other health parameters were similar among treatments. The results of this study indicate that calves did not benefit from fat supplementation during the summer months.

Key Words: calf, fat supplementation, heat stress

301 Effects of feeding milk replacer with increased fat on intake and performance of calves during the summer months in northern New York. K. Hultquist*, C. Ballard, and C. Havekes, William H. Miner Agricultural Research Institute, Chazy, NY.

302 Effects of prebiotic and phytogenic milk replacer additives on growth and feed utilization of Holstein rearing calves. T. Wilke* and H. Westendarp, 1Dr. Eckel Animal Nutrition GmbH & Co KG, Niederzissen, Germany, 2Faculty of Agricultural Sciences and Landscape Architecture, University of Applied Sciences, Osnabrück, Germany.

Various prebiotic and plant feed additives claim to promote growth and proper development of the gastrointestinal tract by different modes of action. Objective of this study was to compare the effects of 2 feed additives for milk replacers on growth and feed conversion of dairy calves. The trial was conducted from October 2015 to May 2016 in North-Western Germany with 80 female Holstein rearing calves of one dairy herd. At d 4 postpartum (BW 44.9 ± 5.2 kg) calves were assigned to 2 treatment groups (A and B). Calves of group A were fed a milk replacer (160 g per liter) enriched with a prebiotic preparation (0.3% dry powder) of calcium gluconate, calcium and sodium carboxylates, fructooligosaccharides (scFOS) and a plant extract (AntaTop MAT, Dr. Eckel Animal Nutrition, Germany). The milk replacer of group B contained a mixture of a plant extract rich in benzophenanthridine alkaloids and organic acids (0.3% dry powder). Milk replacer intake was measured individually (n = 80). Calf starter feed was offered from d 14 (max. Two kg/calf/day) and roughage from d 21 (ad libitum). Body weight was measured at d 4, 14, 40 and 64 postpartum. Data were analyzed using ANOVA (IBM SPSS). During the main feeding period (d 14 - 40) daily weight gain was significantly higher (P < 0.05) in group A (prebiotic) (927 ± 181 vs. 821 ± 252 g/d). Daily weight gain over the whole period (d 4 - 64) was not statistically different (P < 0.05) between treatments A and B (878 ± 119 vs. 860 ± 137 g/d). Calves of group A needed 5.6 kg (±1.58) of milk replacer powder (72.0 vs. 66.4 kg) to achieve this weight gain. Consequently, feed conversion of milk replacer into body mass was significantly lower (P < 0.01) in the prebiotic group (A) than in the alkaloid group (B) (1.28 ± 0.22 vs. 1.41 ± 0.21 kg/kg). Calves of the prebiotic group (A) consumed more roughage (45.8 ± 6.9 vs. 41.7 ± 11.6 kg dry matter) and more calf starter feed (15.2 ± 3.5 vs. 10.9 ± 3.9 kg dry matter). Differences in roughage and starter intake were not significant (P > 0.20). The results indicate that efficiency of dairy calf feeding can be improved by a prebiotic additive in milk replacer.

Key Words: calf feeding, feed additive, prebiotics

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We tested the hypothesis that the uterus of virgin heifers and pregnant cows possessed a resident microbiome. The endometrium of 10 virgin heifers in estrus and the amniotic fluid, placenta, intercotyledonary placenta, cervical lumen, and external cervix surface (control) of 5 pregnant cows were sampled using sterile surgical tools and aseptic surgical techniques. DNA was extracted, and the V4 hypervariable region of the 16S rRNA gene was amplified by PCR with barcode-indexed primers (U515F/806R), and sequenced. Operational taxonomic units were generated from the sequences, and taxonomy was assigned. The effect of tissue on the microbiome within the pregnant uterus was tested using univariate (mixed model) and multivariate (permutational multivariate ANOVA) procedures. Amplicons were generated in all samples supporting the contention that the uterus of virgin heifers and pregnant cows contained a microbiome. On average 53, 199, 382, 382, 525, and 13589 reads annotated as 16, 35, 43, 63, 48, and 176 OTUs in the placenta, virgin endometrium, amniotic fluid, cervical lumen, intercotyledonary placenta, and external surface of the cervix, respectively, were generated. The 3 most abundant phyla in the uteri of the virgin heifers and pregnant cows were Firmicutes, Bacteroidetes, and Proteobacteria and they accounted for approximately 40%, 35%, and 10% of the sequences, respectively. Phyla abundance was similar between the tissues of the pregnant uterus. Principal component analysis, one-way PERMANOVA analysis of the Bray-Curtis similarity index, and mixed model analysis of the Shannon diversity index and Chao1 index demonstrated that the microbiome of the control tissue was significantly different from the amniotic fluid, intercotyledonary placenta, and placenta tissues. Bacteria associated with postpartum uterine disease i.e., Trueperella spp., Fusobacteria spp., and Prevotella spp. were also present in the uterus of virgin heifers and of pregnant cows. The presence of 16S rRNA sequence reads in the samples from the current study suggests that the uterine microbiome is established by the time a female reaches reproductive maturity and that pregnancies are established and maintained in the presence of a uterine microbiome.

Key Words: microbiome, uterus, pregnancy

304 Uterine microbiome during the first week after calving is associated with differences in milk production in the absence of overt signs of disease. S. G. Moore*1, A. C. Ericsson2,3, S. E. Pook4, and M. C. Lucy1, 1Division of Animal Sciences, University of Missouri, Columbia, MO, 2Department of Veterinary Pathology, University of Missouri, Columbia, MO, 3University of Missouri Metagenomics Center, University of Missouri, Columbia, MO, 4College of Veterinary Medicine, University of Missouri, Columbia, MO.

Postpartum uterine disease is associated with reduced milk production and infertility in dairy cows. The reduction in milk production and infertility may be explained by acute disease (metritis) or chronic uterine inflammation (endometritis). Uterine disease may be a response to the uterine microbiome. The objective was to characterize the uterine microbiome at 7, 35, and 63 DIM using 16S rRNA gene sequencing. The hypothesis was that the uterine microbiome would change over time and that the composition would be associated with health and performance. The endometrium of 31 first parity dairy cows of Holstein and Jersey admixture was biopsied at 7, 35, and 63 DIM. DNA was extracted, and the V4 hypervariable region of the 16S rRNA gene was amplified and sequenced. Principal component analysis (PCA) identified a clustering of the samples such that the uterine microbiome at 35 and 63 DIM were similar, but different from the uterine microbiome at 7 DIM. In the same PCA, the 7 DIM samples separated into 2 distinct clusters that defined 2 groups of cows [A (n = 22) and B (n = 9)]. There was a greater number of reads per sample (P = 0.05) but lesser diversity (both Shannon and Chao1 P < 0.0001), and lesser abundance of the Ruminococcaceae family, Treponema spp. (both P < 0.0001), Streptococcus spp., and Prevotella spp. (both P = 0.02) in group A compared with group B. At 7 DIM, group A had a greater vaginal mucus score (VMS; P < 0.01) and 8% greater blood glucose concentrations (P = 0.04) compared with group B. Otherwise, cows from the 2 groups were similar for metabolic status, BW, BCS, body temperature and subsequent VMS. For their first 140 DIM, group A tended to have reduced SCS (3.93 vs. 4.69 units, P = 0.07) and greater ECM yield (25.81 vs. 23.40 kg/d; P = 0.1). The difference in ECM yield was greatest from wk 7 to 20 (Group x wk; P < 0.05). In summary, 2 groups of cows that differed in their uterine microbiome at 7 DIM also differed with respect to ECM yield but were largely similar with respect to other measures indicative of metabolic and uterine disease. These data raise the possibility that the early postpartum uterine microbiome impacts cow milk production in the absence of overt signs of disease.

Key Words: uterus, microbiome, lactation

305 Discovering neutrophil extracellular traps in the bovine endometrium and the effects of feeding a rumen-protected methionine on plasma amino acid concentrations and uterine characteristics. S. L. Stella*1, D. A. V. Acosta2, C. Skendaroie1,3, Z. Zheng1, A. Steelman1, D. Luchini4, and F. C. Cardoso1, 1University of Illinois, Urbana, IL, 2The Colombian Corporation for Agricultural Research (CORPOICA), Bogota, Colombia, 3Texas A&M College of Veterinary Medicine, College Station, TX, 4Adisseo NACA, Alpharetta, GA.

Supplementing methionine, the most limiting amino acid (AA) to dairy cows (NRC 2001), may improve uterine health and reveal that other AA concentrations have been affected. The objective of this study was to observe the effects of feeding rumen-protected methionine (RPM; Smartamine M) on plasma AA concentrations, uterine cytology, neutrophil counts, and to confirm neutrophil extracellular trap (NET) formation in the bovine endometrium. Multiparous Holstein cows (n = 20) were randomly assigned to 2 treatments starting 21d before calving until 73DIM. Treatments were: CON (n = 9, TMR with a Lys:Met = 3.5:1) and MET (n = 11, TMR+RPM with a Lys:Met = 2.8:1). Uterine biopsies and blood samples from the coccygeal artery/vein were obtained at 15, 30, and 73DIM. Biopsy samples were sectioned and stained using immunohistochemistry with Hoechst (DNA) and Anti-Neutrophil Elastase antibody (NE). Biopsy slides were scanned in an automated imaging cytometer to quantify neutrophil numbers and a confocal fluorescent microscope for NET discovery/confirmation via NE and DNA fluorescent antibodies. Endometrial swabs were streaked onto slides, stained with Giemsa, and scanned using whole image.
scanning. Polymorphonuclear neutrophils (PMN) were counted and a percentage was calculated based on the number of PMN to epithelial cells. Statistical analysis was performed using the MIXED procedure of SAS. CON had lower ($P < 0.01$) methionine plasma concentrations (18.05 ± 2.0μg/mL) than MET (30.39 ± 1.6μg/mL). CON had higher ($P < 0.01$) cystine plasma concentrations (3.62 ± 0.3μg/mL) than MET (2.8 ± 0.3μg/mL). A treatment by DIM interaction was observed for PMN and the number of neutrophils in the endometrium: CON (28.28 ± 7.7%) tended to have higher ($P = 0.09$) PMN percentage in swabs than MET (18.19 ± 6.7%) and CON (1423.98 ± 437.9) tended to have higher ($P = 0.06$) neutrophil numbers in the endometrium than MET (192.54 ± 408.2). Supplementation of RPM appears to alter the concentrations of AA and have beneficial effects on uterine immune function.

**Key Words:** rumen-protected methionine, PMN, neutrophil extracellular traps

306 **Ovarian follicular dynamics, endocrinology, and estrous behavior in repeat breeder cattle.** P. Sood1*, H. D. Sarma2, P. K. Dogra1, V. Kadwad1, and S. S. Sachdev3, 1Dr G C Negi College of Veterinary and Animal Sciences, Palampur, Himachal Pradesh, India, 2Bhabha Atomic Research Centre, Mumbai, Maharashtra, India, 3Board of Radiation and Isotope Technology, Mumbai, Maharashtra, India.

Repeat breeding (RB) in cattle is a global and multifactorial problem causing reproductive wastage and economic losses. We investigated follicular dynamics, certain reproductive hormones (FSH, E2, LH and P4) and estrus duration as well as estrus behavior score (intensity) in PGF$_{2α}$ induced estrous cycles in repeat breeding (RB) and normal (C) cows. Depending on the number of follicular waves, the cows were categorized as 2 – wave (RB-2, n = 10 and C-2, n = 10) or 3 – wave (RB-3, n = 6 and C-3, n = 10). The results were analyzed (separately for 2 – and 3 – wave patterns) by ANOVA for repeated measures using the mixed procedure of the SAS and Student’s t-tests. The RB versus C with either wave pattern differed ($P < 0.05$ at least) in terms of (1) shorter interovulatory interval, (2) greater number of recruited follicles, (3) delayed selection of the dominant follicles, and (4) greater count of small and medium follicles. A treatment-by-day interaction of $P < 0.12$ and $P < 0.08$ was observed for FSH in RB-2 and RB-3, respectively; the plasma FSH remained greater, especially around selection of dominant follicles, and failed to exhibit a precipitous drop in peak as observed in the corresponding C-2 and C-3. Except for greater E2 (pg/mL) in RB-2 than C-2 at estrus (15.6 ± 1.1 versus 9.4 ± 0.5), its concentrations were similar at different stages of estrous cycle in RB and C. The plasma LH concentrations did not differ for the RB and C. Progression of P4 toward peak was slower in RB, in spite of which its concentrations at different days of estrous cycle and area under curve did not differ from C. There was no difference in estrus duration (h) (16.5 ± 5.1 in RB-2 versus 17.4 ± 4.5 in C-2 and 18.2 ± 5.8 in RB-3 versus 16.1 ± 3.9 in C-3) and estrous behavior score (1065.6 ± 146.1 in RB-2 versus 615.3 ± 149.1 in C-2 and 951.3 ± 278.4 in RB-3 versus 650.8 ± 110.9 in C-3) between the RB and C. In conclusion, while exogenous P4 can suffice its slow increase in RB, the cause – effect relation between greater FSH and altered follicular dynamics needs to be corrected in RB, which may restore normal reproduction in infertile cows.

**Key Words:** repeat breeder cows, follicular dynamics, endocrinology

308 **Fertility, concentrations of steroid hormones, and antioxidant enzymes during transition period in dairy cows fed organic trace minerals supplement.** V. Kathanasueng*, C. Navanukraw, A. Kraisoon, S. Tongrueng, and T. Bumma, Agricultural Biotechnology Research Center for Sustainable Economy (ABRCSE), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand.

The objective was to evaluate fertility, concentrations of estradiol (E2), progesterone (P4) and antioxidant enzymes in dairy cows fed with organic trace minerals (OTM) supplement during pre- and postpartum. Prepartum Holstein dairy cows (n = 60) were randomly assigned to receive treatments: control or OTM supplemented. Cows were fed ad libitum roughage and dietary concentrate beginning at 21 d before expected calving date and for 21 d after parturition. In OTM supplemented group, cows were supplemented with 5 g/h/d OTM (Bioplex). Cows were timed-AI using a modified Ovsynch as previously described. Serum and follicular fluid (FF) samples were collected via venipuncture and ovum pick-up throughout the experiment for analysis of E2, P4, superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) concentrations. Percentage of retained fetal membranes were not different between the groups ($P > 0.05$), whereas placental expulsion period in control cows was longer than cows fed OTM (13.2 and 6.3 h; $P < 0.05$). Day to first ovulation and estrus in cows fed OTM occurred sooner than those control cows ($P < 0.05$). From d 0 to 8 after timed-AI, serum P4 concentrations did not differ between the groups. However, P4 concentrations in OTM cows were greater ($P < 0.05$) than control.
Most studies have studied the association between cervical and/or uterine size and subsequent fertility in Holstein cows, but none in Jersey cows. The objective of this study was to determine whether cervical and uterine size at 4 weeks postpartum are correlated with subsequent early postpartum cyclicity and fertility at first insemination in Jersey cows. The Missouri commercial dairy farm on study milked cows twice a day with an ME 305 of 7,064 kg. Cows were fed a TMR and were inseminated after estrus. The herd had a 21d-annualized pregnancy rate of 36%. Cows (n = 147) selected were subjected to an ultrasound examination and blood collection for progesterone levels at 4 weeks postpartum. Body condition score at calving, parity and milk production at the 4th week of lactation were recorded. The final statistical analysis included 127 cows with pregnancy status at first insemination. For indicators of cyclicity, [progesterone concentration and presence of corpus luteum (CL) at 4 weeks postpartum] a total of 147 cows were evaluated. Logistic regression models to test the association between cervical and uterine size and presence of CL and fertility were developed. Multivariable regression models for the association of cervical and uterine size at 4 wk postpartum were predictive of cyclicity (either presence of CL or elevated progesterone) and fertility at first insemination in Jersey cows.

Key Words: fertility, organic trace minerals, dairy cow

### 309 The association between cervical and uterine size at 4 weeks postpartum and fertility in Jersey cows

S. Poock1, P. Melendez*1, M. Caldeira2, S. Moore2, L. Mayo2, R. Molina-Coto2, and M. Lucy2, 1College of Veterinary Medicine, University of Missouri, Columbia, MO, 2Department of Animal Sciences, University of Missouri, Columbia, MO.

Several studies have studied the association between cervical and/or uterine size and subsequent fertility in Holstein cows, but none in Jersey cows. The aim of this study was to determine the combined relationship of heat stress and pre-ovulatory follicular size estimated by rectal palpation at fixed-time AI (FTAI) following a progesterone-based estrous synchronization protocol and the subsequent conception rate (CR). The experiment was performed in a commercial dairy herd located in North-Eastern Spain since July to December 2016. A single inseminator palpated the ovaries during AI and assigned the cow to 3 different possible follicle groups: (S) Small (estimated follicular diameter (EFD) < 12mm), (M) Medium (12–16 mm) and (L) Large (>16mm). The sizes were verified by ultrasound within 30 min. The 3 follicle groups had significant different diameters S (n = 56, 11.5 ± 6mm), M (n = 114, 14.7 ± 3) and L (n = 72, 18.3 ± 5mm) according to ANOVA and Tukey’s tests (P < 0.001). Pregnancy diagnosis was performed by ultrasonography on Day 28 post-AI. The mean CR was 58/285 = 21.56%. The single factor affecting CR in the final logistic regression model was the interaction between heat stress (HS) (temperature humidity index (THI) > 72 at AI) and the EFD. Cows in group L with THI ≤72 at AI had a greater probability of becoming pregnant (odd ratio = 4.185; P = 0.013) than the remaining cows (Table 1). Our results suggest that cows with large follicles under HS could suffer ovulation failure resulting in a low CR, and that estimation of pre-ovulatory follicular size at AI may be a good predictor of subsequent CR. The clinical implication of this study is that a trained inseminator can identify which cows are more likely to become pregnant at FTAI.

Key Words: ovulation failure, heat stress, THI

### 310 Pre-ovulatory follicular size and the subsequent conception rate in dairy cows

R. Mur-Novaless*1,2, I. García-Ispierto1,2, B. Serrano-Pérez1,2, V. Cabrera2, and F. López-Gatius2, 1Department of Animal Science, University of Lleida, Lleida, Spain, 2Agrotecnio Center, Lleida, Spain, 3University of Wisconsin-Madison, Madison, WI.

The aim of this study was to determine the combined relationship of heat stress and pre-ovulatory follicular size estimated by rectal palpation at fixed-time AI (FTAI) following a progesterone-based estrous synchronization protocol and the subsequent conception rate (CR). The experiment was performed in a commercial dairy herd located in North-Eastern Spain since July to December 2016. A single inseminator palpated the ovaries during AI and assigned the cow to 3 different possible follicle groups: (S) Small (estimated follicular diameter (EFD) < 12mm), (M) Medium (12–16 mm) and (L) Large (>16mm). The sizes were verified by ultrasound within 30 min. The 3 follicle groups had significant different diameters S (n = 56, 11.5 ± 6mm), M (n = 114, 14.7 ± 3) and L (n = 72, 18.3 ± 5mm) according to ANOVA and Tukey’s tests (P < 0.001). Pregnancy diagnosis was performed by ultrasonography on Day 28 post-AI. The mean CR was 58/285 = 21.56%. The single factor affecting CR in the final logistic regression model was the interaction between heat stress (HS) (temperature humidity index (THI) > 72 at AI) and the EFD. Cows in group L with THI ≤72 at AI had a greater probability of becoming pregnant (odd ratio = 4.185; P = 0.013) than the remaining cows (Table 1). Our results suggest that cows with large follicles under HS could suffer ovulation failure resulting in a low CR, and that estimation of pre-ovulatory follicular size at AI may be a good predictor of subsequent CR. The clinical implication of this study is that a trained inseminator can identify which cows are more likely to become pregnant at FTAI.

### Key Words: Jungle, entity size, fertility

### 311 Associations between inter-service interval and fertility in dairy cows

J. G. Remnant*, M. J. Green, J. N. Huxley, and C. D. Hudson, University of Nottingham, Sutton Bonington, Loughborough, UK.

Studies have suggested that average inter-service (and inter-ovulatory) intervals in dairy cows may be longer than typically expected. Some authors suggest fertility may vary with follicular wave number. This study aimed to identify associations between inter-service-interval (ISI) and the probability of pregnancy in dairy cows. Data from 312 UK dairy herds were analyzed. There were 257,396 insemination records from

<table>
<thead>
<tr>
<th>Factor</th>
<th>n</th>
<th>CR (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFD × HS</td>
<td>S without HS</td>
<td>39</td>
<td>12.8</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M without HS</td>
<td>70</td>
<td>27.1</td>
<td>2.53</td>
<td>2.53–0.86</td>
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<tr>
<td></td>
<td>L without HS</td>
<td>42</td>
<td>38.1</td>
<td>4.18</td>
<td>1.35–12.9</td>
</tr>
<tr>
<td></td>
<td>S with HS</td>
<td>23</td>
<td>8.7</td>
<td>0.64</td>
<td>0.115–3.645</td>
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<tr>
<td></td>
<td>M with HS</td>
<td>57</td>
<td>21.1</td>
<td>1.81</td>
<td>0.583–5.683</td>
</tr>
<tr>
<td></td>
<td>L with HS</td>
<td>37</td>
<td>10.8</td>
<td>0.82</td>
<td>0.203–3.340</td>
</tr>
</tbody>
</table>

1EFD = estimated follicular diameter (S: <12mm, M: 12-16mm, L: >16mm); HS = heat stress; CR = conception rate.
75,745 cows. The intervals between subsequent inseminations in the same cow in the same lactation were calculated. Inseminations with a corresponding calving recorded were deemed successful, allowing the calculation of the average probability of pregnancy at different ISIs. A random effects logistic regression model was constructed to predict the probability of pregnancy for ISIs (16–28 d). Univariable analysis showed a peak probability of pregnancy of 44% with an ISI of 21 and 22 d; the distribution across the range of ISIs is tabulated below. Preliminary multivariable analysis showed that the probability of pregnancy was significantly ($P < 0.05$) lower for inseminations carried out at 16–18 d and significantly higher for those carried out at 21–22 d when compared with 25 d. These results suggest that pregnancy is most likely following inseminations carried out at the expected ISI of 21 d. ISIs of less than 19 d result in a lower probability of pregnancy, suggesting that these ISIs reflect inseminations of cows not truly in estrus or that estrous cycles of shorter length are less fertile. ISIs greater than 24 d are less likely to result in pregnancy than those of 21 d but are more likely to result in pregnancy than ISIs of 18 d or less, suggesting that these ISIs may represent true estrus events at an interval longer than typically expected. These longer ISIs may be a result of embryonic death extending the inter-estrus interval or may represent longer than expected true estrous cycles. These results suggest a need to reconsider the expected cycle length of the modern dairy cow, both in research and when reviewing farm insemination records.

### Table 1 (abstract 311).

<table>
<thead>
<tr>
<th>ISI, d</th>
<th>Probability of pregnancy</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0.27</td>
<td>657</td>
</tr>
<tr>
<td>17</td>
<td>0.24</td>
<td>853</td>
</tr>
<tr>
<td>18</td>
<td>0.30</td>
<td>1,374</td>
</tr>
<tr>
<td>19</td>
<td>0.38</td>
<td>2,844</td>
</tr>
<tr>
<td>20</td>
<td>0.43</td>
<td>5,977</td>
</tr>
<tr>
<td>21</td>
<td>0.44</td>
<td>9,667</td>
</tr>
<tr>
<td>22</td>
<td>0.44</td>
<td>10,515</td>
</tr>
<tr>
<td>23</td>
<td>0.42</td>
<td>9,239</td>
</tr>
<tr>
<td>24</td>
<td>0.41</td>
<td>6,501</td>
</tr>
<tr>
<td>25</td>
<td>0.40</td>
<td>4,682</td>
</tr>
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<td>0.39</td>
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<tr>
<td>27</td>
<td>0.35</td>
<td>2,472</td>
</tr>
<tr>
<td>28</td>
<td>0.37</td>
<td>1,817</td>
</tr>
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</table>

Key Words: estrus, cycle, pregnancy


The bacterial cell wall component lipopolysaccharide (LPS), depletes primordial follicles in ex vivo cultured bovine ovarian cortical strips and in exposed mice. LPS also affects mRNA encoding anterior pituitary hormones. Many studies have characterized negative ovarian effects of acute (bolus) LPS exposure. Our objective was to characterize the effects of chronic endotoxemia on follicular development in lactating cows. To create a more physiologically relevant LPS exposure paradigm, cows were continuously intravenously (i.v.) infused with LPS for 7d. Eleven lactating Holstein cows (164 ± 22 DIM; 676 ± 16 kg BW; parity 3.1 ± 0.4) were acclimated for 3d, and enrolled in 2 experimental periods (P); during P1 (3d) cows consumed feed ad-libitum and baseline serum samples were obtained. During P2 (7d), cows were assigned to either treatment 1) saline-infused and pair-fed (CON-PF; 40 mL/h saline; n = 5) or 2) LPS-infused and ad libitum-fed (LPS-AL; $E. coli$ O55:B5; 0.017, 0.020, 0.026, 0.036, 0.055, 0.088, and 0.148 μg/kg BW/h for d 1–7, respectively; n = 6). CON-PF cows were pair-fed to LPS-AL group to create uniform nutritional status. Estrous cycles were synchronized using a modified Ovsynch protocol before the experiment such that ovulation from the previous estrous cycle occurred on P1D2 and the first wave of follicular growth was monitored using trans-rectal ultrasonography every 24h. Dominant follicles increased in size in CON-PF (33%) and LPS-AL (30%) ovaries between d 4 and d 7 post-induction of ovulation, with no impact ($P > 0.05$) of LPS on either growth rate or size of the dominant follicle on d 7. LPS did not affect ($P > 0.05$) concentration of progesterone in serum or follicular fluid or serum $17\beta$-estradiol. There was a trend for increased $17\beta$-estradiol in serum (44%; $P = 0.1$) in LPS-AL cows. These data do not rule out potential LPS effects on the ovarian follicular reserve, however, demonstrate the surprising capacity of lactating dairy cows to tolerate exponentially increasing chronic LPS exposure without disrupting dominant follicle growth. Interestingly, there is potentially greater circulating $17\beta$-estradiol in LPS-exposed cows, without any impact on progesterone abundance. Supported by Land-O-Lakes fellowship to MJD.

Key Words: LPS, ovary, estradiol


Performing pregnancy diagnosis sooner after TAI could decrease days open in dairy cows if non-pregnant cows are enrolled in resynchronization programs. Methods for accurate pregnancy diagnosis within 3 wk after TAI are based on interferon tau-stimulated gene (ISG) expression in blood but milk samples are often more convenient to obtain than blood samples. The objective was to assess the utility of measuring ISG15 expression in milk somatic cells as a method to diagnose pregnancy in cows after TAI. Blood (10 mL) and composite milk (200 mL) samples were collected from 48 primiparous and 13 multiparous Holstein cows (n = 61; 102 ± 12 DIM; 36 ± 20kg/d) at 18, 20, and 22 d after TAI. Samples were placed on ice after collection and RNA was extracted on the same day. RNA samples were used for cDNA synthesis and cDNA was used in RT-PCR analysis of gene expression for ISG15 and cyclophilin (reference gene). Ratios of ISG15 to cyclophilin (ICR) were calculated. Milk somatic cell expression ratios were log-transformed before analysis to reduce variance. Transrectal ultrasonography diagnosis for pregnancy at 33 d or 35 d after TAI was the reference standard. The ICR of blood and milk cells were tested for the effects of pregnancy status, day, parity, and interactions using the MIXED procedure of SAS 9.4 (Cary, NC). The REG procedure of SAS was used to determine the correlation between milk and blood ICRs. The ICR was greater in blood of pregnant cows (0.58 ± 0.07; n = 28 compared with non-pregnant cows (0.12 ± 0.06; n = 33) on d18, 20, and 22 ($P < 0.0001$). In same cows and on the same days, milk somatic cell ISG15 expression was also greater in pregnant (0.64 ± 0.17) compared with non-pregnant (0.20 ± 0.16) cows ($P < 0.05$). Day of sampling did not affect ISG15 expression for either sample type. Overall, ISG15 expression in both blood and milk somatic cells was greater for pregnant compared with non-pregnant
Holstein cows. Testing milk for ISG15 expression may be an alternative to ISG15 testing in blood.

Key Words: pregnancy, milk, ISG15

Effects of nerve growth factor-β on luteal function and markers of conceptus development in cattle. J. S. Stewart¹, V. R. G. Mercadante², I. F. Canisso¹, and F. S. Lima*¹, ¹University of Illinois, Urbana-Champaign, IL, ²Virginia Tech University, Blacksburg, VA.

Nerve growth factor-β (NGF) is a seminal plasma protein that has been found to improve corpus luteum (CL) function in heifers. The objective of this study was to determine if systemic administration of NGF, purified from bovine seminal plasma, would enhance CL function and conceptus development in cows. Our hypothesis was that NGF administration at artificial insemination (AI) would increase progesterone (P4) production and expression of interferon-stimulated genes (ISGs) and pregnancy-specific protein B (PSPB), markers of conceptus development. NGF was purified from bull seminal plasma using a combination of anion and cation exchange chromatography and gradient elution. Beef cows were randomly assigned to CONT (n = 30) or NGF (n = 30) groups and synchronized using a 7-d Co-Synch + CIDR program. At time of AI (d 0), NGF cows received 296 µg purified NGF, reconstituted in 12 mL phosphate buffered saline intramuscularly. Blood samples were collected from each cow for quantification of peripheral P4 (d 0, 3, 7, 10, 14, 19) and PSPB (d 24) concentrations. Peripheral blood leukocytes were harvested at d 19 for measuring expression of ISGs (ISG15, MX1, MX2, RTP4) by qPCR. Pregnancy diagnosis was performed via ultrasonography exam on d 28 post AI. Statistical analysis was performed using ANOVA with repeated measures (SAS 9.4, Cary NC). NGF cows had increased plasma higher concentration of P4 than CONT cows from d 10–19 (P = 0.04). Pregnancy rates at 28 d were 75% in NGF cows versus 59% in CONT cows (P = 0.13). In pregnant cows, PSPB concentrations were higher in NGF than CONT cows (P < 0.05) at d 24. Additionally, expression of ISG15 and MX2 were greater in pregnant NGF cows than in pregnant CONT cows (P < 0.05) at d 19, but not significantly different for MX1 and RTP4 were present. Collectively, these results demonstrate that NGF administration at AI improved CL function and enhanced markers of conceptus development. Future studies are warranted to investigate whether NGF can be used to improve reproductive efficiency of cattle.

Key Words: conceptus, interferon-stimulated genes, luteotrophic
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Precision Dairy (PD) Management Today


The aim of this summary is to provide new insights into the use of data from estrus events and automated activity monitors as a tool to predict fertility. Recently, more studies have demonstrated how estrus events and intensity is associated with ovulation, ovarian and uterine function, and fertility. In one study, the likelihood of ovulation was greater for high vs. low relative increase estrus, but a more detailed experiment also showed slight differences in the timing of ovulation. Expression of estrus near AI also modified the expression of genes related with the immune system, adhesion molecules and prostaglandin synthesis in the endometrium (MX1, MX2, MYL12A, MMP19, CXCL10, IGFL1, SLPI, OTR and COX-2) and those related with apoptosis, P4 synthesis and prostaglandin receptor (CYP11A, BAx and FPr) in the CL. The expression of estrus (yes vs no) was associated with increased P/AI for timed-AI (38.9 vs. 25.5%) and embryo transfer (46.2 vs. 32.7%) protocols. Moreover, there was a decrease in pregnancy loss in both programs. Data from other recent studies involving spontaneous and induced estrus have shown that greater relative increase and longer duration of estrus, captured by different activity monitors, significantly improve P/AI (over 12% points across different studies). Intensity and duration of estrus were correlated with BCS, parity, milk production and secondary behavior signs as expected, but surprisingly not associated with follicle diameter and concentration of estradiol at estrus. Collectively, ovulation could partly explain the observed reduction in fertility, but it is clear that the endometrium and the CL play an important role. Quantitative information from estrus events could be used to improve estrous detection quality and develop decision-making strategies at the farm level. Further studies in this field should aim to 1) better understand ovarian, embryo and endometrium mechanisms associated with either the expression or intensity of estrus and, 2) refine the collection of phenotypes related to estrus (i.e., relative increase, absolute increase, baseline levels, duration, and repeatability within cow) to improve estrous detection and possibly genetic selection.

Key Words: activity monitor, dairy cow, estrous expression

316 Dairy cattle health and welfare in the precision dairy world. D. Kelton*, University of Guelph, Guelph, ON, Canada.

Precision dairy farming can be defined as the use of sensor technologies to measure the physiology, behavior and production of individual animals for the purposes of managing the herd or individuals within the herd. With increasing herd size and automation, the regular intimate contact between the farmer and his animals is decreasing, and in some cases being replaced by technologies that could serve the function of identifying individuals or groups of animals which need attention because they are diseased or in distress. In the context of dairy cattle health and welfare there are many sensors and systems that have been developed to monitor or detect mastitis, metabolic disease, lameness, calf disease and overall cow comfort. This review will highlight some of the currently available technologies, including their associated opportunities and challenges. The opportunities to use inputs from multiple sensors to inform robust decision support systems are intriguing. However, there are 3 major issues that limit the widespread reliance on these sensor technologies at this time. First, most sensor systems have not been thoroughly evaluated and validated against appropriate reference methods or across a broad range of animal and farm environments. Second, the decision support systems underlying these technologies are often simplistic, not transparent to the user, and don’t make use of the broad range of inputs that are often available. Third, there is a lack of experience, expertise and support among dairy herd advisors who are working with dairy producers trying to incorporate these technologies into their management programs. Until these issues are addressed, the widespread reliance on precision dairy technologies for managing dairy cattle health and welfare will not reach its potential.

Key Words: health, welfare, precision dairy

317 Producer experience with precision dairy. B. Biehl*, Corner View Farm, Kutztown, PA.

Corner View Farm began milking a couple cows in 1915 by Ammon Biehl. It was a typical farm in Berks County, PA, with 14 cows in a tie stall arrangement. The family farmed 93 acres to support the dairy and crop sales. At that time, there was one hired employee to help with the chores. Five generations later, young Blake (age 13) and Baxter (age 11), live on the same property and walk around the same farm with their iPhones in a very different time. They can watch over the cows with IP cameras and control barn functions from their remote touch screens. All of this transformation has been witnessed by second generation Leroy Biehl, who recently turned 92 years old. In December 2011, the Biehls began milking in their new free-flow, 3-row, 120-stall robotic milking facility, equipped with the Astrea 20.20 robot, supplied by AMS Galaxy USA. The foundation of the robot is a standard Motoman HP20 industrial robotic arm that has 6 axis for 6 degrees of freedom allowing it to prep and attach cows in a milking stall on each side of the central milking unit. The single robotic system has milked up to 125 cows at time. Brad not only watches from his smart phone, he has the ability to navigate the Saturnus 20.20 robotic milking and herd software. From anywhere, he can track activity monitoring, milking statistics, sorting sick cows, separating milk for treated cows, and managing herd records. However, it doesn’t stop there. From the touch screen of his phone, Brad can fully control, 5 fans, 2 curtains, sprinkler system, 4 fans, thermostats for floor heating, roll-up doors, and 6 sections of lighting control technology. All of the automation is also controlled by programming that keeps curtains closed when it’s raining, curtains open when the barn is warm, curtains closed when it’s too cold, lighting controlled to maximize cow traffic / production, and fans running only when needed to conserve energy. In 2015 and 2016, Corner View Farm added additional enhancements including the Hetrwin automatic feed pusher, Hetrwin Bedding robot, and Urban Alma Automatic Calf Feeders. Other new precision dairy include the Galaxy Heat Herd Health module that monitors activity, chewing monitoring, and cow position. These additional precision dairy monitors continue to enhance cow health.

Key Words: Corner View Farm, robotic milking
Precision dairy technologies are technologies that collect data by monitoring physiological, behavioral, or production indicators related to health or fertility of individual cows (e.g., automated detection of estrus, mastitis, or lameness). Goals of these technologies are to support management, improve animal health and welfare, and increase profitability. Demands for these technologies are rising, driven by increasing farm management complexity, availability of cheaper technologies, and societal concerns around animal health and welfare. Despite the rising demand, to date adoption of most sensor technologies have been modest. For instance, attempts to automate lameness detection involve automated gait analysis, such as force platforms, 3D-accelerometers or image-based technologies. However, adoption is low since most of these technologies are not (yet) ready to function under practical circumstances. Moreover, there are uncertainties on what exactly needs to be monitored, and what action is required once an alert for lameness is generated. This lack of knowledge inhibits economic calculations on these technologies. Similar adoption issues are seen with clinical mastitis detection in conventional milking parlors. The monitored indicators are proxy measures for clinical mastitis, resulting in suboptimal detection performance (too many cases are missed, and too many false alerts are generated). Also, technical failures are common, and investment costs can be significant. These shortcomings led to the conclusion that investing in automated mastitis detection systems was not profitable for an average-sized pasture-grazed New Zealand farm. The aforementioned examples deduct essential criteria to ensure adoption of precision dairy technologies: indicators have to be associated with events of interest, it should be clear what exactly has to be monitored, reflecting farmers’ needs, and this in turn has to be associated with a clear (autonomous) management action. A positive economic benefit will further fuel adoption, but is not crucial. These criteria are all met by estrus detection systems, and thus, it should be no surprise that these are one of the most successful precision dairy technologies today?

**Key Words:** sensor technologies, economic value, adoption criteria
319 Validation of an accelerometer to monitor rumination, eating and activity in an organic grazing dairy herd. G. Pereira1, S. Ferneborg1, R. A. Black3, S. Agenäs1, M. Thulin2, K. Svensnersten-Sjauinja1, E. Ternman*1, and P. D. Krawczel3, 1University of Minnesota, West Central Research and Outreach Center, Morris, MN, 2University of Minnesota Extension, St. Paul, MN.

The objective of this study was to validate an accelerometer (CowManager SensOor, Agis Automatisering BV, Harmelen, the Netherlands) by direct visual observation in an organic grazing dairy herd. The sensor detects and identifies ear movements and through algorithms can classify data as ruminating, eating, resting or active behaviors. Pasture-based lactating Holstein and crossed-breed cows (n = 24) were observed for 12 h each by a single trained observer who recorded cow behaviors every min for 6h/day. The study was conducted at the University of Minnesota West Central Research and Outreach Center organic dairy in Morris, Minnesota from June to September 2016. Direct visual observation was compared with CowManager sensor data during June and July 2016 (early summer, before a software system update) and during August and September 2016 (late summer; after a software system update) having each minute classified with only one of the following categories: ruminating, eating, resting or activity. Pearson correlations and concordance correlation coefficient (PROC CORR of SAS), bias correction factors (Cb), location shift (V) and scale shift (μ) (epiR package of R software) evaluated associations between sensor data and direct visual observations. Furthermore, pasture fly counts of horn, face and stable flies were used to evaluate associations with sensor data. Correlations between CowManager sensor and visual observations for all 4 behaviors were greater for late summer compared with early summer. For late summer, visual observation correlations were mostly moderate to high (0.72, P < 0.01 for ruminating; 0.88, P < 0.01 for eating; 0.65, P < 0.01 for resting; 0.20, P < 0.01 for activity) compared with sensor data. The active behavior was the most associated with and affected by pasture fly populations (0.22; P < 0.01). The results suggest the CowManager sensor may accurately monitor rumination and eating behavior of grazing dairy cattle. However, it appears that sensor accuracy may be affected by the fly pressure in grazing dairy cattle.

Key Words: precision technology, grazing, rumination

320 Milking efficiency in AMS—Effects of teaser feed and take-off level. S. Ferneborg1, R. A. Black3, S. Agenäs1, M. Thulin2,1, K. Svensnersten-Sjauinja1, E. Ternman*1, and P. D. Krawczel3, 1Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, Uppsala, Sweden, 2Uppsala University, Department of Statistics, Uppsala, Sweden, 3The University of Tennessee, Department of Animal Science, Knoxville, TN.

The objective of this study was to determine the effect of quarter-level take-off settings and feeding during milking on milk flow and efficiency within automatic milking systems (AMS). A total of 30 dairy cows (parity 2.9 ± 1.5, 142 ± 25 DIM, milk yield = 34.0 ± 11.7 kg/d and SCC <115 000 cells/mL) were exposed to treatments using a Latin square with 7d periods. Treatments were take-off (TO) at flow rates of 0.48, 0.30 or 0.06 kg/min on a quarter level using the settings of the AMS, combined with or without teaser feed during milking. Cows for which more than 8h elapsed since the last milking were brought to the AMS holding area, resulting in the milking interval 7.5 ± 0.4h. Data on milk yield and flow, and milking times were automatically collected and the last 2d of each treatment period were used for evaluation of treatment effects. An algorithm was developed for defining the decline phase of milk flow and overmilking was defined when milk flow rate falls below a value of 0.06 kg/min. Data were analyzed by ANOVA for a 6 × 6 Latin square with a 2 × 3 factorial arrangement of treatments in a linear mixed-effects model using repeated measures in SAS (v9.4).

Preliminary data suggest that feeding did not affect milking time, milk yield, mean flow, the length of the decline phase, or overmilking time (P > 0.05). Increasing TO from 0.06 to 0.48 decreased milking time (5.3 and 4.6 ± 0.28 min respectively; P < 0.001). However, milk yield was not affected by TO (P = 0.30). Mean flow was lowest at 0.06 compared with 0.30 and 0.48 (0.72, 0.80 and 0.81 ± 0.004 kg/min respectively; P < 0.001). Decline phase length was longer on 0.06 compared with 0.30 and 0.48 (80.1, 60.9 and 52.2 ± 2.3s respectively; P < 0.001), as were overmilking times (9.3, 1.2 and 0.1 ± 0.05s respectively; P < 0.001). Actual flow at TO was on average 0.0, 0.025 and 0.088 ± 0.093 kg/min for 0.06, 0.30 and 0.48 kg/min respectively (P < 0.001). These data suggest it is possible to reduce milking time by setting a higher take-off level on quarter level in AMS without losing milk yield. Feeding during milking did not affect mean flow or milking efficiency. Overall, milking efficiency of the AMS appears to be linked to take-off level rather than teaser feed.

Key Words: take-off level, milk flow

321 Daily milk production, number of milkings, feed consumption and rumination time for cows in robotic milking systems in the United States. J. M. Siewert*1, J. A. Salfer2, and M. I. Endres1, 1University of Minnesota, St. Paul, MN, 2University of Minnesota Extension, St. Cloud, MN.

Robotic milking systems (RMS) are becoming more common in the USA, but there is yet limited research available. The objective of this study was to compare daily milk production, number of milkings, robot box feed consumption and rumination time between primiparous and multiparous (2nd and > parity) cows in RMS at various stages of lactation. Data were collected daily from 31 farms for approximately 18 mo and analyzed up to 400 DIM. Eight categories of DIM were evaluated: < 7, 8–30, 31–60, 61–90, 91–120, 121–150, 151–250, and >250 DIM (but only some are reported). A mixed model analysis was conducted with parity, DIM category and parity × DIM category included as fixed effects and farm as random effect. Daily milk production (n = 2,703,075 cow-d) differed between primiparous and multiparous cows at all stages of lactation with a parity×DIM detected (P < 0.001). Notably milk production (primiparous and multiparous, respectively) was 15.6 and 24.2 kg for <7 DIM; 30.2 and 43.2 kg for 31–60 DIM (when multiparous cows peaked); 31.3 and 42.4 kg for 61–90 DIM (when primiparous cows peaked); and 25.2 and 24.3 kg for >250 DIM. Number of milkings/d (n = 2,703,075 cow-d) followed a similar pattern and also differed between parities with a parity × DIM detected (P < 0.001). Milkings/d was 1.87 and 2.50 for <7 DIM; 2.58 and 2.99 for 31–60 DIM, 2.64 and 2.96 for 61–90 DIM, and 2.39 and 2.23 for >250 DIM, for primiparous and multiparous cows, respectively. In addition, daily robot pellet consumption (n = 2,697,998 cow-d) also differed between parities with a parity × DIM detected (P < 0.001). Consumption was 2.93 and 3.29 kg for <7 DIM; 5.37 and 6.49 kg for 31–60 DIM and was reduced to 3.76 and 3.74 kg for >250 DIM for primiparous and multiparous cows, respectively. Consumption peaked at 31–60 DIM for both parity categories. Daily rumination (n = 1,465,606 cow-d) averaged 422.1 and 465.3 min for primiparous and multiparous cows (P < 0.001), respectively. It appeared that primiparous
cows in RMS produced less milk in comparison to multiparous cows than expected (mean peak milk ratio of 0.72) which suggests that performance benefits may be achieved by improving management of these cows in RMS.

**Key Words:** robotic milking, milking/day, milk production

**322 Economic and environmental performance of traditional and grass-fed organic dairies using the Integrated Farm System Model.** R. A. V. Santana1, A. F. Britto2, V. E. Cabrera3, F. A. Barbosa3, A. K. Hoshide4, A. F. Benson4, A. N. Hafla5, H. M. Darby5, K. J. Soder7, and R. Kersbergen9, 1Instituto Federal de Educação, Ciência e Tecnologia do Norte de Minas Gerais–Campus Arinos, Arinos, MG, Brazil, 2University of New Hampshire; Department of Biological Sciences, Durham, NH, 3University of Wisconsin; Department of Dairy Sciences, Madison, WI, 4Universidade Federal de Minas Gerais; Departamento de Zootecnia, Belo Horizonte, MG, Brazil, 5University of Maine; School of Economics, Orono, ME, 6Cornell University; Cornell Cooperative Extension, Cortland, NY, 7USDA-ARS; Pasture Systems and Watershed Management Research Unit, University Park, PA, 8University of Vermont; Department of Plant and Soil Sciences, St. Albans, VT, 9University of Maine; Cooperative Extension and School of Food and Agriculture, Orono, ME.

Organic milk production is one of the fastest growing segments of US agriculture. There is an increasing number of US organic farmers who are transitioning to no grain supplementation due to additional premiums paid by some milk processors. However, there is limited information about the economic and environmental performance to support farmers’ decision to make the transition from grain to no grain feeding. Our objective was to compare the economic and environmental performance of traditional (ORG-T) vs. grass-fed (ORG-GF) organic dairy farms using the Integrated Farm System Model over 25 years of daily weather conditions. An average farm with 90 ha of land base, 52 Holstein cows, and 186 d of grazing was constructed using data from 84 organic dairies across 6 states (WI, PA, NY, NH, VT, and ME). The ORG-T diet was characterized by pasture, conserved feed including grass-legume and corn silages, and grain during the grazing season, and conserved feed and grain during the winter season. The ORG-GF was characterized by an all pasture diet during the grazing season, and all conserved feed diet except corn silage during the winter season. Milk price and annual milk production used in the simulations averaged 71.5 vs. 81.7$/100 L and 6,590 vs. 4,879 kg/cow for ORG-T vs. ORG-GF, respectively. The net return/cow was 35% greater in the ORG-GF ($2,766) than ORG-T ($2,051). Additional premiums paid by milk processors to the ORG-GF farm system appear to compensate for its lesser milk when production compared with the ORG-T farm system. Average greenhouse gas (GHG) emission, including biogenic CO2, was 87% greater in ORG-GF (0.56 kg of CO2 eq/kg of ECM) than ORG-T (0.30 kg of CO2 eq/kg of ECM), which is not surprising due to increased fiber intake and lesser milk production in ORG-GF cows. Overall, the ORG-GF farm system seems to be more profitable that its ORG-T counterpart, but at expense of more GHG emissions per unit of ECM. Farmers adopting the ORG-GF management should develop strategies to improve forage quality and milk production to reduce their farm carbon footprint.

**Key Words:** cover crop, organic beef, omega fatty acid

**324 Relationships between protein and energy consumed from milk replacer and starter and first lactation production performance of Holstein dairy cows.** J. Rauba*, B. Heins*, H. Chester-Jones, D. Ziegler, and N. Broadwater, 1Milq Specialties Global, Eden Prairie, MN, 2University of Minnesota West Central Research and Outreach Center, Morris, MN, 3University of Minnesota Southern Research and Outreach Center, Waseca, MN, 4University of Minnesota Extension, Rochester, MN.

The objective was to determine relationships between protein and energy consumed from milk replacer and starter and first lactation performance of Holstein dairy cows. Data were collected from 4,535 Holstein animals from birth year of 2004 through 2014. Calves were received from 3 commercial dairy farms and assigned to 45 different calf research trials at the University of Minnesota Southern Research and Outreach Center from 3 to 195 d. Most calves were fed a 20% CP and a 20% fat milk replacer at a rate of 0.57 kg/calf per day. Milk replacer (MR) metabolizable energy (ME), starter ME, MR protein intake, and starter protein intake consumed from 0 to 8 weeks were (mean ± SD): 102.7 ± 13.2 Mcal/kg, 151.0 ± 24.2 Mcal/kg, 4.8 ± 1.0 kg, and 9.5 ± 2.7 kg, respectively. The MR ME, starter ME, MR protein intake, and starter protein intake consumed from first lactation production data was analyzed for 2,881 cows from the data set, which included 305-d milk, fat, and protein kg. Separate mixed model analyses were conducted with SAS to determine the effect of protein or energy consumed on first lactation production of milk, fat, and protein yield. Birth season, year, 6-week ADG class,
and herd were included in the model with calf trial as a random effect. The 305-d milk and component production were positively affected by early life ME ($P < 0.02$) and protein intake ($P < 0.03$; Table 1). Greater ME and protein intake in the first 8 weeks of life resulted in increased first lactation milk and milk components yield.

Table 1 (abstract 324). Effect of combined protein and starter energy (Mcal/kg) and protein (kg) consumed 0–8 weeks (estimates are regression slopes) on first lactation 305-d milk, 305-d fat, and 305-d protein yield (kg; n=2,880)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
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</tr>
<tr>
<td>MR and Starter ME 0-8wk</td>
<td>4.03</td>
<td>1.55</td>
<td>0.009</td>
</tr>
<tr>
<td>MR and Starter Protein 0-8wk</td>
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<td>10.12</td>
<td>0.011</td>
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<tr>
<td>305-d fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.06</td>
<td>0.005</td>
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<tr>
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<td>0.022</td>
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<td>305-d protein</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>0.001</td>
</tr>
<tr>
<td>MR and Starter Protein 0-8wk</td>
<td>0.87</td>
<td>0.29</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Key Words: milk replacer, starter, first lactation

325 Relationships between protein and energy consumed from milk replacer and starter and growth for Holstein dairy calves. J. Rauba*1, B. Heins2, H. Chester-Jones3, D. Ziegler4, and N. Broadwater4, 1Milk Specialties Global, Eden Prairie, MN, 2University of Minnesota West Central Research and Outreach Center, Morris, MN, 3University of Minnesota Southern Research and Outreach Center, Waseca, MN, 4University of Minnesota Extension, Rochester, MN.

The objective was to determine relationships between protein and energy consumed from milk replacer and starter and calf growth for Holstein dairy calves. Data were collected from 4,534 Holstein animals from birth year of 2004 through 2014. Calves were received from 3 commercial dairy farms and assigned to 45 different calf research trials at the University of Minnesota Southern Research and Outreach Center from 3 to 195 d. Calves were returned to their farms upon completion of the trial. Most calves were fed a 20% CP and a 20% fat milk replacer at a rate of 0.57 kg/calf per day. Milk replacer ME, starter ME, milk replacer protein intake, and starter intake consumed from 0 to 8 weeks were (mean ± SD): 102.7 ± 13.2 Mcal/kg, 151.0 ± 42.2 Mcal/kg, 4.8 ± 1.0 kg, and 9.5 ± 2.7 kg, respectively. Separate mixed model analysis were conducted with SAS to determine the effect of actual ME consumed from both milk replacer and starter and actual protein consumed from both ME and starter and average daily gain of the calves. Year of birth, season of birth and 8-week ADG class (<0.23, 0.23–0.34, 0.34–0.45, 0.45–0.57, 0.57–0.68, 0.68–0.79, > 0.79 kg/d) were included in the model with trial and herd as a random effect. Calves that had greater intake of protein during the first 8 weeks of life resulted in greater growth ($P < 0.01$; Table 1).

Key Words: milk replacer, starter, metabolizable energy


Diets with 2 contents of nonfiber carbohydrate (NFC) containing corn stover were formulated to compare the lactation performance and rumen fermentation characteristics in lactating cows fed alfalfa hay. Twelve Holstein cows in mid-lactation (159 ± 15 d in milk) were randomly assigned to 1 of 3 dietary treatments: (1) low-NFC (NFC = 35.6%, L-NFC), (2) high-NFC (NFC = 40.1%, H-NFC), and (3) alfalfa hay (NFC = 38.9%, AH). In both L-NFC and H-NFC diets, corn stover was included at 15% of total diet DM. The experiment was conducted according to a replicated 3 × 3 Latin square design with 21-d periods each, with the first 14 d for an adaptation. Milk yield and milk composition were recorded during d 15–21, and rumen fluid samples were taken on d 19 of each period. The data were analyzed using PROC MIXED of SAS. Intake of DM was lower for cows fed H-NFC compared with L-NFC and AH (20.1 vs. 21.5, and 21.9 kg/d; $P < 0.01$), while milk yield was higher in AH than in H-NFC and L-NFC (24.8 vs. 22.8 and 23.2 kg/d; $P < 0.01$). Thus, feed efficiency (milk yield/DM intake) were higher for cows fed H-NFC and AH than the L-NFC fed cows (1.15 and 1.15 vs. 1.08; $P < 0.01$). Milk fat content was higher for cows fed H-NFC and L-NFC compared with AH-fed cows (4.11 and 4.25 vs. 3.90%; $P < 0.01$). The NFC digestibility was higher in cows fed H-NFC and AH than those fed L-NFC (92.7 and 92.7 vs. 91.9%; $P = 0.03$). Concentration of milk urea N was lower for cows fed H-NFC and AH than those fed L-NFC (18.3 and 18.1 vs. 20.3 mg/dL; $P < 0.01$), indicating an increased N conversion for cows fed H-NFC and AH. The concentrations of rumen acetate (77.5 vs. 69.5 and 72.7 mM; $P < 0.05$), propionate (24.8 vs. 20.3 and 22.0 mM; $P < 0.01$) and total volatile fatty acids (120 vs. 106 and 111 mM; $P = 0.02$) were higher for cows fed AH than those fed H-NFC and L-NFC, with no difference between cows fed H-NFC and L-NFC ($P > 0.05$). From the results obtained in this study, it is inferred that the increased NFC content can improve feed efficiency in diet containing corn stover, and is beneficial for the N conversion.

Key Words: corn stover, nonfiber carbohydrate, lactation performance

Table 1 (abstract 325). Least square means of ADG class and MR and starter ME (Mcal/kg DM) and protein (kg of DM) consumed 0–8 weeks (n=4534 cows)

<table>
<thead>
<tr>
<th>Variable</th>
<th>P-value</th>
<th>&lt;0.23</th>
<th>0.23–0.34</th>
<th>0.34–0.45</th>
<th>0.45–0.57</th>
<th>0.57–0.68</th>
<th>0.68–0.79</th>
<th>&gt;0.79</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR ME</td>
<td>&lt;0.001</td>
<td>110.7</td>
<td>108.0</td>
<td>102.5</td>
<td>101.4</td>
<td>101.9</td>
<td>102.2</td>
<td>105.0</td>
</tr>
<tr>
<td>Starter ME</td>
<td>&lt;0.001</td>
<td>36.8</td>
<td>52.7</td>
<td>84.1</td>
<td>115.8</td>
<td>143.9</td>
<td>172.7</td>
<td>207.0</td>
</tr>
<tr>
<td>MR Protein</td>
<td>&lt;0.001</td>
<td>5.3</td>
<td>5.0</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.9</td>
</tr>
<tr>
<td>Starter Protein</td>
<td>&lt;0.001</td>
<td>2.3</td>
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<td>7.3</td>
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<td>13.0</td>
</tr>
</tbody>
</table>

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Models originally compiled to predict nutrient absorption from the digestive tract and metabolized in various tissues could be adapted for CH₄ predictions. Numerous empirical equations and mechanistic models to predict CH₄ emission are available. The Molly cow model is a mechanistic, dynamic model describing digestion and metabolism of dairy cattle with the ability to predict the animal-related factors that affect the environment, including CH₄ emission (Hanigan et al., 2013). The Nordic cow model Karoline is a dynamic, mechanistic model describing digestion and metabolism in dairy cows (Danfer et al., 2006), and it was confirmed by Ramin and Huhtanen (2015) to be a useful tool in predicting CH₄ emissions in cattle. The aim was to evaluate these models for predicting CH₄ emissions in cattle using a data set consisting of 267 treatment means from 55 respiration chambers studies. The data set contained DMI, (14.2 ± 5.82 kg/d); ingredient proportions; dietary contents of CP (156 ± 30.8 g/kg) and NDF (356 ± 105.9 g/kg); BW (531 ± 131.1 kg); and CH₄ (303 ± 118.7 g/d) which covers the range of typical cattle diets. The simulations were conducted using observed DMI, BW and dietary nutrient concentrations and digestion rates. Each treatment mean was simulated and predictions of nutrient digestibility and CH₄ output were collected in a database. The relationships between observed and predicted CH₄ (pCH₄) were assessed by regression analysis. Root mean square error (RMSpE) was calculated as: RMSpE = √[(Σ(Obs – Pred)²)/n]. Molly predictions were: CH₄ (g/d) = 0.81 ± 0.018 × pCH₄ (g/d) + 38 ± 6.4 (RMSpE = 54.9 (18.1% of observed mean) CCC = 0.910). The corresponding equation for Karoline was: CH₄ (g/d) = 1.00 ± 0.019 × pCH₄ (g/d) + 5 ± 6.0 (RMSpE = 34.6 (11.4%) CCC = 0.955). Both mean (~27 g/d) and linear bias (~0.19) were significant (P < 0.001) with Molly, but only mean bias (4 g/d) was significant (P = 0.04) with Karoline. Proportions of MSE attributable to mean and linear bias and random error were 23, 24 and 53% for Molly, and 2, 0 and 98% for Karoline, respectively. Based on predictions it can be concluded that both models predicted CH₄ emissions reasonably well in terms of high CCC, but Karoline was more accurate based on smaller RMSE, mean and slope bias.

Key Words: prediction model, DMI, dairy cow

**Development of equations to predict dry matter intake of lactating cows using factors related to the filling effect of rations.**

Our objective was to predict dry matter intake (DMI) by lactating cows using factors related to the filling effect of rations. A database of 156 treatment means from 44 experiments reported in the literature was developed. The database included data for cows ranging from 60 to 309 days postpartum and included diet forage NDF (NDFD) content (23.6 ± 5.8, mean ± SD), diet ADF/NDF (0.61 ± 0.08, mean ± SD), and a laboratory measure (in vitro or in situ) of NDF digestibility (LNDFD) of the sole forage or major forage (51.2 ± 12.1, mean ± SD). Models included the random effect of study as well as its interaction with LNDFD to account for differences in methods used to determine LNDFD. The full model also included linear and quadratic effects of NDFD, ADF/NDF, and LNDFD, as well as their linear and quadratic interactions, mean milk yield (MY) for each study and its interaction with the diet factors. Equations were developed by stepwise regression with backward elimination and treatment means were weighted by the inverse of their variance. The prediction equation for DMI (r² = 0.80, RMSE = 2.76 kg/d) is: DMI (kg/d) = 13.4 - 0.078 × FNDF + 8.264 × ADF/NDF + 0.0126 × LNDFD + 8.453 × (ADF/NDF-0.602)² + 0.178 × MY – 0.172 × (ADF/NDF-0.602) × (MY-33.1) + 0.0060 × (LNDFD-48.2) × (MY-33.1) + 8.183 × (ADF/NDF-0.602)² × (MY-33.1). DMI was positively related to MY and ADF/NDF and negatively related to FNDF, while LNDFD was negatively related to DMI for cows with low MY but positively for cows with high MY. Response to higher ADF/NDF was greater as MY increased. ADF/NDF was included to represent differences in forage fragility between grasses and legumes but it is also affected by the fraction of cereal grain in the diet. The following equation was developed for when LNDFD data is not available (r² = 0.77, RMSE = 3.01 kg/d) using the same database: DMI = 11.6 - 0.097 × FNDF + 8.31 × ADF/NDF + 0.268 × MY. DMI was related positively to MY and ADF/NDF and negatively to FNDF with no interactions detected with MY. These...
equations might be useful to evaluate DMI response to factors related to the filling effects of rations.

**Key Words:** rumen fill, diet formulation, forage

### 330 A meta-analysis of starch concentration in dairy calf feeds on growth and digestibility

W. Hu, T. M. Hill*, F. X. Suarez-Mena, T. S. Dennis, J. D. Quigley, and R. L. Schlatterbeck, Nurture Research Center, Provinci, Brookville, OH.

There are limited published studies evaluating the concentration of starch in a dry starter diet on growth and digestibility in Holstein dairy calves through 16 wk of age. Our objectives were to improve this understanding using a database developed from published studies from our lab that included 4 trials from 0 to 8 wk of age (10–43% starch), 8 trials from 8 to 16 wk of age (19–50% starch), and 3 digestibility trials from 6 to 16 wk of age (13–50% starch). A meta-analysis was conducted to examine potential relationships between starch concentration in the starter (% dry matter, DM; STA) and ADG (kg/d), hip width change (cm/d; HWC), or DM digestibility (%; DMD). Factors such as ME intake (Mcal/d), CP intake (g/d), DM intake (% of body weight), and ambient temperature, which might have influences on the responses to STA, were also evaluated using mixed-effects models. Milk replacers (4.7–4.8 Mcal ME/kg) were fed at an equal rate among starter treatments within a trial. Chopped grass hay (5%) was blended with the starter from 8 to 16 wk. Starters were 18–20% CP and 2.8–3.0 Mcal ME/kg. Within a trial, starters were equal in CP with a high starch (>38%) control. Depending upon the trial, one or more low starch ingredients (cottonseed hulls, soybean hulls, wheat middlings, distillers dried grains with solubles) replaced corn to reduce the starch and ME concentrations of the feed. Linear relationships were: 0–8 wk HWC = 0.000262 × STA + 0.0562 (P = 0.01; R² = 0.75); 0–8 wk ADG = 0.001345 × STA + 0.4817 (P = 0.13; R² = 0.42); 8–16 wk HWC = 0.000724 × STA + 0.7432 (P < 0.0001; R² = 0.76); 8–16 wk ADG = 0.00575 × STA + 0.8608 (P = 0.003; R² = 0.61); 6–16 wk DMD = 0.2777 × STA + 69.988 (P = 0.02; R² = 0.89). Increasing intake of ME (P < 0.0001) improved ADG from 8 to 16 wk along with STA. Intake of CP and DM and ambient temperature did not impact growth or digestibility. From this, changing a feed from 20 to 38% starch (within a typical range in the US industry) was predicted to increase DMD from 6 to 16 wk by 6%, HWC from 0 to 8 wk of age by 7%, and HWC from 8 to 16 wk of age by 14%. Increasing starch concentration of feeds increased digestibility and calf growth through 16 wk of age.

**Key Words:** calf, formulation, starch

### 331 Effects of transition nutrition on the fertility of lactating dairy cattle: A meta-analysis

R. M. Rodney1,2, P. Celi3,4, W. Scott1, K. Breinholt1, and I. J. Lean*1,2, 1Schibus, Camden, NSW, Australia, 2School of Life and Environmental Sciences, Faculty of Veterinary Science, University of Sydney, Camden, NSW, Australia, 3DSM Nutritional Products, Animal Nutrition and Health, Columbia, MD, 4Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC, Australia.

This meta-analysis of 39 studies containing 118 individual diets explored the effects of early lactation diets on the proportion of cows pregnant (PP) to service and calving to pregnancy interval. It was hypothesized that diet, intake of nutrients, and production after calving would affect these reproductive measures. Four negative binomial multivariable models, that included a random effect of study, were used to examine the effects of diet and production on proportion pregnant. They examined: i) output of products, ii) balance or duodenal intakes of nutrients, iii) oral intake of nutrients, or iv) dietary percentage of nutrients. The multivariable models identified that increased milk protein yield (kg/d; Relative Risk (RR) 0.45, 95% CI 0.281 to 0.703), sugar intake (kg/d; RR 0.813, 95% CI 0.713 to 0.927), and sugar % (% DM; RR 0.96, 95% CI 0.930 to 0.990) were associated with a decrease in RR for PP. Metabolizable energy balance (MJ/d; RR 1.004, 95% CI 1.001 to 1.008), duodenal c14:0 intake (g/d; RR 1.008, 95% CI 1.005 to 1.015), increased fatty acid intake (kg/d; RR 1.0003, 95% CI 1.000 to 1.001), and starch intake (kg/d; RR 1.061, 95% CI 1.006 to 1.118) were associated with improved RR for PP. Unvariably, increased metabolizable energy balance (MJ/d) was associated with a shorter calving to pregnancy interval, while increased milk production (kg/d) was, and increased intake of lysine and threonine (g/d) tended to be, associated with longer time to pregnancy. Nutritional management was associated with reproductive success. The study identified associations of specific carbohydrate fractions to proportion pregnant. The importance of dietary fats and positive energy and protein balances in early lactation for improved fertility outcomes was also supported.

**Key Words:** fertility, transition, nutrition

### 332 Repeatability of residual feed intake across diets with two levels of dietary protein content

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Our objective was to examine the repeatability of residual feed intake (RFI) across diets with different levels of dietary protein. Mid lactation Holstein cows with initial MY 42 ± 10 kg/d (n = 88, in 3 blocks) were fed either a high protein diet (18% CP; 18P) or a low protein diet (14% CP; 14P) in a crossover design with 2 28-d treatment periods. Diets were similar and both contained at least 10% RDP (DM basis). The 18P diet contained expeller soybean meal, which was replaced by soybean hulls and ground corn in the 14P diet; 18P diet provided 4 percentage units more CP, 2 units less starch and 2 units less NDF than 14P. Cows were milked 2×/d; DMI and MY were recorded daily. Milk composition was measured during 4 consecutive milkings each week and BW was measured 3× weekly. Fixed effects of experiment, parity, diet and period nested within experiment and random effect of cow were included in the model to compare intake and production performance of treatments. RFI value was calculated for each cow on each treatment based on her actual intake, milk energy output, metabolic BW, and body energy change. Cows were ranked as high (>0.5 SD), medium (±0.5 SD) or low (<−0.5 SD) RFI. Compared with the 14P diet, the 18P diet increased DMI by 1.3 kg/d (27.1 vs. 25.8 kg/d; P < 0.01), milk yield by 5.1 kg/d (43.8 vs. 38.7 kg/d; P < 0.01), and BW gain by 0.22 kg/d (0.49 vs. 0.27 kg/d; P < 0.01). Ranking of cows for RFI was relatively repeatable (r = 0.64; P < 0.01). Of all cows, 64% maintained their group ranking across treatments whereas 33% changed ranking by 1 group. Only 2% moved in the ranking from the high to the low RFI group or vice versa. Compared with the previous studies where RFI repeatability was 0.73 across starch levels and 0.44 across forage levels, we presume that nutrient digestibility and protein efficiency are the 2 main sources for RFI variation. In conclusion, RFI was relatively repeatable across 2 diets varying in protein content enough to cause marked changes in production. We suggest this supports the use of RFI as part of a genomic breeding index to enhance feed efficiency.

**Key Words:** deficient dietary protein, residual feed intake, breeding index
333 Early lactation meal size, but not meal frequency, is positively associated with whole-lactation milk production and retention in the dairy herd. A. J. Carpenter, M. Wood, and B. J. Bradford*, Kansas State University, Manhattan, KS.

The importance of feed intake for a successful transition to lactation in dairy cattle is frequently cited, but little is known about whether feeding patterns predict key lactation outcomes. To explore this, data from 185 cows in 4 transition studies (10 treatments) were collated for meta-analysis. Cows were housed in tie stalls for > 20 d postpartum, with continuous monitoring of feed bunk weight to determine size and duration of each meal. Unique meals were ≥ 0.2 kg DM and were separated by ≥ 12 min. Additional data collected included treatment, parity, month of calving, year of calving, time to removal from herd (only if before subsequent calving), and 305-d mature equivalent milk yield (305MEM, kg). Associations with 305MEM were assessed in mixed models with predictors tested as linear and quadratic effects, and with treatment(study) as a random effect; model selection was by minimum Bayesian Information Criterion. A similar approach was used to model risk of removal from the herd in a proportional hazards model. DMI through 20 d in milk was positively associated with 305MEM (P < 0.001), but only accounted for 10% of the variation in 305MEM, respectively (both P < 0.001). Backward stepwise regression generated the following model (all factors P < 0.05, model P = 0.02, r² = 0.53): 305MEM = 12508 + 1.71 × MilkPTA + 229 × MS − 43.5 × (MS − 5.46)², where MS is expressed in kg DM. In contrast to these findings, meal frequency was negatively associated with 305MEM (P = 0.02, r² = 0.03). Survival analysis revealed significant associations with 305MEM, year, parity, and ML; a 1-min increase in mean ML was associated with an 11% decrease in risk of removal from the herd (risk ratio 95% CI: 0.82–0.97; P < 0.01). Meal frequency and MS were not associated with altered risk of removal. Increased 305MEM and longer retention in the herd were associated with longer, but not more frequent, meals in the first 20 d of lactation.

Key Words: transition cow, feeding behavior, appetite


The objectives of this study were to evaluate the effects of particle size and source of undigested neutral detergent fiber (uNDF) on dry matter intake (DMI), milk production and composition, and chewing behaviors. Fifty-six Holstein cows averaging 102 (SD = 28) DIM were fed one of 3 brown midrib (BMR) corn silage based diets balanced for equal uNDF240 intake (2.32 kg/d) and varying straw length (0.82 vs 0.66 physical effectiveness factor; p<ef) in a completely randomized block design with a 2 wk covariate period and 4 wk treatment period. Treatments were 1) 42% BMR corn silage (1.01 kg/d of uNDF240) and 7% long straw (0.74 kg/d of uNDF240;LS), 2) 42% BMR corn silage (1 kg/d of uNDF240) and 7% short straw (0.73 kg/d of uNDF240;SS), and 3) 60% BMR corn silage (1.54 kg/d of uNDF240) and 2% long straw (0.23 kg/d of uNDF240;BMR). Cows were housed in freestall pen, fed TMR daily with Calan bins, and milked 3x/d. Feed intake and milk yield were collected daily. Milk composition and body weight were collected weekly. Chewing behavior was collected for 72 h the last week of both periods. Data were summarized by week and analyzed using MIXED procedure in SAS with cow as experimental unit. The uNDF240 intake for the LS, SS, and BMR diets were 2.32, 2.28, and 2.36 kg/d. Results reveals that particle size and source of uNDF will affect milk fatty acid profile and chewing behaviors.

### Table 1 (abstract 334).

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Within a row, different superscripts differ at P ≤ 0.05.

Within a row, different superscripts differ at P ≤ 0.10.

Key Words: particle size, undigested NDF, ruminations

335 Impact of ration nutrient density on the energy balance and inflammatory response of dairy cows during and after dry-off. K. M. Dancy*, E. S. Ribeiro, and T. J. DeVries, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.

The objective of this study was to assess the effects of ration nutrient density on the energy balance and inflammatory response of dairy cows during and following dry-off. Forty-eight Holstein dairy cows (1.7 ± 0.9 lactations) were dried-off over a 5-d period, approximately 60 d before their expected calving dates. At the start of dry-off (d 1), cows were randomly assigned to either 1 of 2 TMR: control diet (1.48 Mcal/kg, 13.3% CP and 32.0% NFC) and treatment diet (1.44 Mcal/kg, 12.6% CP and 29.7% NFC), which was diluted with extra straw. During dry-off, cows were milked 1x/d on d 1, 2, 3 and a final time on d 5. Before the start of dry-off, cows were producing 26 ± 5.5 kg/d, milking 2x/d, and consuming a TMR (1.65 Mcal/kg, 16.1% CP and 41.7% NFC). Blood samples were collected from the coccygeal vein on d 1, 5, 7, 13, 19 and 25, and analyzed to determine serum concentrations of NEFA, glucose, and haptoglobin. Data were summarized by cow and day and analyzed in a repeated measures linear mixed-effect regression model. From d 5 to 25, NEFA concentrations were lower (P < 0.009) than at d 1 for both treatments. Cows fed the diluted ration tended to have lower NEFA on d 5 (0.08 vs 0.10 mmol/L; P = 0.09) and d 7 (0.07 vs 0.09 mmol/L; P = 0.06) than cows fed the control ration. Higher BW at dry-off was associated (P = 0.02) with greater haptoglobin during and after dry-off. These results suggest energy balance and inflammatory response of dairy cows during and after dry-off may be altered by controlling cow body weight, milk yield, and the magnitude change, from lactation to the dry period, in nutrient density of the ration consumed.

Key Words: dry-off, energy balance, inflammation
The objective of this study was to determine if behavior, reticulorumen health, and production in early lactation dairy cows varies with TMR straw particle size. For 28 d after calving, 40 multiparous Holstein cows, housed in free-stalls, were individually fed (with 24 h/d feed access) either 1 of 2 TMR with 8.8% wheat straw (DM basis) chopped to: 1) 2.54 cm (Short; n = 20) or 2) 5.08 cm (Long; n = 20). Enrollment in the trial was on a rolling basis; cows were evenly distributed between treatments by parity and previous 305-d milk yield. Wireless telemetry boluses were used to measure reticulorumen pH. Automated systems recorded TMR DMI, milk yield, and rumination activity. TMR and orts samples were collected every 3 d to determine feed sorting. A particle separator was used to separate feed samples into 4 fractions: long (>19 mm), medium (<19 mm, > 8 mm), short (<8 mm, > 4 mm), and fine (<4 mm) particles. Feed sorting was calculated as: actual intake of each particle fraction expressed as a % of its predicted intake. Data were analyzed in mixed-effect linear regression models and were modeled with polynomials of order 3 over the 28 d of observations. The fitted data indicated treatment differences in linear slopes (P < 0.01), quadratic coefficients, and cubic coefficients (P < 0.01), and cubic coefficients (P < 0.01). Relative to PF cows, circulating LPS-binding protein and serum amyloid A in LPS cows increased 2 and 5-fold, respectively (P < 0.01). Between 12 and 48 h post-bolus, neutrophils increased (12%; P = 0.04) in LPS-Cr vs. LPS-CON cows. In summary, Cr increased the neutrophil response following an LPS challenge, but had minimal effects on circulating energetic metabolites.

**Key Words:** LPS, chromium, neutrophil

337 Effects of dietary chromium on circulating energetic metabolites and leukocyte parameters following a lipopolysaccharide challenge in lactating cows. E. A. Horst*1, S. K. Kvidera1, E. J. Mayorga1, C. S. Shouse1, M. Al-Qaisi1, M. J. Dickson1, J. A. Ydstie1, H. A. Ramirez1, K. E. Griswold2, and L. H. Baumgard1, Iowa State University, Ames, IA, 2Kemin Industries Inc., Des Moines, IA.

Activated immune cells avidly utilize glucose and are insulin sensitive. Chromium (Cr) increases insulin sensitivity and thereby may affect immune function. Objectives were to evaluate supplemental Cr (KemTRACE Chromium 0.04%, Kemin Industries Inc., Des Moines, IA) effects on circulating metabolites and leukocytes following an i.v. lipopolysaccharide (LPS) challenge in non-pregnant lactating Holstein cows. Cows were enrolled in a 2x2 factorial design and assigned to 1 of 4 treatments: 1) pair-fed (PF) and control (PF-CON; 5 mL saline; n = 5), 2) PF and Cr (PF-Cr; 5 mL saline; n = 5), 3) LPS-euglycemic clamp and control (LPS-CON; 0.375 μg/kg BW LPS; n = 5), and 4) LPS-euglycemic clamp and Cr (LPS-Cr; 0.375 μg/kg BW LPS; n = 5). Cows received experimental diets throughout the trial. After acclimation (3 d) baseline values were obtained during period 1 (3 d). At the initiation of period 2 (2 d), a 12 h LPS-euglycemic clamp was conducted or cows were PF to their respective dietary counterparts. Circulating NEFA in PF cows increased (95%) from 6 to 18 h post-bolus while LPS cows increased from 12 to 18 h (63%); thereafter, NEFA decreased in all treatments (P < 0.01). Circulating BUN was increased in LPS vs. PF cows throughout P2 (31%; P = 0.02). Circulating β-hydroxybutyrate tended to decrease in LPS vs. PF cows (16%; P = 0.07) and increased in all cows throughout the 2 d (P < 0.01). Relative to PF cows, circulating LPS-binding protein and serum amyloid A in LPS cows increased 2 and 5-fold, respectively (P < 0.01). Overall, Cr-supplemented cows had increased (22%; P = 0.03) monocytes. Relative to PF cows, WBC and neutrophil counts in LPS cows initially decreased, then progressively increased 58 and 163%, respectively (P < 0.01). Between 12 and 48 h post-bolus, neutrophils increased (12%; P = 0.04) in LPS-Cr vs. LPS-CON cows. In summary, Cr increased the neutrophil response following an LPS challenge, but had minimal effects on circulating energetic metabolites.

**Key Words:** LPS, chromium, insulin
The effects of prepartum fatty acid supplementation on colostrum and milk fatty acid profiles and production. D. Coleman* and A. Relling, The Ohio State University, OARD, Wooster, OH.

The objective of the present study was to evaluate the impact of supplementing ewes with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) during late gestation on ewe colostrum, and milk production and composition. Eighty-four gestating ewes (28 pens) were blocked and randomly assigned to a diet with an addition of 0.4% added fat during the last 50 d of gestation. The fat sources were rich in the monounsaturated fatty acid (MUFA) oleic acid, or the polyunsaturated fatty acids (PUFA) EPA and DHA. After lambing, ewes and lambs were placed on the same pasture. Colostrum samples were taken at lambing for fatty acid analysis. Milk yield, composition and fatty acid profile were analyzed 30 d postpartum. Data was analyzed using a mixed model (SAS 9.4) with treatment, time and their interaction as fixed variables and ewe within pen as a random variable. There was a treatment by time interaction (P < 0.05) with PUFA yielding greater EPA and DHA concentrations than MUFA at lambing, but not at 30 d in lactation. A treatment by time interaction (P < 0.05) was also observed for trans-9 18:1, trans-10 18:1, cis-15 18:1 and conjugated linoleic acid (CLA) isomers other than trans-10, cis-12 18:2, and cis-9, trans-12 18:2, with PUFA ewes having significantly greater concentrations than MUFA ewes at lambing, but not at d 30. A treatment by time interaction (P < 0.05) was also observed for cis-12 18:1, with PUFA ewes having lower concentrations than MUFA ewes in colostrum and at d 30. The concentrations of fatty acids with 6–10 carbons were significantly increased (P < 0.05) in colostrum and milk of PUFA ewes. Neither milk yield nor milk protein, fat, lactose, solids, somatic cell count, milk urea nitrogen, energy corrected milk or net energy of lactation were different (P > 0.05) among treatments. These results suggest that supplementation of EPA and DHA during late gestation alters the fatty acid profile of colostrum and milk, even 30 d after stopping the supplementation, but not milk yield or composition. Future research should investigate the effects of supplementing higher doses of EPA and DHA.

Key Words: fatty acids, milk, colostrum

Why and when should dairy ewes be shorn: Open, pregnant, or neither? G. Caja*1, L. Cordón1, S. González-Luna2, A. A. K. Salama1, X. Such1, E. Albanell1, A. Contreras-Jodar1, and J. Teixeira*1, A. P. Souza1, X. Such1, E. Albanell1, A. Contreras-Jodar1, and J. Teixeira*1, A. P. Souza1, X. Such1, E. Albanell1, A. Contreras-Jodar1, and J. Teixeira*1, A. P. Souza1, X. Such1, E. Albanell1, A. Contreras-Jodar1, and J. Teixeira*1, A. P. Souza1, X. Such1, E. Albanell1, A. Contreras-Jodar1, and J. Teixeira*1, A. P. Souza1, X. Such1, E. Albanell1, A. Contreras-Jodar1, and J. Teixeira*1. 2The Ohio State University, OARD, Wooster, OH. 3University Autonoma of Barcelona, Bellaterra, Barcelona, Spain, 2University Nacional Autonoma of Mexico, Cuautitlán, México.

Lactational responses to summer shearing were studied in 73 dairy ewes of 2 breeds (MN, Manchega, n = 43; LC, Lacaune, n = 30). Ewes were electronically identified and managed under intensive conditions, grazing during the day (6 h/d) followed by night shelter (straw bedded pens) and fed hay and concentrate. Treatments were: SO (shorn open, d −15 mating), SP (shorn pregnant, d 100 pregnancy) and FW (full wool, not shorn). Ewes lambed once-a-year (September), suckled their lambs (28 d) and were milked twice daily (d 29 to 180) in a 2 × 12 milking parlour with electronic milkmeters (DeLaval, Tumba, SE). Milk recording was done at each milking and milk samples collected (DIM 5, 14, 28, 35, 49, 63 and 160) for composition (NIR system; Foss, Nordersted, DE) or coagulation traits (Optigraph; Ysebaert, Frepillon, FR). According to usual breed traits, the milk of MN being richer in components whereas LC ewes yielded more milk and lambs were heavier. Regarding shearing treatments, no differences (P = 0.99 to 0.54) were found in prolificacy (1.64 ± 0.13 lambs/ewe), lamb birth weight (4.20 ± 0.14 kg), ADG (248 ± 12 g/d) nor yield (2.69 ± 0.10 kg/d) and composition of sucked milk. Ewe BW after lambing was also similar between treatments (69.0 ± 0.12 kg; P = 0.99), but BCS of SP ewes was greater than for SO and FW, that did not differ (2.66 ± 0.07 vs. 2.43 ± 0.07; P < 0.001). Although milk yield throughout lactation did not vary by treatments (1.71 ± 0.09 kg/d; P = 0.99), shearing response varied by breed. The LC SP ewes produced 19% more milk (P < 0.001) than the SO or FW ewes, which was not observed in MN ewes (P = 0.99). No differences in milk composition, BW and BCS were detected during lactation (P = 0.99 to 0.23). Moreover, cheese-yield indexes did not differ between treatments but, in both breeds, the SP ewes had numerically richer milk (P = 0.99 to 0.58) and higher indexes (P = 0.23 to 0.01) than SO and FW ewes. In conclusion, shearing dairy ewes at late-pregnancy (d 100), during summer, may be a recommended practice for increasing milk yield of high yielding ewes, without negative effects on milk composition nor cheese-yielding traits. Acknowledgment: Project AGL-2013-44061-R (MINECO, Spain).

Key Words: dairy ewe, shearing, milk

Net protein and energy requirements for growth according to the degree of maturity of Saanen goats. I. A. A. K. Salama1, X. Such1, E. Albanell1, A. Contreras-Jodar1, and J. Teixeira*1, A. P. Souza1, N. R. St-Pierre2, M. H. M. R. Fernandes1, A. K. Almeida1, J. A. C. Vargas1, and K. T. Resende1, 1Universidade Estadual Paulista (Unesp), Jaboticabal, Sao Paulo, Brazil, 2Ohio State University, Columbus, OH.

We conducted a meta-analysis to develop equations for predicting net protein (NP) and energy (NE) requirements for growth of different sexes in dairy goats using the degree of maturity as predictor. A data set from 7 comparative slaughter studies including 238 individual records of Saanen goats (i.e., fed ad libitum and slaughtered at different BW) was used. We performed the study in 2 steps: first, using the traditional approach (i.e., allometric equations to determine protein or energy contents in the empty body weight (EBW) as dependent variables, and EBW as the allometric predictor, where the net requirements were estimated as the first partial derivative); second, we evaluated the relationship between protein or energy content of the EBW gain (g or kcal/kg EBW gain) and degree of maturity (calculated as a ratio between EBW and mature EBW of Saanen goats, considering mature EBW of 42.6, 34.9, and 26.0 kg for intact male, castrated male, and female, respectively). Parameter estimates were obtained using the MIXED procedure of SAS. The model included the random effect of the study, and the fixed effects of sex (intact male, castrated male, and female; n = 94, 73, and 71, respectively). Using the allometric equations, sex affected the NP (P = 0.08) and the NE (P < 0.01), where the NP for males were greater than for females, and the NE of castrated males were greater than intact males, and lower than females. On the other hand, considering the degree of maturity, sex no longer affected the NP (P = 0.26) and NE (P = 0.05). The NP general model was: Protein (g/kg EBW gain) = 176 (±12.8) + 3.25 (±19.0) × (EBW/mature EBW) (s² = 156.2; s² = 2,237), with an overall NP of 176 (±12.8) g/kg EBW gain irrespective of degree of maturity. The NE general model was: Energy (kcal/kg EBW gain) = 1,265 (±234) + 2,312 (±316) × (EBW/mature EBW) (s² = 110,722; s² = 459,166). The NE (mean ± SD) increased from 1,726 ± 188 to 3,575 ± 197 kcal/kg EBW gain as degree of maturity
ranged from 0.2 to 1.0. Including the degree of maturity as predictor of NP\textsubscript{O} and NE\textsubscript{G} canceled out the differences across sexes in Saanen goats.

**Key Words:** mature weight, nutritional requirements, sex

**342 Effects of dietary nitrogen sources and nisin on nutrient digestibility, rumen fermentation, nitrogen utilization, plasma metabolites, and growth performance in growing lambs. J. Shen*1,2, Y. Chen1, W. Zhu1, and Z. Yu2, 1Nanjing Agricultural University, Nanjing, Jiangsu, China, 2The Ohio State University, Columbus, OH.

This study was conducted to investigate the effects of dietary N sources and nisin on nutrient digestibility, rumen fermentation, N utilization, growth performance, and plasma metabolites in growing lambs. Thirty-two male Hu lambs (23.1 ± 1.66 kg initial BW) were assigned to 4 dietary treatments in a randomized block design with a 2 × 2 factorial arrangement. Two N sources, soybean meal (SBM) and distillers dried grains with solubles (DDGS), and 2 levels of nisin, 0 and 30.5 mg of nisin/kg of diet, were used to formulate 4 diets. Growth performance of lambs fed different N sources diet responded differently with time. From wk 1 to 4, DDGS resulted in lower DMI and ADG than SBM (P < 0.05), but G:F was not affected (P > 0.05). In contrast, from wk 5 to 8, DDGS did not affect DMI or ADG (P > 0.05) but resulted in a higher G:F than SBM (P < 0.05). In wk 4, the SBM-fed lambs had a trend to increase BW relative to those fed DDGS (P < 0.07), while the final BW did not differ between SBM and DDGS (P > 0.05). Ruminal acetate, butyrate, and BCVFA concentrations were greater (P < 0.05) and total VFAs concentrations tended to be greater (P = 0.08) for the SBM-fed lambs than for the DDGS-fed lambs. The SBM-fed lambs had higher ruminal ammonia-N, BUN, and urinary N excretion than those consuming DDGS (P < 0.05), while N retention was similar between SBM and DDGS (P > 0.05). Compared with the DDGS-fed lambs, SBM-consuming lambs had higher DM, OM, and CP but lower ADF digestibility (P < 0.05). Nisin supplementation did not affect growth performance, rumen fermentation, nutrient digestibility, plasma metabolites, or N utilization (P > 0.05).

It was concluded that DDGS can substitute SBM to grow Hu without adverse effects on animal performance and to reduce production cost, but nisin supplementation probably has little no benefits.

**Key Words:** nitrogen source, nisin, growing lamb

**343 Effects of algae supplementation on milk performance and rumen fermentation in lactating Xinong Saanen dairy goats. P. Wang*1, Y. Xue2, X. Zhang1, A. Koontz2, and J. Luo1, 1Alltech-NWAFU Animal Science Research Alliance, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China, 2Alltech China, Beijing, China.

The objective was to evaluate the effects of algae supplementation on milk performance and rumen fermentation in lactating dairy goats. Eight multiparous Xinong Saanen dairy goats in late lactation (3.9 ± 0.4 Parities; 208.5 ± 2.7 DIM; 61.5 ± 6.2 kg BW) were individually penned and randomly assigned to a replicated 4 × 4 Latin square design with 4 periods of 18 d. Four levels of algae power (Alltech International, Inc.) were supplemented in the basic ration: 0, 10, 20 and 40 g/d per goat. Algae powder was mixed with a small portion of concentrate and provided for goats before each feeding. Then left concentrate was given to goats followed by corn silage and alfalfa hay which was ad libitum. Goats were fed and manually milked twice daily in the pens before each feeding. DM intake (DMI) and milk performance were measured for 4 d following a washout period of 14 d in 18 d-period. Rumen fluid was collected for measuring pH value, NH\textsubscript{3}-N concentration and VFAs profile at the last day of each period. Data were run by MIXED linear procedure and treatment means were compared by LSD test (P < 0.05).

No significant dietary × time interaction effects was shown on DMI and milk performance. Goats fed 40 g/d algae produced 29.5% more milk than goats fed with 0 g/d algae, although milk yield was not affected by algae supplementation. Neither DMI nor milk components were changed by diets. In addition, no apparent milk fat depression was observed in goats with algae supplementation. Although pH value, NH\textsubscript{3}-N concentration and total VFAs (TVFAs) in rumen were not influenced, changes of VFAs profile were provoked by algae supplementation. Lower molar proportion of acetate and higher of propionate were induced by algae (P < 0.05), decreasing the ratio of acetate to propionate (P < 0.05). In conclusion, goats with 40 g/d algae showed the potential to produce more milk than those with 0/d g algae, while not provoking milk fat depression. Considering VFAs profile was changed by algae supplementation, further analysis for the rumen microbial process and milk fatty acids profile should be measured.

**Key Words:** Xinong Saanen goats, algae supplementation, rumen VFA


Eight rumen cannulated dairy goats in early lactation (3.7 ± 0.2 kg/d) from the INRA-AgroParisTech experimental farm (Thiverval-Grignon, FR), were provided with wireless bolus sensors (KB1001 Kahne, Aukland, NZ) of pH and temperature to study the relationship between intake and subclinical acidosis for 35 d. After adapting to a TMR control diet (CO, 20% concentrate) for 12 d, goats were brusquely changed to an acidogenic diet (AC, 50% concentrate) for 23 d. Diets were fed ad libitium twice daily (a.m. 1/3, p.m. 2/3) according to milking intervals. Rumen pH and temperature data were captured every 15 min and intake measured every 2 min by weighing scales. Rumen samples were collected (h 0, 1, 2, 4 and 6 post feeding) to measure pH by pH-meter before (d 8 and 11) and after (d 13, 14, 15, 16, 20, 26 and 34) the change. One sensor failed and was discarded, the rest of data being modeled by logistic regression with Solver of Microsoft Excel. Data were analyzed by MIXED for repeated measurements and GLM procedures of SAS. Values of pH-meter vs. sensors correlated (r\textsuperscript{2} = 0.86; P < 0.01) and were used for sensor recalibration. Mean rumen pH varied markedly by hour and diet; on average, it was higher in CO vs. AC (6.34 ± 0.06 vs. 6.10 ± 0.03; P < 0.001). Despite the high concentrate percentage of AC diet, rumen pH was shortly under pH 6.0. Feed intake reached plateaus during the day when pH was closer to the a.m. or p.m. nadirs, and correlated negatively (r\textsuperscript{2} = 0.77 to 0.87; P < 0.01) during the periods in which pH dropped. Correlations between rumen temperature and pH were very poor (r\textsuperscript{2} < 0.1), except for the nightly resting period (r\textsuperscript{2} = 0.93; P < 0.001), the rumen being slightly colder in AC goats (CO vs. AC, 39.73 ± 0.09 vs. 39.61 ± 0.09°C; P < 0.001). Temperature and pH data from sensors fit logistic models (r\textsuperscript{2} = 0.97 to 0.99; P < 0.001). Pattern of pH logistic models and time spent under pH 6.0, allowed us to classify the goats as sensitive (3/7, 43%) or tolerant (4/7, 57%) to acidosis, which was related to individual feeding behavior. In conclusion, daily intake measurement and wireless sensors proved to be useful for monitoring rumen function, which allow for an individual separation of sensitive and tolerant goats to rumen acidosis.

**Key Words:** rumen sensor, SARA, goat
Evaluation of two bulk tank milk paratuberculosis tests in dairy goats and sheep. C. Bauman*1, A. Jones-Bitton1, J. Jansen2, P. Menzies1, and D. Kelton1. 1University of Guelph, Guelph, ON, Canada, 2Ontario Ministry of Agriculture Food and Rural Affairs, Guelph, ON, Canada.

The objective of this study was to evaluate the ability of 2 bulk tank milk tests to correctly classify dairy goat and dairy sheep farms as containing lactating animals infected with Mycobacterium avium ssp. paratuberculosis. Twenty-nine dairy goat herds and 21 dairy sheep flocks in Ontario, Canada were visited in 2011 to collect blood and fecal samples from 20 randomly selected animals and a bulk tank milk sample. The fecal samples underwent fecal culture (Bactec MGIT) and fecal PCR testing (Tetracore), the serum was tested using the Parachek ELISA and the bulk tank milk was tested using PCR (IS900 PCR) and the IDEXX ELISA utilizing the enhanced hyper-ELISA protocol (conjugate incubation time and color development phase were doubled). Sensitivity (Se) and specificity (Sp) for both tests were estimated using the individual animal tests, interpreted at the herd-level, as the reference test. A herd or flock was classified as positive if one or more of the 20 animals tested positive on the specific test (Table 1). No goat herds tested positive on the bulk tank milk PCR test (Se 0%; Sp 100%) while 8/29 (27.6%) herds tested positive on the hyper-ELISA. Of the dairy sheep flocks tested, 3/19 (15.8%) were positive using the PCR-based testing (Se 25%; Sp 100%) while 8/21 (38.1%) were positive using the hyper-ELISA test. The hyper-ELISA yielded higher Se in dairy sheep than in dairy goats (Table 1). In summary, the hyper-ELISA test demonstrated higher Se and Sp than the PCR test. However, further analysis, using a larger sample size, is needed to determine if randomly sampling 20 animals may give more accurate herd-level status.

<table>
<thead>
<tr>
<th>Reference Test</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Goats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal culture</td>
<td>34.8% (16.4–57.3)</td>
<td>100% (64.9–100)</td>
</tr>
<tr>
<td>Fecal PCR</td>
<td>33.3% (15.3–56.8)</td>
<td>100% (63.2–100)</td>
</tr>
<tr>
<td>Serum ELISA</td>
<td>43.8% (24.2–67.1)</td>
<td>92.3% (65.2–99.1)</td>
</tr>
<tr>
<td>Dairy Sheep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal culture</td>
<td>50.0% (21.1–78.9)</td>
<td>77.8% (40.0–97.2)</td>
</tr>
<tr>
<td>Fecal PCR</td>
<td>46.7% (21.3–73.4)</td>
<td>83.3% (35.9–99.6)</td>
</tr>
<tr>
<td>Serum ELISA</td>
<td>87.5% (47.4–99.7)</td>
<td>92.3% (64.0–99.8)</td>
</tr>
</tbody>
</table>

Key Words: dairy goats, dairy sheep, paratuberculosis
346  A rational evaluation of the dairy fat debate. L. Baumgard*, Iowa State University, Ames, IA.

Despite the public dogma that fats of animal origin, particularly from ruminants, cause human disease (primarily cardiovascular and cancer), the topic has been far from axiomatic within the scientific community. The general hypothesis is more than 70 yr old and, importantly, there are actual studies demonstrating a link between animal fat intake and a specific disease. These reports receive considerable attention from the mainstream media. However, these associations (mostly epidemiological) are based upon differences in relative risk and not absolute risk. If some environmental factor causes disease frequency to increase from 1/100 to 2/100, media report the relative risk difference as being a 100% increase, without providing context of actual disorder incidence. In reality, the absolute risk difference is 1 percentage unit. Appreciating how these 2 simple arithmetic calculations markedly influence data interpretation is key to putting the aforementioned trials into sensible perspective. When evaluated on an absolute risk the increased chance of acquiring a disease in the abovementioned studies is typically below 2 percentage units (a statistical difference most people would presumably consider biologically insignificant). Further, there are a much larger number of scientific articles that do not support the causal relationship between animal fat and human disease. Noteworthy is the fact that these also include some very large and randomly controlled long-term intervention trials. Interestingly, these scientific publications rarely receive media exposure. Since Gary Taubes first eloquently exposed the controversy in 2001 (Science 291:2536–2545), the number of papers disagreeing with the animal fat-human disease dogma has markedly increased. Thus, most scientific evidence does not corroborate the hypothesis that animal fat causes human disease, and in the epidemiological experiments that do, rational people would contextualize if results were presented as absolute risks instead of relative risks. In summary, the perceived link between animal fat intake and human health disorders was always tenuous, but it is becoming increasingly ambiguous and this is especially true with regards to ruminant-derived products.

Key Words: dairy fat, disease

347  Dietary fats: The saturated vs. unsaturated controversy. G. D. Lawrence*, Long Island University, Brooklyn, NY.

The low fat, low saturated fat mantra has been chanted so loudly and so often that many people believe it must have solid scientific support (it does not). There will be a brief description of the historical development of the saturated fat-cholesterol hypothesis that begat the low fat doctrine in popular diet and nutrition circles and presentation of the scientific evidence that shows the inaccuracies and false assumptions of those hypotheses. Numerous studies in recent years have shown that saturated fatty acids, palmitic acid in particular, can increase levels of several inflammatory markers in vitro, although these studies have not shown exacerbation of inflammatory diseases in vivo. There will be some discussion of the role of saturated vs omega-3 and omega-6 polyunsaturated fatty acids in inflammatory syndromes, metabolic disorders and cardiovascular disease. The effect of high sugar diets on all of these metabolic consequences will also be discussed in the context of human health.

Key Words: inflammation, saturated fat, polyunsaturated fatty acid

348  Scientific evidence and gaps: A systematic review of dietary cholesterol and cardiovascular disease. G. Raman*, Tufts Medical Center, Boston, MA.

Established in the 1960s, the dietary guidelines recommended no more than 300 mg/day of cholesterol for healthy populations in the US. The objective of this presentation is to identify scientific evidence and gaps using a systematic review to examine the effects of dietary cholesterol on cardiovascular risk in healthy adults. A systematic review is a form of research that provides a summary of studies on a specific clinical question, using explicit methods to search, critically appraise, and synthesize the literature systematically. It is particularly useful in bringing together several separately conducted studies and synthesizing their results. Following a systematic review, meta-analyses can be conducted in which data from individual studies are pooled quantitatively and reanalyzed using established statistical methods. Systematic reviews and meta-analyses are considered to provide the most robust evidence for evaluating scientific questions related to human health. Of the 40 eligible studies, 19 prospective observational cohorts with 361,923 subjects found no association between dietary cholesterol intake and chronic heart disease or cerebrovascular stroke. In 21 clinical trial articles with 632 subjects, as compared with control, intervention doses of 500 to 900 mg/day of dietary cholesterol interventions increased serum lipids including, total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol. Our systematic review identified that there is a lack of long-term data (observational or trials) in healthy adults to support a recommendation of lower intake of dietary cholesterol of no more than 300 mg/day of cholesterol. Additional clinical trials are needed to examine the role of dietary intake of cholesterol between 300 and 500 mg/day on clinical outcomes. These data are based on the Original Publication: Berger S, Raman G*, Vishwanathan R, Jacques PF, Johnson EJ. Dietary cholesterol and cardiovascular disease: A systematic review and meta-analysis. Am. J. Clin. Nutr. 2015;102:276–294.

Key Words: dietary cholesterol, cardiovascular disease, serum cholesterol

349  Nutritional significance of milk fat membrane composition and structure. R. Jimenez-Flores*, The Ohio State University, Columbus, OH.

The milk fat globule membrane (MFGM) is avidly studied by many groups of scientists around the world and is yielding very important new information. Its structure, complex and heterogeneous, doesn’t fit into the norms of physical and chemical studies. The structure of the MFGM is not static, it changes constantly with its surroundings and, in particular, it changes with each different step in processing. From the simple process of cooling milk to the drastic homogenization and UHT treatments, the fate of the MFGM and its components is poorly understood in terms of its influence on digestion and nutrient delivery. The MFGM was initially described in the 1970s and 1980s as the membrane that surrounds fat globules in milk, preventing coalescence and rancidity of lipids. However, in the last 2 decades, its biologically active properties have been explored in greater detail and in different models. In fact, research has ascribed to MFGM anticancer and anti-hypercholesterolemic activities, antimicrobial and antiviral properties such as inhibition of the ulcer-forming bacterium Helicobacter pylori and rotavirus, and suppression of diseases such as multiple sclerosis. In
addition, in clinical studies, complementation of infant food with MFGM and micronutrients has led to new products with great potential for the health and wellness of consumer, especially babies. We propose that the composition and structure of the MFGM in milk plays a central role in the digestion of fat both the rate and extent of digestion. The structural studies presented here are based on the phospholipid characterization, on protein analysis, bacterial binding and microscopy observations on native and processed MFGM. Bacterial interactions have been studied by a combination of gradient centrifugation procedures, fluorescent tagging and binding, and confocal microscopy. In addition, some of the changes to the MFGM proteins during milk processing have been followed by proteomic techniques, particle size distribution and surface charge. We present also an important part of the milk lipids, the ectosomes and exosomes, that recently have been linked with functions in nutrition and health. Results of these studies have proven useful in finding relevant information from this complex system.

Key Words: milk fat globule membrane (MFGM), nutrition, fat digestion
Evaluating the interaction of stocking density and the feeding environment is the next step in furthering dairy cow well-being and ruminal health. The objective of this study was to determine the short-term effects of stocking density and feed access on ruminal pH. Multiparous (n = 16, 4 cows/pen) ruminally cannulated Holstein cows were assigned to 1 of 4 pens as a part of a larger study (n = 17 cows/pen). Treatments were assigned to pens in a 4 × 4 Latin square with 14-d periods using a 2 × 2 factorial arrangement. Two stocking densities (STKD; 100 or 142% of stalls and headlocks) and 2 levels of feed access (FA; no restriction; NR and 5 h restriction from 19 to 24 h post-feeding; R) resulted in 4 treatments: (1) 100NR, (2) 100R, (3) 142NR, and (4) 142R. A mixed model was delivered 1×/day at approximately 0600 h. Ruminal pH was measured on d 12–14 of each period using indwelling pH loggers. Data were averaged into 10-min intervals across days and among cows into a pen average. Data were analyzed using a mixed model in JMP with pen as the experiment unit. Overstocking significantly reduced daily time spent below pH 5.8 and tended to increase area under the curve (AUC) below pH 5.8. While time spent below pH 5.8 tended to increase with overstocking 9–16 h post-feed delivery, no other time periods were significantly different. This indicates no singular time period accounted for the increased daily time spent below pH 5.8 with overstocking but rather a culmination throughout the day. Daily ruminal pH was not affected by FA, but an interaction was found between STKD and FA on time spent below pH 5.8. Overstocking negatively impacts ruminal pH and R exacerbates this effect.

Key Words: overstocking, feed restriction, ruminal pH

This study investigated the interaction of stocking density and feed availability on short-term behavioral responses of dairy cattle. Multiparous (n = 48) and primiparous (n = 20) lactating Holstein cows were assigned to 1 of 4 pens (n = 17 cows/pen). Pens were balanced for parity (2.3 ± 1.1; mean ± SD), DIM (121 ± 38), and milk production (47 ± 8 kg/d). Treatments were assigned to pens in a 4 × 4 Latin square with 14-d periods using a 2 × 2 factorial arrangement. Two stocking densities (STKD; 100 or 142% of stalls and headlocks) and 2 levels of feed access (FA; no restriction, NR and 5 h restriction from 19 to 24 h post-feeding, R) resulted in 4 treatments: (1) 100NR, (2) 100R, (3) 142NR, and (4) 142R. Pen intake was measured on d 8–14 of each period. Time spent lying, feeding, and ruminating were measured using 10-min scan sampling for 72-h from d 8–10 of each period. Data were analyzed using a mixed model in JMP with pen as the experiment unit. Overstocking decreased daily lying time. Overstocking tended, and R decreased, daily feeding time. Intake (25.8 kg/cow/d, SEM = 0.3) did not differ (P > 0.10). While STKD did not alter total rumination time, overstocking decreased rumination within the free-stall, implying a shift in location of rumination. Feeding and rumination times were shifted with R, increasing feeding and decreasing rumination 0 to 8 h post-feed delivery while decreasing feeding and increasing rumination 17 to 24 h post-feed delivery. In response to reduce feed access, cows altered their feeding and rumination patterns to maintain total chewing activity. An additive effect of overstocking and feed access was not evident in these behaviors.

Key Words: overstocking, feed access, chewing response

Clinical mastitis detection—Development of an accurate detection method for automatic milking systems. M. Khatum*, P. C. Thomson, K. Kerrisk, J. Molfino, and S. C. García, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camden, NSW, Australia.

This study investigated the potential for accurate detection of clinical mastitis (CM) in an automatic milking system (AMS) using electronic data from the support software. Data from 358 cows were used to develop the model which was then tested on 2 independent data sets; one with 311 cows (same farm different year) and one with 568 cows (from a different farm). Data from a common period was captured for healthy cows (n = 1066), single quarter (n = 101) and multi-quarter (n = 70) CM cows. Clinical mastitis was determined by visual inspection of

### Table 1 (abstract 350).

<table>
<thead>
<tr>
<th>Item</th>
<th>100%</th>
<th>142%</th>
<th>SEM</th>
<th>STKD</th>
<th>FA</th>
<th>STKD × FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pH</td>
<td>5.96</td>
<td>6.03</td>
<td>5.98</td>
<td>5.89</td>
<td>0.06</td>
<td>0.14</td>
</tr>
<tr>
<td>Minimum pH</td>
<td>5.42</td>
<td>5.50</td>
<td>5.51</td>
<td>5.39</td>
<td>0.07</td>
<td>0.81</td>
</tr>
<tr>
<td>Maximum pH</td>
<td>6.49</td>
<td>6.61</td>
<td>6.48</td>
<td>6.53</td>
<td>0.04</td>
<td>0.25</td>
</tr>
<tr>
<td>AUC &lt;5.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH units × h/d</td>
<td>1.66</td>
<td>1.24</td>
<td>1.73</td>
<td>2.55</td>
<td>0.63</td>
<td>0.97</td>
</tr>
<tr>
<td>pH &lt; 5.8, h/d</td>
<td>6.62</td>
<td>5.23</td>
<td>6.78</td>
<td>8.77</td>
<td>1.27</td>
<td>0.32</td>
</tr>
<tr>
<td>pH &lt; 5.8, h</td>
<td>0-8 h post-feed delivery</td>
<td>1.79</td>
<td>1.31</td>
<td>1.56</td>
<td>2.12</td>
<td>0.31</td>
</tr>
<tr>
<td>9-16 h post-feed delivery</td>
<td>2.28</td>
<td>1.75</td>
<td>2.56</td>
<td>3.25</td>
<td>0.59</td>
<td>0.08</td>
</tr>
<tr>
<td>17-24 h post-feed delivery</td>
<td>2.55</td>
<td>2.44</td>
<td>2.67</td>
<td>3.11</td>
<td>0.56</td>
<td>0.16</td>
</tr>
</tbody>
</table>
suspect quarters for presence of redness, heat, swelling with flakes/clots in the milk, having prior elevated electrical conductivity (mS/cm, EC), and treated with antibiotic. All data was assessed using logistic mixed models. Twelve parameters were included in the initial model before a backward elimination which resulted in the following 6 parameters being included in the final model: quarter level milk yield (kg, MY), EC, average milk flow (kg/min), and occurrence of incompletely-milked quarters in each milking session; MY per hour and EC per hour between successive milking sessions. All measurements were assessed to determine their ability to detect CM, both as individual variables, but also as combinations of the 12 abovementioned variables. These were assessed by producing a receiver operating characteristic curve and calculating the area under the curve (AUC) for each model. Overall, 6 measurements of final model had significant ($P < 0.05$) mastitis detection ability as separate predictors. The best mastitis prediction was possible by incorporating 6 measurements as well as the random cow/quarter effects in the model resulted in sensitivity (Se) of 90%, specificity (Sp) of 91% and AUC (0.96). Assessment of the model found robust results with sound Se (90% to 100%), Sp (87% to 96%) and excellent AUC (>0.9). This study demonstrated that improved mastitis status prediction can be achieved by using multiple measurements and any new index based on multiple measurements is expected to result in improved accuracy of mastitis alerts thereby improving the detection ability and practicality on farm.

**Key Words:** mastitis, dairy cow, automatic milking system (AMS)

<table>
<thead>
<tr>
<th>Item</th>
<th>100%</th>
<th>142%</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lying time, min/d</td>
<td>NR</td>
<td>R</td>
<td>13</td>
<td>0.02</td>
</tr>
<tr>
<td>Feeding time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily total, min/d</td>
<td>784</td>
<td>786</td>
<td>13</td>
<td>0.02</td>
</tr>
<tr>
<td>0-8 h post-feeding, min</td>
<td>87</td>
<td>98</td>
<td>6</td>
<td>0.08</td>
</tr>
<tr>
<td>17-24 h post-feeding, min</td>
<td>60</td>
<td>39</td>
<td>2</td>
<td>0.41</td>
</tr>
<tr>
<td>Ruminating time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily total, min/d</td>
<td>510</td>
<td>524</td>
<td>9</td>
<td>0.90</td>
</tr>
<tr>
<td>0-8 h post-feeding, min</td>
<td>162</td>
<td>155</td>
<td>3</td>
<td>0.73</td>
</tr>
<tr>
<td>17-24 h post-feeding, min</td>
<td>185</td>
<td>200</td>
<td>5</td>
<td>0.77</td>
</tr>
<tr>
<td>% total ruminating</td>
<td>85.0</td>
<td>84.4</td>
<td>80.0</td>
<td>78.2</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>&lt;0.01</td>
<td>0.18</td>
<td>0.53</td>
</tr>
</tbody>
</table>

No subclinical mastitis (n = 3,553). Generalized estimating equations using logit link function was used to account for repeated measures from the same cow. Variables with $P < 0.10$ in the univariable models were included in the multivariable models. Lactose, protein, electrical conductivity, DIM, ruminating time, lying time, and number of lying bouts were included in the NPMIX multivariable model. Lactose, protein, electrical conductivity, DIM, reticulorum temperature, activity, lying time, and number of steps were included in the GPOS model. Lactose, fat, milking order, DIM, activity, feeding time, ruminating time, and parity were included in the NOGROW model. Variables with $P > 0.04$ in the multivariate models were eliminated through backward elimination. However, no variables in any multivariable model were significant. The ideal combination of variables to predict subclinical mastitis were not found.

**Key Words:** mastitis, precision dairy, subclinical

### 354 Cows at high risk of Johne’s disease spend less time lying down around peak lactation.


Johne’s disease (JD) is a fatal chronic enteritis which causes detrimental effects on production and health and significantly reduces animal welfare. Control of JD is highly desirable, but single milk ELISA testing may not be sensitive enough to identify all affected animals, particularly in the early stages of the disease. The objective of this study was to compare the activity of cows with a low risk of JD to cows with a high risk of JD around calving and peak lactation. The study was conducted at Harper Adams University, UK, using 42 multiparous (3.1 ± 0.22 (Mean ± SEM); range: 2–7 lactations) Holstein Friesian cows, fitted with an IceQube accelerometer (IceRobotics Ltd., Edinburgh, UK) on the back left leg. The sensors recorded lying duration (h/d), frequency of lying bouts (LB/d), average lying bout duration (LBD; min/bout) and steps (S/d). Four times during 2015 and 3 times during 2016 the cows were milk sampled, and subsequently tested for JD using an ELISA. Cows in infection groups JD0-JD2 were classed as low risk and cows in infection groups JD3-JD5 were classed as high risk. Low risk cows (JD0; n = 21 (repeat ELISA –iv)) were matched to high risk cows (JD5; n = 19 (repeat ELISA +ve), JD4; n = 2 (last ELISA +ve) based on parity and age and there was no difference in milk yield between low and high risk JD cows (P > 0.05; 41.8 ± 1.11 vs. 43.1 ± 1.13 kg/d, respectively). Activity data were averaged and analyzed 14d before calving and peak lactation (8 wks post calving). One way ANOVA (Genstat, 18th edition, VSN International Ltd., UK) revealed no significant differences
(P > 0.05) in activity between low vs. high risk cows around calving. However, at peak lactation low risk JD cows spent, on average 93 min/d longer lying down than high risk cows (P = 0.019; 11:05 vs. 09:32 hh:mm/d, respectively) and they had more LB/d (P = 0.027; 12.7 vs. 10.2 LB/d, respectively). There was no difference in average LBD (P = 0.392; 00:55 vs. 00:59 hh:mm/bout) or total S/d (P = 0.388; 1586.4 vs. 1796.9 S/d, respectively) around peak lactation. The results show that activity data from leg-mounted accelerometers have the potential to help identify cows at a higher risk of JD although more research is required.

**Key Words:** Johne’s disease, activity, accelerometer

### Table 1 (abstract 355). List of precision dairy monitoring technology (PDMT) and variables measured

<table>
<thead>
<tr>
<th>PDMT (company name and country)</th>
<th>Variables measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>IceQube (IceRobotics Ltd., Edinburgh, Scotland)</td>
<td>Total motion (units/d)</td>
</tr>
<tr>
<td>Afimilk MPC Analyzer (Afimilk, Kibbutz Afikim, Israel)</td>
<td>Yield (kg/d)</td>
</tr>
<tr>
<td>Track a Cow (ENGS Systems Innovative Dairy Solutions, Israel)</td>
<td>Body weight (kg/d)</td>
</tr>
<tr>
<td>Smartbow (Smartbow GmbH, Jutogasse, Austria)</td>
<td>Activity (h/d)</td>
</tr>
<tr>
<td>Feedbunk visits (bouts/d)</td>
<td></td>
</tr>
<tr>
<td>Time at feedbunk (h/d)</td>
<td></td>
</tr>
<tr>
<td>Feetbunk visits (bouts/d)</td>
<td></td>
</tr>
<tr>
<td>Lying bouts (no./d)</td>
<td></td>
</tr>
<tr>
<td>Steps (no./d)</td>
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<tr>
<td>IceQube (IceRobotics Ltd., Edinburgh, Scotland)</td>
<td>Total motion (units/d)</td>
</tr>
</tbody>
</table>
How has the veterinary feed directive changed dairy production medicine? P. J. Gorden*, Iowa State University, Ames, IA.

Antimicrobial residues in milk and meat from dairy cattle have long been scrutinized by the US public and governmental agencies as well as by the consumer. There are also increased concerns about the presence of elevated levels of antimicrobial resistance in both veterinary medicine and human medicine. Additionally, there is heightened fear that certain antimicrobial use practices in veterinary medicine are leading to decreased treatment efficacy in human medicine. As a result, the US Food and Drug Administration has issued several guidance policies in attempts to protect the effectiveness of medically important antimicrobials in human medicine. Due to the limited number of antimicrobials currently developed, many of these products crossover into veterinary medicine. One of the biggest changes the dairy industry has experienced in recent times related to antimicrobial use is the implementation of the Veterinary Feed Directive. As we move forward, the FDA expects that veterinarians will be more highly involved in decisions regarding antimicrobial stewardship on food production farms. Dairy farmers and their veterinarians must be progressively vigilant to make sure that public health is protected following consumption of products from dairy animals and that perception of milk and dairy beef remains as high as possible.

Key Words: Veterinary Feed Directive, antimicrobial stewardship

The status of antimicrobials for dairy practice: An update on efficacy and resistance. P. Ruegg*, University of Wisconsin, Madison, WI.

Concern about antimicrobial usage (AMU) in food animals and development of antimicrobial resistant microorganisms continues to grow and regulators are increasing supervision of AMU on dairy farms. The recently enacted Veterinary Feed Directive is an example of increased restrictions on previously OTC drugs and increased veterinary oversight of antimicrobials given in feed and water. On dairy farms, antimicrobials are used to treat bacterial diseases and treatments for mastitis, lameness, respiratory diseases and reproductive and digestive disorders are the most common reasons dairy cows receive antimicrobials. Studies conducted in North America and Europe have quantified AMU using standardized indices (Defined Daily Doses). While calculations vary, most researchers have reported that dairy cows receive about 5 – 8 DDD of antibiotics per cow per year and about 35–85% of the doses are given via intramammary (IMM) infusion, with the remainder given parenterally or orally (to calves). In most studies, mastitis is the most common reason for AMU. Increased regulations on AMU are based on assumptions that reduced usage will result in decreased selection of resistant organisms and reduced threats to human health. However, on dairy farms, AMU has been relatively restricted and evolution and maintenance of resistant pathogens in dairy cows or farm environments has not been well described. Increased resistance of fecal E. coli has been reported after systemic administration of ceftiofur, but susceptible bacterial populations rapidly rebounded after treatment ended. Similar trends have been noted in farm environments. Some researchers have demonstrated variation among farms and organisms in resistance to some drugs and some associations of resistance with AMU has been noted but these associations have been limited to pathogens recovered from milk. While AMU for mastitis consumes the greatest quantity of antimicrobials, several reviews have stated that there is relatively little evidence to suggest that widespread resistance or lack of efficacy is emerging or progressing. However, more research on how to improve AMU on dairy farms is needed and mechanisms to increase involvement of veterinarians in treatment decisions are warranted.

Key Words: antimicrobial usage, antibiotics, dairy

Enhancing animal health through multiple modes of action. K. C. Jeong*, Department of Animal Sciences, Emerging Pathogens Institute, University of Florida, Gainesville, FL.

The emergence of infections caused by antimicrobial resistant microorganisms (AMRs) is currently one of the most important challenges to public and animal health. More than 23,000 deaths have been attributed to infections from AMRs in the United States; and an estimated 10 million people may die every year by 2050 around the globe due to increased resistance. With microorganisms acquiring antibiotic resistance, previously potent antibiotics are becoming ineffective and antibiotics are unable to sustain the demand to effectively treat microorganisms. Consequently, the choices of antibiotics used to treat human and animal pathogens are decreasing, leading to a health crisis. The number of AMRs is increasing and will continue to increase due to the slow development of new antibiotics and lack of alternative therapy for infectious diseases. However, developing new antibiotics in the 21st century has slowed down considerably after the over-screening of microorganisms. In addition, advanced antibiotic discovery programs including genomics, high-tech chemical approaches, and high-throughput screening methods have not been successful to develop new antibiotics and many companies have halted their antibiotic research programs. In this presentation, I will summarize major antibacterial targets and pathways and mechanisms by which AMRs survive in the presence of antibiotics. Current efforts to discover and develop antibiotics using unculturable microorganisms and new antibiotic molecular frameworks as well as development of biological therapy with bacterial phages. In addition, I will present some of our own studies that evaluated the efficacy of nanoparticles in antimicrobial activity. Our findings suggested that CM, derived from natural biopolymer chitosan, can treat infectious diseases caused by AMRs and CM harbored strong antimicrobial activity against both gram-positive and gram-negative bacteria in different environments due to broad targets, including OmpA and LPS in gram-negative bacteria and teichoic acid in gram-positive bacteria. Furthermore, risk assessment of CM revealed by the normal function of the rumen indicates that CM unlikely cause side effects. In addition, we found CM exerted antimicrobial activity in cows with metritis, resulting in cure of this disease. These studies emphasize options for alternative treatment to both human and animal disease caused by bacterial infections.

Key Words: antibiotics, antimicrobial resistance, alternative antimicrobial agent
Holstein heifers (n = 40) were allocated to 5 groups: (1) control; (2) virginiamycin (10 g/d); (3) monensin (2.2 g/d) + tylosin (0.44 g/d); (4) monensin (2.5 g/d) + yeast (Levucell SC Direct 25 g/d); (5) sodium bicarbonate (200 g/d) + magnesium oxide (30 g/d). Heifers were fed a 62% forage:38% concentrate total mixed ration for a 20-d adaptation. On d-21 heifers were fed with 1.0% of BW dry matter wheat and 0.2% of BW fructose plus additive(s). Rumen samples were taken weekly and 5 times over 3.6 h after challenge and analyzed for pH, and ammonia, D- and L-lactate, and VFA concentrations. Relative abundance of bacteria and archaea were determined using Illumina MiSeq. Rumen metabolites were analyzed to produce an eigenvector that indicates the risk of ruminal acidosis. The DNA was sequenced using the Geneseek Genomic Profiler Bovine 150K illumina SNPhip from 34 heifers. Genome-wide association used an additive model and linear regression with PCA population stratification and a Bonferroni correction for multiple comparisons. Few genome associations were found with rumen pH, acetate, propionate, total VFA, or ammonia concentration or relative abundance of Firmicutes and Bacteroidetes phyla pre-challenge or challenge. Metabolites and microbial phyla that had associated markers and genomic regions identified were: acetate:propionate (A:P), D-, L- and total lactate, butyrate, acidosis eigenvector, Actinobacteria, Chloroflexi, Archaea, Fibrobacteria, Proteobacteria, and Tenericutes. A putative genomic region overlapped for Actinobacteria, Archaea, and Fibrobacteria and the region that codes for matrix extracellular phosphoglycoprotein. Other overlapping regions were found for: (1) Chloroflexi, Tenericutes, and A:P; (2) L- and total lactate and Actinobacteria, and (3) Actinobacteria, Archaea, Fibrobacteria, and A:P. Genome-wide associations with the metabolome and microbiome were present despite a small population size, suggesting markers for ruminal acidosis susceptibility exist.

Key Words: genome-wide association, lactic acid, ruminal acidosis

Table 1 (abstract 361).

<table>
<thead>
<tr>
<th></th>
<th>Shannon</th>
<th>Control</th>
<th>SARA</th>
<th>P-value</th>
<th>Control</th>
<th>SARA</th>
<th>P-value</th>
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<tr>
<td>Rumen</td>
<td>8.74</td>
<td>6.09</td>
<td>0.04</td>
<td>2,991</td>
<td>1,196</td>
<td>0.001</td>
<td>1,603</td>
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<tr>
<td>Duodenum</td>
<td>8.24</td>
<td>6.37</td>
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<td>1,049</td>
<td>838</td>
<td>0.68</td>
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<tr>
<td>Jejunum, prox.</td>
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<td>5.77</td>
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<td>888</td>
<td>577</td>
<td>0.88</td>
<td>720</td>
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<td>Jejunum, middle</td>
<td>7.52</td>
<td>6.55</td>
<td>0.22</td>
<td>612</td>
<td>675</td>
<td>0.88</td>
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<tr>
<td>Jejunum, distal</td>
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<td>0.04</td>
<td>805</td>
<td>492</td>
<td>0.05</td>
<td>618</td>
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<tr>
<td>Ileum</td>
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<td>4.98</td>
<td>0.01</td>
<td>999</td>
<td>713</td>
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<td>501</td>
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<tr>
<td>Cecum</td>
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<td>0.001</td>
<td>1,468</td>
<td>722</td>
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<tr>
<td>Colon</td>
<td>8.48</td>
<td>6.01</td>
<td>0.02</td>
<td>2,405</td>
<td>993.4</td>
<td>0.001</td>
<td>1,388</td>
<td>603</td>
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</tbody>
</table>

8 Nonlactating Danish Holstein cows were fed a control diet with a forage-to-concentrate ratio of 78:22 (DM basis) for several weeks, before a SARA challenge was conducted in 4 cows. This challenge was conducted by gradually substituting 45% of the DM of the control diet with pellets containing 50% wheat and 50% barley over a 4 d. Control cows received the control diet throughout. All cows were slaughtered after the SARA cows had received the full challenge for 2 d. Biopsies of epithelia were collected from different sites of the digestive tract. Extracted DNA from these biopsies were analyzed using MiSeq Illumina sequencing of the V4 region of the bacterial 16S rRNA gene. Rarefaction analysis was performed on observed species and Chao1 estimates of species richness and Shannon estimate of evenness at an even sequencing depth of 8000 per sample. The SARA challenge reduced (P < 0.01) the mean daily rumen pH from 6.6 to 5.8, and increased (P < 0.01) the duration of the rumen pH below 5.6 from 15 to 509 min/d, and the daily average rumen concentration l-lactate from 0.45 to 27.57 nmol/L, without affecting this concentration of total VFA. The challenge decreased the α-diversity along the digestive tract, with the exception of the middle jejunum. Principal coordinate analyses (PCoA) of weighted UniFrac distances and hierarchical clustering of the abundant taxa (≥0.1%) revealed distinct clustering patterns (P < 0.05) by diet for all sites. On the rumen epithelium, the challenge increased (P < 0.05) the relative abundance of Streptococcaceae, Bifidobacterium and Lactobacillales taxa, whereas it reduced (P < 0.05) Clostridia, Butyribrio, Ruminococcus, Bacteroidales and Fibrobacter taxa. In conclusion, the challenge caused mostly adverse changes in epimural microbiota throughout the digestive tract.

362 Associations of productivity and supplemental feed consumption with subclinical ketosis in dairy cows in robotic milking herds. K. J. Sparkman, M. T. M. King*, and T. J. DeVries, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.

Robotic milking systems provide the capability of offering different amounts of supplemental feed to cows based on parity, DIM, and milk size, suggesting markers for ruminal acidosis susceptibility exist.

Key Words: genome-wide association, lactic acid, ruminal acidosis

Table 1 (abstract 361).
yield, but settings often do not take milk yield into account until 20 to 70 DIM, past the peak risk period for developing subclinical ketosis (SCK). To determine associations between SCK, milk yield, and supplement intake in robotic milking systems, we monitored 607 cows from 9 robotic herds, testing blood BHB 1x/wk for the first 3 wk of lactation. Positive cases of SCK (incidence = 33%) were defined by BHB ≥ 1.2 mmol/L at ≥ 1 of 3 tests. Milk yield and supplement intake data were obtained. In mixed linear regression models, we analyzed parity and week of lactation (wk 1 = −7 DIM, wk 2 = −8 −14 DIM, wk 3 = −15 −21 DIM) separately due to 3-way interactions with SCK status. The only differences in supplement intake between SCK and healthy cows was for 3+ lactation cows in wk 3 (supplement consumption was 0.1 kg/d lower with SCK; P = 0.04). Milk production and milk yield per kg supplement were greater for SCK cows, particularly for primiparous cows in wk 1−2, and for 2nd and 3+ lactation cows in wk 1. From 0 to 21 DIM, in linear regression models, milk yield and milk/supplement were positively associated (P < 0.01) with BHB, but supplement intake was not (P = 0.9). The difference in milk yield and milk/supplement between 7-d forward- and backward-moving averages from the day of blood sampling were negatively associated with BHB (P < 0.01), showing that higher BHB values were associated with slower milk inclines. Accounting for parity in logistic regression models, smaller differences between the 2 moving averages for milk yield and milk/supplement were associated with greater risk of having BHB ≥ 1.2 mmol/L (P < 0.01), such that reducing the difference in milk yield by 4 kg and the difference in milk/supplement by 1 unit were associated with 1.35 (95% CI = 1.21−1.50) and 1.44 (CI = 1.29−1.61) times higher odds, respectively. These results highlight the differences in milk production (per day and relative to supplement consumed) associated with SCK and the potential for feed tables in robotic milking systems to reduce negative energy balance by accounting for milk production of fresh cows.

Key Words: robotic milking, dairy cow, hyperketonemia

363 Postpartal subclinical ketosis can be predicted by monitoring prepartal standing behavior in transition dairy cows. S. Rodriguez-Jimenez*1, K. J. Haer2, E. Trevisi3, J. S. Osorio1, J. J. Loor2, and F. C. Cardoso2. 1South Dakota State University, Brookings, SD, 2University of Illinois, Champaign, IL, 3Università Cattolica del Sacro Cuore, Milan, Italy.

Subclinical ketosis has emerged as one of the most important metabolic diseases in dairy cows since it is often undiagnosed. Twenty-four multiparous Holstein cows were monitored from −30 to 30 d relative to parturition with data loggers (Onset; Pocasset, MA) to record 3D acceleration every 60-s to elucidate cow behavior such as standing and lying time. Blood samples collected at −30, −15, and 3 d relative to parturition were used to analyze biomarkers of metabolism, inflammation, and liver function. Blood BHB was measured with the Precision Xtra at 21 DIM, in linear regression models, milk yield and milk/supplement were positively associated (P < 0.01) with BHB, but supplement intake was not (P = 0.9). The difference in milk yield and milk/supplement between 7-d forward- and backward-moving averages from the day of blood sampling were negatively associated with BHB (P < 0.01), showing that higher BHB values were associated with slower milk inclines. Accounting for parity in logistic regression models, smaller differences between the 2 moving averages for milk yield and milk/supplement were associated with greater risk of having BHB ≥ 1.2 mmol/L (P < 0.01), such that reducing the difference in milk yield by 4 kg and the difference in milk/supplement by 1 unit were associated with 1.35 (95% CI = 1.21−1.50) and 1.44 (CI = 1.29−1.61) times higher odds, respectively. These results highlight the differences in milk production (per day and relative to supplement consumed) associated with SCK and the potential for feed tables in robotic milking systems to reduce negative energy balance by accounting for milk production of fresh cows.

Key Words: ketosis, behavior, transition cow

364 Glucagon-like peptide 2 administration improves biomarkers of inflammation and intestinal morphology in feed restricted lactating Holstein cows. S. K. Kvidera*1, E. A. Horst1, M. V. Sanz Fernandez1, A. Abuaamjah1, S. Ganesan3, P. J. Gorden1, H. B. Green2, K. M. Schoenberg2, W. E. Trout2, A. F. Keating1, and L. H. Baumgard1, 1Iowa State University, Ames, IA, 2Elanco Animal Health, Greenfield, N.

The gastrointestinal tract is continuously exposed to pathogens, thus effective barrier function is important to prevent infiltration. Glucagon-like peptide 2 (GLP2) improves intestinal integrity in various models. Objectives were to characterize effects of GLP2 administration on inflammation and intestinal morphology in feed restricted (FR) cows; a model of leaky gut. Thirteen lactating Holstein cows (153 ± 12 DIM; 732 ± 16 kg BW; parity 2−4) were enrolled in 2 experimental periods. Period 1 (5 d of ad libitum feed intake) served as baseline for period 2 (5 d), during which cows received 1 of 3 treatments: 1) 100% of ad libitum feed intake (AL100; n = 3), 2) 40% of ad libitum feed intake + saline (AL40; 3 mL s.c. 2×/d; n = 5), or 3) 40% of ad libitum feed intake + GLP2 (AL40G; bovine GLP2; Elanco Animal Health, Greenfield, IN; 75 µg/kg BW s.c. 2×/d; n = 5). Milk yield decreased (~34%) similarly in both AL40 and AL40G cows compared with AL100 controls. Relative to AL40, AL40G cows had similar circulating endotoxin and lipopolysaccharide binding protein, decreased haptoglobin (76%; P = 0.01), and tended to have reduced serum amyloid A (57%; P = 0.11). Relative to AL100, AL40 cows had or tended to have decreased jejuneum villus width and crypt depth and ileum villus height, crypt depth, mucosal surface area, and cell proliferation (36, 14, 22, 28, 12, and 14%, respectively P ≤ 0.08). Goblet cell area was or tended to be decreased in jejunum, ileum, and colon of AL40 relative to AL100 cows (52, 25, and 17%, respectively; P ≤ 0.06). Compared with AL40, AL40G cows had 46 and 51% increased jejunum and ileum villus height, respectively and increased ileum crypt depth (17%; P ≤ 0.01). In jejunum and ileum, AL40G cows had increased villus height to crypt depth ratios, mucosal surface area, and cellular proliferation (38 and 35%, respectively, 30 and 27%, respectively, and 43 and 36%, respectively; P < 0.01) compared with AL40 cows. Goblet cell area from AL40G cows was increased 59, 17, and 48% in jejunum, ileum, and colon, respectively, relative to AL40 cows (P < 0.01). In conclusion, FR had detrimental effects on intestinal barrier integrity and administrating GLP2 mitigated these negative effects.

Key Words: feed restriction, glucagon-like peptide 2, gut health
Fatty liver (FL) is a common condition affecting dairy cows during the peripartum period, with no diagnostic test that is accurate, fast, and low cost. FL can be determined by TG extraction, histopathology or flotation of liver biopsies from live animals. However, these methods are invasive and at higher risk of hemorrhages, infection and adhesions. Cytology for detection of FL in cattle has not been extensively evaluated. It is feasible in clinical practice, being minimally invasive and inexpensive, and having a rapid turnaround time compared with biopsy. The objective was to estimate the sensitivity (Se) and specificity (Sp) of needle aspiration cytology and histopathology for FL using TG concentrations as the gold standard. Liver samples were harvested from a slaughterhouse in WI, USA. Livers were visually inspected for gross evidence of fat accumulation and classified as follows. (1): Normal—homogeneous maroon color (n = 20), (2): Moderate—moderate yellow-pale color (n = 20) and (3): Severe—more extreme yellow color (n = 20). Biopsies and needle aspiration were carried out from the parietal upper portion of the caudate lobe. Two samples of liver tissue were harvested with a 16 g x 15 cm biopsy needle for histological and TG concentration assessment. A third sample was harvested for cytology using a 18 g x 3.81 cm needle. The content of the needle was transferred to a glass slide, spread and air-dried. TG were assessed by a kit based on colorimetry/fluorimetry. Concentrations <2% were considered normal. Histological and cytological evaluation was blind to the visual classification and conducted by 2 pathologists. Se, Sp, and area under the receiver-operating curve [ROC] were calculated. For cytology, Se and Sp were 73% (95% CI 55.9–86.2) and 85% (95% CI 62.1–96.8), respectively. The area under ROC was 0.79 (95% CI 0.68–0.89). For histopathology, Se and Sp were 45.9% (95% CI 29.5–63.1) and 100% (95% CI 83.2–100), respectively. The area under ROC was 0.73 (95% CI 0.64–0.81). This data suggests that cytology may be a viable less invasive tool for detecting FL in dairy cattle.

**Key Words:** fatty liver, cytology, histology

### 367 Effects of chloride and sulfate-based diets fed to grazing prepartum dairy cows on postpartum plasma calcium.

P. Melendez*1, V. Zaror2, P. Gaul2, S. Poock1, and J. Goff3, 1College of Veterinary Medicine, University of Missouri, Columbia, MO, 2Tribute Dairy, Benton, MO, 3College of Veterinary Medicine, Iowa State University, Ames, IA.

Hypocalcemia is a common metabolic disorder affecting dairy cows during the postpartum period. Lowering dietary cation-anion difference (DCAD) by feeding supplemental chloride or sulfate has been shown to reduce the incidence of hypocalcemia. Different sources of anions have not been extensively compared under practical conditions. The aim of this study was to compare the effect of chloride (Cl) (SoyChlor; commercial chloride supplement) and sulfate (S) (magnesium sulfate) fed to grazing prepartum cows on postpartum plasma Ca. The study was conducted in southeast Missouri, USA in the fall of 2016. Breeds consisted of 20% Holstein, 20% Jersey and 60% crossbred Jersey × Holstein. Cows had been synchronized for breeding such that approximately 600 cows were due to calve in a short period of time. At 30 d before expected parturition, 2 groups of 200 cows each were moved into paddocks where they were fed each day a partial mixed ration (PMR) containing the anionic supplements. Cows had free access to pasture. The PMR would constitute half of the daily DMI. Estimated DCAD for the 2 diets was −25 mEq/kg DM and −26 mEq/kg DM for Cl and S, respectively. Urine samples were collected each week prepartum and checked for pH. Average pre-partum urine pH was 7.71 ± 0.12 and 7.81 ± 0.10 (P = 0.06) for Cl and S, respectively. At calving, 42 cows per group were matched by breed and parity (primiparous, multiparous), and their blood was sampled on d 1, 2, 3, and 7 postpartum. Plasma total Ca was determined by atomic absorption spectroscopy. Data for blood Ca concentration were analyzed by developing a mixed model ANOVA for repeated measures and the best covariance structure to fit the model. It is concluded that cows fed a TMR based on chloride had a higher calcium concentration at d 1 than cows fed sulfur as a source of anions.

**Key Words:** Jersey cow, hypocalcemia

### 373 Factors associated with subclinical hypocalcemia at calving on multiparous Jersey cows. A. Valdecabres*1, J. A. A. Pires2, and N. Silva-del-Río1,1Veterinary Medicine Teaching and Research Center, University of California Davis, Tulare, CA, 2Unité Mixte de Recherche sur les Herbivores, INRA, VetAgro Sup, Saint-Genes-Champanelle, France.

The objective was to evaluate factors associated with calcemic status at calving on 527 multiparous Jersey cows from 2 commercial dairies. Study variables included: lactation number (Lact; continuous, or 2, 3, ≥ 4); previous lactation 305-d mature equivalent milk production (Pr305ME) and somatic cell count (PrSCC); calving body condition score (BCS; ≤ 2.5, 2.75, ≥ 3) and locomotion score (LS; ≤ 2, 3, ≥ 4); calf number, sex, and stillbirth; and dry period length (dDry). Blood samples for serum Ca analysis were collected from the coccygeal vessels 3 h 14 min (±2 h 04 min) after calving. Previous lactation information and calving outcomes were obtained from DairyComp305 herd records. Based on serum Ca concentration at calving cows were classified as hypocalcemic (SHC; Ca ≤8.5 mg/dL; n = 180). Associations among variables were studied using spearman rank correlations and mixed models for continuous, and chi-squared and exact fisher tests for categorical data (CORR, MIXED, GLIMMIX and FREQ procedures of SAS). A significant positive correlation was observed between Lact and BCS, and both were negatively correlated with serum Ca concentration at calving. Cows with SHC had greater (P < 0.001) Lact (3.6 vs. 2.7), frequency of Lact ≥4 (40.4 vs. 14.4%), dDry (68.8 vs. 64.6 d), and BCS (43.4 vs. 58.2%, 31.2 vs. 27.7% and 25.4 vs. 14.1% for BCS ≤2.5, 2.75 and ≥3, respectively; P = 0.02[NI1]) than NC cows. Twinning rate was higher for SHC than NC (5.8 vs.1.1%; P = 0.01). The frequency of male calves (n = 98) was higher for SHC than NC cows (22.4 vs. 14.0%, P = 0.02), however, sexed semen was selectively used on both dairies and these results may derive from herd reproductive management. There was no association of Pr305ME, PrSCC, LS at calving or frequency of stillbirth with calcemic status. These complex associations among SHC, Lact, BCS, dDry, twinning and calf sex, could be used for the decision-making concerning prophylactic SHC treatment at calving.

**Key Words:** Jersey cow, hypocalcemia
Dairy cows are typically fed high concentrate diets. This feeding practice reduces chewing activity and leads to a high risk of subacute ruminal acidosis (SARA). The excess of concentrates in the diet may further deteriorate cow health and productivity. Because of their potential to modulate ruminal pH, phytogenics (PHY) and autolyzed yeast (AY) may counteract negative consequences of high-concentrate diets. This study investigated, if supplementing concentrate-rich diets with PHY and AY modulates chewing behavior and reticular pH. Eight ruminally cannulated nonlactating Holstein cows were arranged according to an incomplete double 4 × 3 Latin square design with 4 runs and 3 treatments (PHY (Digestarom Dairy, Biomin Holding GmbH), AY (Lebanon Rumen E, Biomin Holding GmbH) or no additive (CON) (n = 8 per treatment). The treatments were mixed and fed with the respective concentrates. During each run cows were fed a pure forage diet (FD1) before receiving a 65%-concentrate diet for one (CONC1) or 2 (CONC2) weeks, divided by a week FD2. Chewing activity was measured using noseband sensors (RumiWatch System, ITIN + Hoch GmbH) for 2 d during FD1, CONC1 and CONC2, while ruminal pH was evaluated continuously using wireless pH sensors (eCow, Decon). Statistical analysis was performed using the MIXED procedure of SAS. For each variable tested, the time (feedless pH sensors (eCow, Decon). Statistical analysis was performed using the MIXED procedure of SAS. For each variable tested, the time (feed-

### Table 1 (abstract 367). Mean (± SEM) plasma calcium (mg/dL) in grazing cattle fed a TMR containing sulfate or chloride

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<th>Sulfate</th>
<th>Chloride</th>
<th>(P)-value</th>
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<tr>
<td>d 1</td>
<td>7.83 ± 0.3</td>
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<tr>
<td>d 2</td>
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<tr>
<td>d 3</td>
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<tr>
<td>d 7</td>
<td>8.79 ± 0.3</td>
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<td>Multiparous</td>
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<td>d 1</td>
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<tr>
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<tr>
<td>d 7</td>
<td>8.07 ± 0.1</td>
<td>8.33 ± 0.1</td>
<td>0.85</td>
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Key Words: calcium, DCAD, grazing

### Table 1 (abstract 369). Summary of mycotoxin analysis

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<th>Parameter</th>
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<th>Afla</th>
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<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Mean of positives (ppb)</td>
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<td>412</td>
<td>20</td>
<td>271</td>
<td>0</td>
</tr>
<tr>
<td>SEM(^1) of positives (ppb)</td>
<td>116</td>
<td>669</td>
<td>135</td>
<td>8</td>
<td>7</td>
<td>NA</td>
</tr>
<tr>
<td>Maximum contamination (ppb)</td>
<td>11,700</td>
<td>119,400</td>
<td>19,000</td>
<td>139</td>
<td>401</td>
<td>0</td>
</tr>
<tr>
<td>Risk threshold (ppb)</td>
<td>900</td>
<td>2,000</td>
<td>200</td>
<td>20</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^1\) Standard error of mean.

Key Words: mycotoxin, deoxynivalenol, fumonisin

### 369 Mycotoxin survey in 2016 US corn. P. N. Gott\(^1\), E. G. Hendel\(^1\), T. Jenkins\(^2\), and G. R. Murugesan\(^3\), \(^1\)Biomin America Inc., San Antonio, TX, \(^2\)Biomin Holding GmbH, Getzersdorf, Austria.

Many common molds which are able to infect most types of grains and forages are capable of producing toxic secondary metabolites called mycotoxins. The negative effects on livestock are dependent on the level, type, and duration of mycotoxin exposure as well as animal factors such as age, species, and general health status. The objectives of the current study were to determine the occurrence of mycotoxins in the 2016 US corn crop and to assess the potential risk posed to livestock species. A total of 387 corn samples were collected from August 2016 to January 2017 from 26 states as part of the annual Biomin PROcheck mycotoxin survey. Samples were analyzed via the highly sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) technique for the presence of 17 mycotoxins from the following 6 major mycotoxin groups: Type B trichothecenes including deoxynivalenol (DON), fumonisins (FUM), zearalenone (ZEN), aflatoxins (Afla), Type A trichothecenes including T-2 toxin (T-2), and ochratoxin A (OTA). Ninety percent (90%) of the corn samples analyzed were contaminated with at least one mycotoxin while 67% of samples contained more than one mycotoxin. The presence of more than one mycotoxin may intensify the negative effects of each mycotoxin present, reducing animal performance to a greater extent than would be expected when a single mycotoxin is present. The percent of positive samples, mean of positives (ppb), standard error of the mean (SEM) of positives (ppb), and maximum of positives (ppb), as well as risk threshold (ppb) for the 6 major mycotoxin groups are presented in Table 1. Fumonisins and ZEN were frequently detected, often in combination with DON (28% of samples tested positive for all 3 toxins). The high prevalence as well as large number of samples above the FDA recommended level positions DON as the highest threat to livestock from the US corn harvest.

Key Words: mycotoxin, deoxynivalenol, fumonisin

### 370 Mycotoxin survey in US corn distillers dried grains with solubles. P. N. Gott\(^1\), E. G. Hendel\(^1\), T. Jenkins\(^2\), and G. R. Murugesan\(^3\), \(^1\)Biomin America Inc., San Antonio, TX, \(^2\)Biomin Holding GmbH, Getzersdorf, Austria.

Mycotoxins are toxic secondary metabolites produced by a variety of molds which may infect most crops. Severity of the negative effects associated with these toxins is dependent on the level, type, and duration of exposure and age, species, and health status of animals. Distillers dried grains with solubles (DDGS), a by-product of the ethanol industry,
are a common feed ingredient for various livestock species. During ethanol production, mycotoxins become more concentrated in DDGS than in the original corn. The objectives of the current study were to determine the occurrence of mycotoxins in US corn DDGS samples and to evaluate the potential risk posed to livestock. Seventy-nine corn DDGS samples from 14 states were analyzed between 2015 and 2016 as part of the Biomin PROcheck mycotoxin survey. Samples were analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS) technique for the presence of 17 mycotoxins from the following 6 major mycotoxin groups: Type B trichothecenes including deoxynivalenol (DON), fumonisins (FUM), zearalenone (ZEN), aflatoxins (Afla), Type A trichothecenes including T-2 toxin (T-2), and ochratoxin A (OTA). Each DDGS sample in the current survey was contaminated with at least one mycotoxin while 96% of samples contained more than one mycotoxin. The presence of more than one mycotoxin may result in an intensification of the negative effects of each mycotoxin present. The percent of positive samples, mean of positives [ppb], standard error of the mean (SEM) of positives [ppb], and maximum of positives [ppb], as well as risk threshold [ppb] for the 6 major mycotoxin groups are presented in Table 1. Deoxynivalenol was detected in 100% of samples and FUM and ZEN were also highly prevalent. Due to the high prevalence (100%) and large number of samples contaminated beyond the FDA recommended level (84%), DON poses the greatest threat from US corn DDGS.

**Key Words:** mycotoxin, deoxynivalenol, fumonisin

| Table 1 (abstract 370). Summary of mycotoxin analysis |
|-----------------------------------|---|---|---|---|---|---|
| Parameter                        | DON | FUM | ZEN | Afla | T-2 | OTA |
| Positive samples (%)              | 100 | 88  | 71  | 7    | 0   | 1   |
| Mean of positives (ppb)          | 2,681 | 3,393 | 230 | 12   | 0   | 19  |
| SEM\(^1\) of positives (ppb)     | 207 | 428 | 24  | 6    | NA  | NA  |
| Maximum contamination (ppb)      | 9,800 | 18,500 | 666 | 37   | 0   | 19  |
| Risk threshold (ppb)             | 900 | 2,000 | 200 | 20   | 100 | 100 |

\(^1\) Standard error of mean.
371 Calving ease, stillbirth, and gestation length of ProCROSS calves compared to pure Holsteins in two research herds. E. S. Houdek*1, A. R. Hazel1, B. J. Heins2, and L. B. Hansen1, 1University of Minnesota, Saint Paul, MN, 2West-Central Research and Outreach Center, Morris, MN.

ProCROSS calves sired by Montbéliarde (MX), Holstein (HX), and Viking Red (VX) bulls were compared with pure Holstein (HO) calves sired by Holstein bulls for calving ease score (CE; 1 to 5 scale), stillbirth (SB), and gestation length (GL). Calves were born in 1 of 2 research herds at the University of Minnesota, the high-input dairy at St. Paul (HO = 324, HX = 57, MX = 92, VX = 77) and the low-input dairy at Morris (HO = 239, HX = 89, MX = 156, VX = 196), from August 2009 to July 2016. Twin births were removed. Independent variables for statistical analysis of all traits included the fixed effects of herd, year of calving within herd, parity (primiparous or multiparous), sex of calf, breed, breed of sire nested within breed (breed group), interaction of sex of calf and breed, interaction of sex of calf and breed group, interaction of parity and breed group, and the random effect of cow nested within breed group. Female calves (1.3) had lower (P < 0.01) CE compared with male calves (1.6); however, SB was not significantly (P = 0.35) different between female (4%) and male (5%) calves. Furthermore, female calves across breed groups and parities (279 d) had significantly (P < 0.01) shorter GL than male calves (280 d). For first calving, the HO (1.6), HX (1.4), and VX (1.5) were similar for CE, and were significantly lower (P < 0.01) than MX (2.0) calves. However, for multiparous cows, CE was not significantly different between breed groups. For first calving, HX (2.5%), MX (6.6%), VX (5.7%) did not differ from HO (8.1%) calves for SB because standard errors were large. Furthermore, The HO calves (279 d) had significantly shorter (P < 0.05) GL than MX (282 d) and VX (280 d) calves across parities. Results indicate the 3 types of ProCROSS calves were similar to HO calves for CE and SB; however, GL of ProCROSS calves was 1.6 d longer than HO calves.

Key Words: crossbreeding, calving trait, Montbéliarde

372 The Finnish dairy farmers’ usage of AI bulls: Variation in realized trait preferences. E. P. Paakala*1,2, D. Martin-Collado3, A. Mäki-Tanila1, and J. Juga1, 1University of Helsinki, Department of Agricultural Sciences, Helsinki, Finland, 2Faba Co-op, Vantaa, Finland, 3Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain.

AI bulls in the Nordic joint dairy breeding program are selected primarily on Nordic Total Merit Index (NTM). Traits in the NTM are weighted based on their economic importance. Individual farmers’ preferences may differ from NTM weighting. The aims of this study were to investigate (1) the variation in the usage of AI bulls among Finnish dairy herds and its economic implications, and (2) the association of the variation with herd characteristics. Data consisted of 104,670 insemination records of 1,279 pure Ayrshire (AY) herds and 544 pure Holstein (HOL) herds within one year (2015), estimated breeding values (EBV) of the used AI bulls (176 AY and 232 HOL) and farm characteristics. Herds’ breeding profile was defined as the mean of the EBVs of the AI bulls used in the herd, weighted by the number of inseminations per bull. Herds were grouped according to the breeding profile using cluster analysis. Analysis was done separately for the breeds. The economic implications of breeding choices in each cluster were estimated, based on the traits’ genetic standard deviation and economic value in Finland. The result was compared with the expected response to the NTM based selection. Four herd clusters in both breeds were identified. Clusters were named according to the main focus in the breeding choices; Pure-AV herds: (1) Yield and functionality (452 herds), (2) Longevity, fertility and health (393), (3) All-rounders (377), (4) Conformation (57); Pure HOL herds: (1) Longevity, health and fertility (280), (2) Production (169), (3) Yield, conformation and longevity (48), (4) Conformation (47). In the first 3 clusters of both breeds their average herd breeding profiles were close to the NTM based selection indicating that the vast majority of farmers followed NTM in their breeding decisions. In the Conformation clusters, the traits other than conformation were almost neglected, which expectedly lead to poorer economic result than the NTM based selection. The Conformation focused herds were in a minority, were large herds and had recently made investments.

Key Words: farmers’ breeding preferences, AI bull, economic implications

373 Comparative performance of Holstein-Friesian dairy cows of contrasting Economic Breeding Index. M. O’ Sullivan*1,2, S. McParland1, K. M. Pierce2, and F. Buckley1, 1Teagasc Moorepark Animal & Grassland Research and Innovation Centre, Fermoy, Cork Ireland, 2School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland.

The objective of this study was to evaluate the phenotypic performance of 2 genetic groups of Holstein-Friesian cows based on the Irish national dairy index, the Economic Breeding Index (EBI). Data were available from 2013 to 2016. Ninety ELITE cows representative of the top 1% and 45 National Average (NA) cows, representative of the mean nationally; EBI €249 and €133, respectively (www.ICBF.com, April 2016), were evaluated annually across 3 contrasting seasonal pasture-based feeding treatments; Control, High Concentrate and Low Grass Allowance. All cows were parity 1 in 2013, 33.3% parity 1 and 66.6% parity 2 in 2014, in 2015, 27% of animals were parity 1, 27% parity 2 and 46% parity 3, and in 2016 27% of animals were parity 1, 20% parity 2, 20% parity 3 and 33% parity 4. Milk volume 5757kg v 5979kg was significantly lower (P = 0.0003) but milk fat (45.2g/kg v 42.2g/kg) and milk protein content (37.4g/kg v 35.5g/kg) was significantly higher (P < 0.001) throughout lactation. Pregnancy rate to first service (60% v 46%; P < 0.002), 6-week in-calf rate (73% v 58%; P < 0.0002), 13-week in-calf rate (92% v 81%; P < 0.0002) was higher, and number of services (1.57 v 1.78; P < 0.0002) was lower for ELITE compared with NA. Based on the 4 years data there was no genotype x feeding treatment interaction implying a similar milk response to additional concentrate and similar reduction in milk yield due to a lower grass allowance expressed by both genotypes. The results obtained provide confidence to the Irish dairy industry that genetic selection based on EBI delivers more productive and more fertile dairy cows. In addition, the study confirms that the decline in fertility evidenced in the Holstein-Friesian population, caused by selection mostly for milk production, may be reversed when appropriate selec-
tion pressure on suitable fertility traits is applied while simultaneously improving production.

**Key Words:** Economic Breeding Index, Holstein-Friesian, genotype

374 *Combined use of test-day model and principal component analysis to obtain heat tolerance phenotypes in dairy cattle.* N. P. P. Macciotta*1, S. Biffani2, U. Bernabucci3, N. Lacetera3, A. Vitali3, P. Ajmone-Marsan3, and A. Nardone3, 1University of Sassari, Sassarì, Italy, 2Associazione Italiana Elevatori, Rome, Italy, 3University of Tuscia, Viterbo, Italy, 4University of the Sacred Heart, Piacenza, Italy.

Tolerance to heat stress is one of the most relevant traits in defining the ability of animals to cope with environmental challenges. Phenotypes for measuring heat tolerance could be obtained by combining production and climate variables. Principal Component Analysis (PCA) carried out on test day records previously corrected for systematic effects except for THI is able to derive 2 principal components (PC) that summarize the individual patterns of corrected production data across different THI levels. However, PCA does not allow for missing data. To overcome this problem, a new method was proposed. In this work, 35,992 TD records of for milk yield, fat percentage and somatic cell score (SCS) measured on Italian Holstein cows sired by 3,697 bulls were analyzed. The fixed effects included the fixed effects of calving month nested within year, age at calving, herd nested within year, and the interaction between days in milk and parity, and the random effect of the sire. Sire (co)variance was modeled by 11 × 11 unstructured matrix, where 11 were the classes of THI into which TD records were grouped. PCA was then carried out on the estimated matrices. Finally, PC scores for each trait were calculated by multiplying the eigenvector matrix for the matrix of standardized data. As expected, the first principal component (PC1) was a measure of the level at which the pattern of corrected TD records across THI level is located, whereas the second (PC2) summarized the shape of this curve. Heritabilities of the PC1 scores were 0.39, 0.44, and 0.37 for milk yield, fat percentage, and SCS respectively. Values for PC2 were 0.07, 0.05, and 0.06 respectively. Genetic correlations between PC1 and PC2 were lower than 0.20 for milk yield and fat percentage, 0.39 for SCS. The combined use of mixed linear model and PCA allowed the derivation of 2 phenotypes able to summarize describe the overall level and the shape of patterns of milk production traits across different levels of THI index.

**Key Words:** heat tolerance, mixed model, principal component analysis

375 *Single-step genomic evaluation of digital dermatitis in Canadian Holsteins.* F. Malchiodi*1, D. A. L. Lourenco2, I. Misztal2, A.-M. Christen3, J. Jamrozik1,4, F. S. Schenkel1, D. F. Kelton5, and F. Miglior1,4, 1Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada, 2Department of Animal and Dairy Science, University of Georgia, Athens, GA, 3Valacta, Sainte-Anne-De-Bellevue, QC, Canada, 4Canadian Dairy Network, Guelph, ON, Canada, 5Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

This study aimed to assess the gains in the breeding value reliability using single step genomic evaluation for digital dermatitis. Hoof lesions were recorded by 51 hoof trimmers during routine trimming visits in 1,080 herds located in Alberta, British Columbia, New Brunswick, Ontario, and Quebec between 2009 and 2016. The final data set contained 249,801 observations from 105,557 cows. The final pedigree file contained 351,215 animals. Of those animals 10,924 (8,934 bulls and 1,990 cows) also had genomic information and 3,637 cows had both phenotypic and genomic information. Animals were genotyped with either the 50K SNP panel or the low-density panel. Imputation to 50K SNP for animals genotyped with the low-density panel was done using FImpute software. Digital dermatitis was analyzed as a binary trait, where 1 was assigned to the presence of the lesion. Traditional pedigree-based and genomic evaluations were computed using the BLUPF90 software. The daughter deviation (DD) from complete data set was used as the expected future progeny performance of validation bulls to assess predictive ability based on the reduced data set, in which only phenotypes recorded up to 2011 were included. The coefficient of determination ($R^2$) from the regression of DD on parent average or GEBV in 2011 was used to assess the validation reliability of the models. Digital dermatitis had a prevalence of 18.5% and a heritability (SE) of 0.08 (0.005). As expected, predictions based on genomic information had higher $R^2$ than traditional evaluations (0.11 and 0.07, respectively, for sires with more than 10 daughters with recorded lesions in the complete data set). Also, the inflation of the evaluation for bulls in the validation population was lower when genomic information was included in the analyses. For sires with more than 10 daughters with recorded lesions in the complete data set, the regression coefficients for traditional and genomic evaluation were 0.86 and 0.99, respectively. Results indicate that the use of genomic selection for digital dermatitis can improve the accuracy of selection for young bulls compared with using selection based on parent average information.

**Key Words:** digital dermatitis, single-step

376 *Genetic control of health treatment cost and the correlation of health treatment cost with production and conformation of first-lactation Holstein cows.* M. R. Donnelly*1, A. R. Hazel2, B. J. Heins2, and L. B. Hansen2, 1University of Minnesota, St. Paul, MN, 2West-Central Research and Outreach Center, Morris, MN.

Heritability and correlations were estimated for the cost of health treatments during first lactations of Holstein cows (n = 2,214) in 8 high-performance dairy herds. A fixed treatment cost (including veterinary fees, pharmaceutical expense, and labor cost) was assigned to 14 types of health treatment cost. Treatments were grouped into 5 categories: mastitis (MAST; including diagnostic tests), reproduction (REPRO); cystic ovary, retained placenta, metritis), lameness (LAME; hoof treatments), metabolic (META; milk fever, displaced abomasum, ketosis, digestive), and miscellaneous (MISC; respiratory, injury, other). Total health cost (THC) was summed for each cow. The fixed effect of herd and the random effect of cow nested within herd were fitted with linear animal models using ASReml. The estimates of heritability were significant for MAST, LAME, META, and THC (Table 1). Genetic correlations between categories of health treatment cost were largest for MAST and REPRO (0.85 ± 0.20); however, phenotypic correlations between all categories were small (r <0.16). The THC had a large genetic correlation with 305-d milk production (0.44 ± 0.18) and somatic cell score (0.93 ± 0.13). The genetic correlation (−0.60 ± 0.16) between THC and udder depth indicated shallower udders were genetically associated with less THC. Genetic selection for reduced health treatment cost should be possible from the use of producer-recorded health treatment records that are supplemented with treatment cost.
The objectives of this research included estimating variance components for ketosis susceptibility and identification of genomic regions associated with ketosis in Jersey cattle. Ketosis is one of the most commonly reported metabolic diseases in dairy herds. Genetic analyses of ketosis have been conducted previously, but few focus specifically on Jersey cattle. Voluntary producer-recorded health event data related to ketosis were available from Dairy Records Management Systems (NCSU, Raleigh, NC). Standardization was implemented to account for the various acronyms used by producers to designate an incidence of ketosis. Ketosis events were restricted to the first reported incidence within 60 d of calving in first through fifth parity. After editing, there were a total of 42,233 records from 23,865 cows. A total of 1,750 genotyped animals were used for analyses using 60,671 markers. Given the binary nature of the trait, a threshold animal model was fitted using THRG-IBBS1F90 (version 2.110) using only pedigree information and then also incorporating genomic information using a single-step genomic BLUP approach. postGSD90 (version 1.38) was used to calculate SNP effects as well as variance explained by 10-SNP windows. Heritability of ketosis susceptibility was 0.083 (SD = 0.021) and 0.078 (SD = 0.018) in pedigree-based and genomic analyses, respectively. The marker with the largest estimated SNP effect was located on chromosome 10 at 66.3 Mbp. Additional peaks were identified on chromosomes 11, 14, and 23. The region explaining the largest proportion of variance (0.70%) was located on chromosome 6 at 56.1 Mbp. Additional regions explaining large proportions of variance were located on chromosomes 11 (0.51%), 3 (0.45%), and 25 (0.40%). Genes located in these regions were investigated for having a role in ketosis susceptibility. Results indicate that ketosis susceptibility in Jerseys has a significant genetic component, making feasible the selection for animals more resistant to ketosis. Associated genomic regions could be incorporated into genomic evaluations in the future, as well as used to further understand the underlying biology of this disease.

Key Words: genomic analysis, Jersey, ketosis

Table 1 (abstract 376). Estimates of heritability (in bold), genetic correlations (above diagonal), and phenotypic correlations (below diagonal) for the treatment costs of 5 health categories and total health treatment cost for first parity

<table>
<thead>
<tr>
<th></th>
<th>MAST</th>
<th>REPRO</th>
<th>LAME</th>
<th>META</th>
<th>MISC</th>
<th>THC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAST</td>
<td>0.13*</td>
<td>0.85*</td>
<td>0.34</td>
<td>0.52</td>
<td>0.66</td>
<td>0.92*</td>
</tr>
<tr>
<td>REPRO</td>
<td>0.00</td>
<td>0.04</td>
<td>0.41</td>
<td>0.73*</td>
<td>0.59</td>
<td>0.91*</td>
</tr>
<tr>
<td>LAME</td>
<td>0.03</td>
<td>–0.01</td>
<td>0.10*</td>
<td>0.56</td>
<td>0.21</td>
<td>0.65*</td>
</tr>
<tr>
<td>META</td>
<td>0.02</td>
<td>0.14*</td>
<td>0.02</td>
<td>0.12*</td>
<td>0.40</td>
<td>0.85*</td>
</tr>
<tr>
<td>MISC</td>
<td>0.04*</td>
<td>0.02</td>
<td>–0.05*</td>
<td>0.16*</td>
<td>0.04</td>
<td>0.72*</td>
</tr>
<tr>
<td>THC</td>
<td>0.34*</td>
<td>0.66*</td>
<td>0.27*</td>
<td>0.63*</td>
<td>0.39*</td>
<td>0.27*</td>
</tr>
</tbody>
</table>

*Estimate significantly different from zero based on 95% CI.

Key Words: health treatment cost, Holstein


The objectives of this research were to estimate variance components for 6 common health events recorded by producers on US dairy farms, as well as investigate correlations with fitness traits currently used for selection. Producer-recorded health event data were available from Dairy Records Management Systems (NCSU, Raleigh, NC) for 6 common health events occurring in US dairy herds: hypocalcemia (CALC), displaced abomasum (DSAB), ketosis (KETO), mastitis (MAST), metritis (METR), and retained placenta (RETP). Standardization and editing constraints were applied to ensure data validity. After editing, the number of phenotypic records ranged from 1.2 million for CALC up to 2.5 million for MAST. Traditional predicted transmitting abilities (PTA) were calculated for 63.1 million Holsteins using a linear animal model accounting for year-season, age-parity, herd-year, and permanent environmental effects, as well as a regression on inbreeding. Heritability estimates on the observed scale were 0.6%, 1.1%, 1.2%, 3.1%, 1.4%, and 1.0% for CALC, DSAB, KETO, MAST, METR, and RETP, respectively. Genomic PTA were calculated using 60,671 markers for 1.36 million Holsteins. For bulls with >90% reliability (>75% for CALC and RETP), health trait PTA had low correlations with PTA protein (~0.03 to 0.23) but much higher correlations with official PTA for several fitness traits included in net merit. Largest correlations for each health trait were ~0.68 for MAST with somatic cell score (SCS), 0.59 for KETO with daughter pregnancy rate (DPR), 0.47 for DSAB with livability, 0.46 for METR with DPR, –0.29 for CALC with SCS, and 0.17 for RETP with productive life (PL). An economically weighted sum of all 6 health trait PTA was correlated by 0.56 with PL, 0.55 with livability, 0.50 with DPR, and ~0.45 with SCS, using estimated costs per case of $72 for MAST, $178 for DSAB, $105 for METR, $64 for RETP, $38 for CALC, and $28 for KETO. Young animal reliabilities averaged 11–18% from the pedigree model vs. 40–49% from genomic predictions. The standard deviation of lifetime net merit is $193 compared with $8 for the sum of health trait PTA that could be included in the near future.

Key Words: genomic evaluation, health, fitness


The objectives of this research included estimating variance components for ketosis susceptibility and identification of genomic regions associated with ketosis in Jersey cattle. Ketosis is one of the most commonly reported metabolic diseases in dairy herds. Genetic analyses of ketosis have been conducted previously, but few focus specifically on Jersey cattle. Voluntary producer-recorded health event data related to ketosis were available from Dairy Records Management Systems (NCSU, Raleigh, NC). Standardization was implemented to account for the various acronyms used by producers to designate an incidence of ketosis. Ketosis events were restricted to the first reported incidence within 60 d of calving in first through fifth parity. After editing, there were a total of 42,233 records from 23,865 cows. A total of 1,750 genotyped animals were used for analyses using 60,671 markers. Given the binary nature of the trait, a threshold animal model was fitted using THRG-IBBS1F90 (version 2.110) using only pedigree information and then also incorporating genomic information using a single-step genomic BLUP approach. postGSD90 (version 1.38) was used to calculate SNP effects as well as variance explained by 10-SNP windows. Heritability of ketosis susceptibility was 0.083 (SD = 0.021) and 0.078 (SD = 0.018) in pedigree-based and genomic analyses, respectively. The marker with the largest estimated SNP effect was located on chromosome 10 at 66.3 Mbp. Additional peaks were identified on chromosomes 11, 14, and 23. The region explaining the largest proportion of variance (0.70%) was located on chromosome 6 at 56.1 Mbp. Additional regions explaining large proportions of variance were located on chromosomes 11 (0.51%), 3 (0.45%), and 25 (0.40%). Genes located in these regions were investigated for having a role in ketosis susceptibility. Results indicate that ketosis susceptibility in Jerseys has a significant genetic component, making feasible the selection for animals more resistant to ketosis. Associated genomic regions could be incorporated into genomic evaluations in the future, as well as used to further understand the underlying biology of this disease.

Key Words: genomic analysis, Jersey, ketosis

379 Genome-wide association study for clinical mastitis, metritis, and ketosis in US Holstein cattle. A. Sigdel*1, C. K. Mak1,2, R. Abdollahi-Arpanah1,3, K. Galvão1, and F. Pehagiganico1.

Health traits impact the profitability of dairy production and affect animal welfare. The objective of this study was to perform a genome-wide association study (GWAS) to identify genomic regions, and preferably candidate genes associated with Clinical Mastitis (CM), Metritis (MET) and Ketosis (KET) in US Holstein cattle. Data consisted of 28,000 producer-recorded health event records from 14,000 cows in one large commercial dairy farm. Whole-genome single nucleotide polymorphism (SNP) data were available for 7,500 animals. The association analyses were performed using the single-step genomic BLUP approach combining all the available phenotypic, pedigree and genotypic data into threshold models. These models included year-season and parity as fixed effects, and animal and permanent environmental as random effects. Candidate regions were identified based on the amount of genetic variance explained by 2-Mb SNP windows. Several genomic regions were associated with these 3 relevant postpartum diseases. For instance, 4 regions located on BTA5, BTA6, BTA8 and BTA 29 explained together more than 3.0% of the genetic variance for CM. These regions harbor many candidate genes, such as CXCL13, SPTLC1, and FADD that are involved in mammary gland inflammation. Similarly, different regions on BTA9, BTA13, BTA14 and BTA29 explained between 0.75% and 1.0% of the genetic variance for MET. These regions harbor several interesting genes, including GSDMC and CCR6 that are directly
involved in inflammation of uterine tissues. Two other potential candidate genes in these regions, RASSF2 and WDR37, induce cell arrest and apoptosis of the uterine tissue. For KET, strong genetic signals were found on BTA8, BTA14 and BTA16. Interestingly, the genomic region on BTA14 explained more than 2.5% of the genetic variance and contains 2 candidate genes, DENND3 and PTK2, that are directly implicated in the metabolism of ketone bodies. Overall, our findings could contribute to a better understanding of the genetic control of these 3 important postpartum diseases. In addition, our findings may provide opportunities for improving these health traits in dairy cattle through marker-assisted selection.

**Key Words:** genetic dissection, postpartum diseases, wellness traits

### 380 Genome-wide DNA methylation patterns and differential methylation in leukocytes from Holstein cattle

C. D. Dechow* and W. S. Liu, *Pennsylvania State University, University Park, PA.

The objectives of this study were to describe DNA methylation patterns in leukocytes from dairy cows with variable milk yield, determine associations with gene expression from an independent population, and to identify regions with differential methylation. DNA was extracted from blood samples of 8 lactating Holstein dairy cows with 305 d milk yields ranging from 10,948 kg to 23,105 kg. Following methylated DNA immunoprecipitation, approximately 100 million pair-ended reads per cow were sequenced and aligned to the bovine genome. There were differences among chromosomes \( P < 0.05 \) with the lowest methylation levels observed for the X chromosome and chromosome 6, whereas chromosome 19 had the highest methylation levels. Methylation patterns were described for 13,677 protein coding genes and associations with expression from a separate population determined for 9,750 genes. Gene rich regions had more unique reads mapped than gene poor regions \( P < 0.05 \), but repetitive elements were not considered. Methylation levels were generally low upstream of genes with the nadir occurring 95 base pairs before the transcription start site. Methylation was lower over the first exon than later exons and, for introns, highest near intron-exon junctions. Gene expression became increasing negative as methylation increased until 35 base pairs upstream of the transcription start site, and for higher methylation across the first exon. Regions of differential methylation were evident, including in an immune related gene family on chromosome 19 (**SECTMI**). Approximately 27% of the genome was associated with 100 kb or larger regions of suppressed methylation, or partially methylated domains (PMD). Functional annotation identified olfactory transduction related genes as enriched in PMD. 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Market insights and consumer trends in fluid milk and beverages. M. Wilcox*, Significant Outcomes LLC, Pandora, OH.

Ninety-five percent of US households purchase fluid milk at retail and are increasingly demanding wholesome, nutritious, and sustainable products. These shifts in consumer demand present a growth opportunity for the dairy industry to create value-added products for the fluid milk and beverage categories beyond the traditional gallon jug. Based upon data from the IRI DMI Custom Dairy Milk Database, flavored milk, lactose-free milk, and whole milk retail sales increased 6.6%, 15.8% and 5.3% respectively, in 2016. While much of the increase in whole milk sales has occurred from switching away from lower fat versions, some whole milk households are increasing their overall milk usage. Whole milk now represents over a third of the fluid milk category. Whole milk’s gaining acceptance may be tied to consumers’ interest in real and natural and emerging science finding no link between saturated fat and increased risk of heart disease, stroke, or diabetes. Refuel milks focused on consumer interest for additional protein have also increased 36.7%.

Understanding the evolving marketplace and emerging nutritional findings is important when considering which advancements in dairy product and process research will need to occur to meet the needs of consumers today and in the future.

Key Words: fluid milk, whole milk, protein

The influence of protein and fat on sensory properties and consumer perception of fluid milk. M. A. Drake*1 and D. M. Barbano2, 1North Carolina State University, Raleigh, NC, 2Cornell University, Ithaca, NY.

Sensory properties of milk are crucial to its consumer appeal. Milk heat treatment and composition (fat and protein) impact sensory properties and consumer liking. Higher heat treatments associated with extended shelf life (ESL) are not preferred over traditional high temperature short time (HTST) pasteurized milk by most consumers, conceptually or by tasting. The fat content of milk is the most readily adjusted compositional factor of milk. Fat plays a multi modal role in the sensory properties of milk, providing appearance cues but also mouthfeel and flavor properties that have varying appeal to different consumer segments. Finally, the sensory properties of milk can also be adjusted by altering protein content and casein: true protein ratio. Cooked/sulfur and cardboard flavors, viscosity and throat cling can be increased with protein content (3.00, 3.67, 4.34, and 5.00%; \( P < 0.05 \)) while increased casein as a percentage of true protein (5, 25, 50, 75, and 80%) can be applied to decrease cardboard flavor and astrinency \( P < 0.05 \) and to increase cooked/milky, cooked/sulfur and throat cling \( P < 0.05 \). These results demonstrate that heat treatment and fat influence sensory properties of traditional milk, but also that membrane fractionation can be applied to optimize physical and sensory properties of milk beverages.

Key Words: milk, sensory properties, consumer liking

Preserving milk freshness in retail environment. S. E. Duncan*, Virginia Tech, Blacksburg, VA.

“Freshness” is a valued quality attribute for fluid milk but interpretation and assumptions associated with the term may be different to scientists, processors, retailers, and consumers. Dairy production and processing professionals influence fluid milk freshness, from cow to cup, based on production and processing decisions and practices. Consumer perception of fluid milk freshness of fluid milk begins at the point of purchase and continues through their experience with the product in their home. Storage conditions and handling in the retail environment can have significant effect on consumer perception of freshness of fluid milk, but has received very limited attention. Retailer decisions on dairy retail case design and management, employee training, and interaction with customers can influence freshness and customer satisfaction with fluid milk quality. This presentation will explore the (1) influence of retail display case lighting and milk packaging on consumer perception of freshness and acceptability; (2) opportunity for protecting freshness and quality through the training of retail dairy managers; and (3) potential influence of such decisions and actions on consumer perception of milk freshness and quality. In our recent studies, we verified that controlled LED lighting positively influenced milk acceptability in contrast to the traditional fluorescent lighting. Preliminary evidence from our laboratory illustrates that dairy retail managers are not familiar with the influence of lighting on milk quality and relationship to consumer perception of freshness. Retail case lighting and case design innovation benefits from controlled research studies and discussion with experts in packaging, milk processing and quality, retail economics and marketing, and consumer insights to protect milk freshness.

Key Words: milk, quality, consumer
This will improve the efficiency and sustainability of milk production while providing consumers with a demonstration of what the dairy industry is doing to meet their expectations.

**Key Words:** fatty acids, blood NEFA, blood BHB

**387  Impact of post-pasteurization contamination on milk quality.** N. Martin*, A. Alles, S. Reichler, K. Boor, and M. Wiedmann, Cornell University, Ithaca, NY.

Fluid milk quality in the US has improved steadily over the last 2 decades, in large part due to the reduction in post-pasteurization contamination (PPC). Despite these improvements, nearly 50% of fluid milk is still spoiled (i.e., reaching bacterial levels > 20,000 cfu/mL) due to PPC. Over 30% of all dairy products are lost each year before consumption, in part due to bacterial contamination. Gram-negative spoilage bacteria when introduced as PPC grow rapidly at refrigeration temperatures and lead to spoilage within 7–10 d of processing. Other notable organisms that cause PPC are psychrotolerant coliforms and Enterobacteriaceae. These organisms are known to produce a variety of enzymes that lead to flavor, odor and body defects which can ultimately impact consumer perception and willingness to buy. Detecting PPC in freshly pasteurized HTST fluid milk can be challenging because many times PPC occurs sporadically and at low levels. Additionally, indicator organisms typically used in fluid milk (i.e., coliforms) have been shown to represent only a fraction of the total PPC. Recent studies indicate that coliforms account for less than 20% of the total gram-negative organisms introduced into fluid milk post-pasteurization. In contrast, *Pseudomonas*, which is not a coliform and therefore is not detected using coliform media, is the most commonly isolated genus in PPC fluid milk. To reduce PPC processors must begin to use testing methods that can both detect coliforms as well as non-coliform gram-negatives (i.e., *Pseudomonas*). This “total Gram-negative” approach will allow the industry to detect and respond to PPC, thereby providing consumers with the highest quality product.

**Key Words:** post-processing contamination, quality, fluid milk
Genomes of Advenella, Psychrobacter and Psychroflexus strains into ripening and flavor generation. S. Schmitz-Esser1,2, E. Nischler2, M. Dzieciol2, E. Mann3, and M. Wagner. Iowa State University, Department of Animal Science, Ames, IA, 2University of Veterinary Medicine Vienna, Institute for Milk Hygiene, Vienna, Austria.

Vorarlberger Bergkäsé (VB) is an artisanal raw milk washed-rind hard cheese manufactured in Western Austria without adding external ripening cultures. The composition of the VB rind microbiota has been described recently by our group. To learn more about the functional contribution of abundant gram-negative bacteria to cheese ripening, we performed genome sequencing of Advenella (Betaproteobacteria), Psychrobacter (Gammaproteobacteria) and Psychroflexus (Bacteroidetes) isolated from VB rinds. Furthermore, total and species-specific bacterial numbers during ripening were determined using quantitative PCR (qPCR). Cheese rind samples were taken from ripening cellars of 2 cheese producing facilities in Austria at the day of production and after 14, 30, 90 and 180 d of ripening. Genome sequencing resulted in 17 to 27 contigs with assembly sizes of 2.7 Mb (Psychrobacter), 3 Mb (Psychroflexus), and 4.3 Mb (Advenella). Our results reveal that each genome harbors enzymes shown to be important for cheese ripening such as: Cystathionine-Methionine β or gamma-Lyases, many proteases and peptidases (including proline imminopeptidases), aminotransferases, and lipases. Thus, all 3 isolates have the potential to contribute positively to cheese ripening. Psychrobacter and Psychroflexus increased significantly during the first 30 d, then decreased to their initial abundance during the rest of ripening. Advenella decreased significantly in the first month of ripening, but increased significantly throughout ripening. In conclusion, the 3 species quantified were stable community members throughout the ripening process and their abundance on cheese rinds together with the results from genome sequencing suggests an important contribution of these bacteria to cheese ripening. Although Advenella, Psychrobacter and Psychroflexus have been found on cheese rinds in recent studies in various different cheeses, our knowledge about their possible contributions to cheese ripening is still limited. Here, we provide first insights into their genomic potential with a particular focus on cheese ripening.

Key Words: cheese rind bacteria, genome

Lactoferrin protect Caco-2, HEK, Hep-G2 and SK-N-SH cell lines inhibits aflatoxin-cytotoxicity and oxidative DNA damage. H. Zhang1,4, N. Zheng1,2, J. Liu2, Y. N. Gao1,2, and J. Q. Wang1,2. 1Ministry of Agriculture-Key Laboratory of Quality & Safety Control for Milk and Dairy Products, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2Ministry of Agriculture-Laboratory of Quality and Safety Risk Assessment for Dairy Products, Beijing, China, 3Ministry of Agriculture-Milk and Dairy Product Inspection Center, Beijing, China, 4College of Food Science& Engineering, Ji Lin University, Chang Chun, China, 5China National Research Institute of Food and Fermentation Industries, Beijing, China.

Aflatoxin is a natural potent carcinogen produced by Aspergillus flavus and A. parasiticus that can be detected in milk. It is a major cause of liver cancer. However, the involved molecular mechanisms and intervention methods remain largely unexplored. Lactoferrin (LF) was examined with regard to its potential role as a scavenger against radical oxygen. Moreover, no one has studied whether LF would inhibit the cytotoxicity of aflatoxin B1 (AFB1) and aflatoxin M1 (AFM1). The purpose of this study was to evaluate the AFB1 and AFM1 induced cell toxicity by determining cell viability, membrane permeability and genotoxicity, and then investigate the capacity of LF to protect cells against AFB1 and AFM1. Cell viability, lactate dehydrogenase (LDH) release, TBARS and glutathione production and DNA damage were determined. In the Comet assay, DNA damage was quantified by assessing tail moment (TEM; arbitrary unit) and tail length (TL; arbitrary unit), as indicators of DNA strand breaks in single cell gel electrophoresis. Data showed that 4 µg/mL AFB1 or AFM1 could significantly inhibit Caco-2 cells, HEK cells, Hep-G2 cells, and SK-N-SH cell growth, increase lactate dehydrogenase (LDH) and cause genetic damage (P < 0.05). In comparison, AFB1 was found to be more toxic than AFM1 on all 4 cells especially on Hep-G2 cells, indicating liver is more sensitive than other organ as an important detoxification system. All these cytotoxic outcomes might be associated with intracellular ROS generation, leading to membrane damage and DNA strand break. Significant reductions in cytotoxicity and oxidative DNA damage were observed when cells were pretreated with 10, 100 or 1000 µg/mL LF then exposed to 4 µg/mL AFB1 or AFM1. Our data suggested that AFB1 or AFM1 induces DNA damage in Caco-2 cells, HEK cells, Hep-G2 cells, and SK-N-SH cells, whereas that the antioxidant activity of LF may contribute to the alleviation of AFB1 or AFM1-induced cytotoxicity and DNA damage by reducing oxidative stress, it proved that LF plays an active role in inhibiting the toxicity of mycotoxins.

Key Words: aflatoxin, lactoferrin, oxidative DNA damage

Impact of the addition of exopolysaccharides containing β (1→ 4), and β (1→ 3) linkages isolated from Streptococcus thermophilus into milk prior to fermentation on physical and rheological properties of fermented milk gels. S. N. Khanal*1 and J. A. Lucey1,2. 1University of Wisconsin, Department of Food Science, Madison, WI, 2Wisconsin Center for Dairy Research, Madison, WI. Streptococcus thermophilus strains St-143 and DGCC7785 are known to produce exopolysaccharides (EPS), where sugars in the repeating units are linked mainly by β (1→ 4), and β (1→ 3) types of glycosidic linkages, respectively. We investigated the impact of addition of these isolated EPS to milk before fermentation on the properties of milk gels. Milk samples were fermented (at a similar rate of acidification) by these strains and these EPS solutions were isolated by ultrafiltration. Skim milk was rehydrated in these isolated EPS solutions containing 60, 120 and 200 mg of EPS/L, heated to 85°C for 30 min, cooled, and fermented at 40°C by a non-EPS producing S. thermophilus strain ST-1UWM until pH 4.6. Milk gels formed without any EPS, and formed by the respective EPS producing S. thermophilus strains were used as 2 types of controls. Rheological properties of the gels during fermentation were determined using small-strain dynamic oscillatory measurements. Yield stress and yield strain of the gels were determined at pH 4.6. Whey separation and permeability of the milk gels were measured at pH 4.6. The fermentation times for all the milk samples were similar (~250 min). The pH of gelation of the milk samples ranged from pH 5.1 - 5.3. The storage modulus (G’) values at pH 4.6 of the gels made with in situ production of the β (1→ 3) linked EPS (72 Pa), and the in situ β (1→ 4) linked EPS (35 Pa) were significantly different from the control (non-EPS producer) (53 Pa). Addition of isolated EPS to milk did modify the rheological, whey separation and permeability properties, however, additional of isolated
EPS could not exactly replicate the properties of the gels where this type of EPS was produced in situ. Other possible factors like the time during fermentation when EPS was produced, or the distribution of EPS within the gel network could play a role in explaining why isolated EPS could not better replicate the physical properties of acid milk gels.

**Key Words:** exopolysaccharide, glycosidic linkages, rheological properties

**Transcriptomic analysis of high exopolysaccharide-producing dairy starter bacterium *Streptococcus thermophilus* ASCC 1275 in milk.** Q. Wu and N. P. Shah*, The University of Hong Kong, Pok Fu Lam Road, Hong Kong.

*Streptococcus thermophilus* ASCC 1275, a typical dairy starter bacterium that has been completely sequenced, could produce the highest known amount (~1000 mg/L) of exopolysaccharide (EPS) in milk under the optimal condition among the species of *Str. thermophilus*. However, little is known about the mechanistic insights into the effects of environmental factors such as pH, temperature and whey peptides on its EPS production. In this study, RNA-seq was applied to this organism cultivated in skim milk under different conditions: (1) Cd1 – pH 6.5 and 37°C; (2) Cd2 – pH 5.5 and 37°C; (3) Cd3 – pH 5.5 and 40°C; (3) Cd4 – pH 5.5 and 37°C with whey peptide supplementation. TopHat and Cufflinks pipelines were used for differential gene expression analysis. Results showed that there were 526 genes that have been significantly changed in Cd2 compared with that in Cd1; specifically, ribosomal protein synthesis and EPS assembly genes were significantly upregulated in Cd2 which may explain the enhanced EPS production in Cd2. As for temperature effect, only 49 genes were significantly changed in Cd3 compared with that in Cd2; purine and lactose metabolism were enhanced in Cd3 suggesting that more nucleotide sugars (UDP-glucose and UDP-galactose) may have been synthesized for EPS assembly in Cd3. Whey peptide supplementation induced the significant changes of 16 genes in Cd4 compared with that in Cd2; it was found that cysteine and methionine metabolism and some unidentified amino acid/peptide transporters were improved for bacterial growth fitness. This study indicates that regulation of nucleotide sugar synthesis, EPS assembly and bacterial fitness are responsible for an enhanced EPS production from *Str. thermophilus* ASCC 1275.

**Key Words:** *Streptococcus thermophilus*, exopolysaccharide, environmental factors

**Un-shielding biofilm forming bacteria of protective extracellular matrix provides novel mean to improve dairy products microbial quality.** M. Shemesh*, N. Ben-Ishay1,2, D. Inbar1,3, R. Refin2, and D. Steinberg3, 1Department of Food Quality and Safety, Institute for Postharvest Technology and Food Sciences, Agricultural Research Organization, Rishon LeZion, Israel, 2The Robert H. Smith Faculty of Agriculture, Food and Environment, The Institute of Biochemistry, Food Science and Nutrition, The Hebrew University of Jerusalem, Rehovot, Israel, 3Biofilm Research Laboratory, Institute of Dental Sciences, Faculty of Dental Medicine, Hebrew University-Hadassah, Jerusalem, Israel.

Microbial damages caused by biofilm forming bacteria in the dairy industry are a fundamental threat to safety and quality of milk products. *Bacillus* species, which are common contaminants of dairy products, can form structured multicellular communities known as biofilms on contact surfaces as well as within the milk products themselves. We investigated the role of extracellular matrix (EM) produced through biofilm formation in bacterial survival during milk processing. We used molecular genetics and food microbiology methodology to show that EM, produced by *Bacillus subtilis* during biofilm formation in milk, have a major role in bacterial survival during the milk processing. Noticeably, the mutant strains of *B. subtilis*, which cannot produce EM during biofilm formation, showed hypsersensitivity to milk processing procedures such as heat pasteurization. This finding indicates that the EM could serve as a protective material for biofilm forming bacteria and un-shielding it would lead to increased sensitivity of bacterial cells to stress environments encounter during milk processing. Consequently, we aimed to mitigate biofilm formation using the ability of divalent cations such as Mg²⁺ of blocking EM production. Our further findings indicate that in the presence of Mg²⁺ bacterial cells are hypsersensitive to the heat pasteurization applied during milk processing. We therefore suggest that un-shielding biofilm forming bacteria of protective EM may provide novel mean to improve dairy products microbial quality.

**Key Words:** biofilm formation, extracellular matrix, dairy food
393 **An introduction and overview of the emerging field of microbial endocrinology.** M. Lyte*, Iowa State University, Ames, IA.

Microbial endocrinology represents the intersection of 2 seemingly disparate fields, microbiology and neurobiology, and is based on the shared presence of neurochemicals that are exactly the same in structure in the host as well as in the microorganism. The ability of microorganisms not only to respond to but also to produce many of the same neurochemicals that are produced by the host, such as during periods of stress, has led to the introduction of this evolution-based mechanism which has a role in the pathogenesis of infectious disease as well as the microbiota-gut-brain axis. Production of neurochemicals by microorganisms usually employs the same biosynthetic pathways as those utilized by the host, indicating that acquisition of a neurochemical-based signaling system in the host may have been acquired due to lateral gene transfer from microorganisms. Such recognition of a shared signaling system suggests that there is a common mechanism by which the host may interact with the microbe in a bidirectional fashion influencing aspects of both disease and health. In the case of infectious disease pathogenesis, the consideration of a microbial endocrinology–based mechanism in which infectious bacteria can directly respond to host-derived neurochemicals such as those present during periods of stress has demonstrated, for example, that the prevalent use of catecholamine-based synthetic drugs in the clinical setting contributes to the formation of biofilms in indwelling medical devices leading to increased morbidity and mortality. At the same time, the ability of the microbe to produce neurochemicals that constitute the host’s own neuronal signaling systems means that a common pathway exists for the microbiota to influence host neurophysiology. Currently, we have little understanding of the neurochemical environment of ruminants, whether that within the rumen microbiota or the host tissue. Application of a microbial endocrinology-based approach may lead us to the identification of novel mechanisms by which the ruminant interacts with its microbiota and thereby provide new approaches to health and disease management.

**Key Words:** neurochemicals, bacteria, stress

394 **Microbes, epithelial cells and chemical signals in the digestive tract.** D. R. Brown*, University of Minnesota, Dept. of Veterinary & Biomedical Sciences, St. Paul, MN.

The rumen and intestines in the cattle digestive tract house 2 complex, symbiotic microbial communities, which are established early in a calf’s life and have complementary roles in cattle health and productivity. It is unclear how these vital relationships between microbes and host cells are maintained throughout life, but the ability of prokaryotic and eukaryotic cells to communicate with one another by direct contact or through secreted or shed chemical substances may be a factor. Commensal microflora of the intestinal tract shape gut immunity and enteric nervous system development, and are essential for feed assimilation and energy production. Some bacteria produce neurotransmitter substances, and microbial products such as exotoxins and pathogen-associated molecular patterns are recognized by host cells in the intestinal mucosa. The host in turn may influence establishment and maintenance of ruminal and intestinal microbial communities. Neurotransmitters and other chemical signals secreted into the intestinal lumen may influence bacterial growth and virulence. Epithelial cells in the rumen wall and intestinal mucosa are key points of microbe-host contact regulated by nerves and hormones. In the intestinal mucosa, the sympathetic neurotransmitter and stress hormone norepinephrine increases bacterial internalization into Peyer’s patches, the inductive site for gut immunity. Norepinephrine enhances non-intimate adherence of Escherichia coli to colonic epithelial cells and promotes vectorial transport of secretory immunoglobulin A across the small and large intestinal mucosae, which stabilizes mucosa-associated bacteria. Stress activation of the sympathetic-adrenomedullary system has profound effects on the digestive tract and on the microbial communities housed within it. Knowledge of the bidirectional communication between host cells and prokaryotic bacteria is steadily increasing for the intestinal tract, but is lacking with respect to the rumen. By understanding the cellular and chemical factors that impact ruminal and intestinal microbial populations, we can potentially identify targets and develop strategies for maintaining the well-being and enhancing the productivity of cattle and other ruminants.

**Key Words:** microbe-host interaction, norepinephrine, epithelium
Serotonin (5HT) regulates mammary gland physiology and Ca dynamics during lactation. Our lab’s transition period rodent research revealed that 5HT is involved with Ca transport to and within the mammary epithelial cell (MEC). Additionally, 5HT increased parathyroid hormone-related peptide (PTHRP) production and bone mobilization in these rodents. The objective of the following cell culture experiment was to evaluate the effect of 5HT and Ca on PTHrP production and Ca transport in the bovine MEC, providing the first steps in evaluating the 5HT, Ca, and PTHrP axis in bovine mammary epithelium. Immortalized bovine mammary epithelial cells (MAC-T) were grown to 95% confluency and then plated on a 24 well plastic plate coated with Matrigel (n = 3/treatment). Cells were allowed to grow for 6 d in standard growth media. On d 7, cells were treated with lactogenic media only (control), lactogenic media + 5HT (200 µM), lactogenic media + EGTA (0.6 µM), or lactogenic media + 5HT + EGTA. After 48 h, cells were harvested and extracted for qPCR analysis. Expression of β-casein was measured to confirm the cells were lactogenic. Relative expression of calcium release-activated calcium channel protein 1 (ORAI1) was increased 0.6-fold in 5HT + EGTA treated cells compared with control (P = 0.06) and 0.8-fold compared with EGTA treated cells (P = 0.03). Relative expression of plasma membrane Ca ATPase 2 (PMCA2) tended to increase in cells treated with 5HT + EGTA compared with control (P = 0.06) while secretory pathway Ca ATPase 1 (SPCA1) tended to increase in cells treated with EGTA (P = 0.07). Relative expression of PTHrP tended to increase in cells treated with 5HT and 5HT + EGTA compared with control (P = 0.06). These results are consistent with our rodent data in that they support a role for 5HT in the transport of Ca to and within the MEC as well as into the milk. In addition, an increase in PTHrP potentiates increased bone resorption, thus increasing Ca mobilization. The apparent synergistic effect of 5HT and EGTA on the expression of some genes warrants further investigation. We believe with additional replication we will provide evidence that 5HT is altering Ca dynamics in the dairy cow.

Key Words: serotonin, MAC-T cells, parathyroid hormone related peptide (PTHRP)

Serotonin may be an effective therapy for hypocalcemia. Pre-partum infusions of the serotonin precursor 5-HTP increased circulating calcium in postpartum Holstein dairy cows. However, mammary gland calcium flux in response to 5-HTP infusion has not yet been determined. Elucidation of serotonin’s effects on calcium flux in the transition and dairy cow mammary gland may reveal the molecular mechanisms underpinning hypocalcemia. Multiparous Holstein cows were intravenously infused daily with either 1 L of saline (CON; n = 6) or 1 mg/kg bodyweight of 5-hydroxytryptophan (5-HTP; n = 6) for 5.67 ± 0.78 d pre-partum until parturition. Biopsies were performed before treatment, on the d (d1) and d7 after parturition on opposite rear quarters using a punch tool. RNA was extracted from all samples and real-time PCR was performed. Data were analyzed using the delta-delta CT method and statistical analysis was conducted using a 2-way ANOVA for time, treatment, and the interaction on mRNA abundance of calcium sensing receptor (Casr), plasma-membrane ATPase 2 (Pmc2a), calcium release-activated calcium channel protein 1 (Orai1), tryptophan hydroxylase 1 (Tph1), serotonin reuptake transporter (Sert), and serotonin receptor 7 (5HTR7). mRNA abundance of Casr was elevated in 5-HTP cows on d1 and d7 (P = 0.0002) compared with CON, and mRNA was more abundant on d1 and d7 postpartum compared with pre-partum (P = 0.001), with a time and treatment interaction (P = 0.007). While Pmc2a abundance increased postpartum compared with pre-partum (P = 0.03), there was no effect of treatment. Orai1 was elevated in CON cows over 5-HTP cows on d1 (P = 0.03), and on d1 and d7 compared with baseline (P = 0.0001), with an interaction of treatment and time (P = 0.03). There was increased abundance of Tph1 (P = 0.03), Sert (P = 0.02), and 5htr7 (P = 0.02) transcripts in 5-HTP compared with CON cows on d1 and d7 postpartum. mRNA abundance of Tph1 (P = 0.03), Sert (P = 0.003), and 5htr7 (P = 0.007) was increased on d1 and d7 relative to pre-partum. These findings demonstrate that 5-HTP infusion pre-partum alters serotonin and calcium axes in the cow mammary gland. We also show these axes are activated postpartum, establishing a molecular profile for calcium and serotonin signaling in early-lactation dairy cows.

Key Words: serotonin, calcium, mammary gland

The effects of cabergoline, as dopamine receptor agonist and suppressor of prolactin (PRL), were studied in a total of 30 dairy ewes of 2 breeds (Manchega, MN, n = 15; Lacaune, LC, n = 15). Ewes were in lactation (185 ± 3 DIM), had similar BW (70.6 ± 0.3 kg) and different milk yield (MN vs. LC, 1.02 ± 0.03 vs. 2.27 ± 0.05 kg/d; P < 0.001). They were penned indoors, fed a TMR (forage:concentrate, 55:45%) ad libitum. Milking was done twice-daily in a 2 × 12 parlor with electronic flowmeters (Delaval, Tumba, SE) during all the experiment. Treatments consisted of a single i.m. injection of cabergoline per ewe (Velactis, 1.12 mg/mL of cabergoline; Ceva, Libourne, FR) at 3 doses: A (1 mL), B (0.5 mL) and C (saline). Milk yield was recorded daily (d −14 to 14) and milk (d −2, −1, 1, 2, 5, 7 and 14) and blood (d −1, 1, 2, 5, 7 and 14) samples were collected for milk composition (ALLIC laboratory, Cabrils, ES) or plasma PRL (DIAsource Immunoassays, Luvain-la-Neuve, BE). Udder traits were measured on d −2, −1, 5, 7 and 14. Data were analyzed by the PROC MIXED for repeated measurements of SAS v.9.4. No reactions to cabergoline injection were detected. Milk yield fell rapidly after treatment in both breeds, reaching a nadir at d 4 (P < 0.001). The effect of B dose was greater than A (A vs. B, −0.62 ± 0.20 vs. −0.75 ± 0.25 kg/d; P < 0.05). Despite the size of the effect, milk production recovered from d 5, the differences between treatments were no detectable at d 7 (P > 0.05). Milk fat and protein contents increased (P < 0.001), whereas lactose content decreased (P < 0.01) after treatment, agreeing with the milk yield change, but resume thereafter. Values of PRL did not change in C, whereas dramatically decreased in A and B (P < 0.001). PRL was undetectable (< 0 ng/mL) from d 1 to 4 after cabergoline injections, whereas ranged between 15 and 28 ng/mL in C ewes. No PRL differences between treatments were detected on d 14 (P > 0.05). Udder volume varied by breed (MN vs. LC, 1.65 ± 0.05
Absorption of glucose across the luminal membrane of absorptive enterocytes occurs via the Na+/glucose co-transporter-1 (SGLT1), while GLUT1 is the known major glucose transporter for glucose uptake by mammary acinar cells. AIMS: to compare i) SGLT1 expression and activity in intestinal tissues and ii) glucose transporter expression in the mammary gland of dry vs lactating cows. Three lactating and 3 dry Holstein cows were used. Multi-parous lactating cows were fed a TMR ad libitum and averaged (±SEM) 707 ± 26 kg BW, 21.8 ± 0.8 kg/d DMI, 356 ± 129 DIM, and 27.9 ± 9.9 kg/d milk yield. Dry cows were fed a grass silage and straw ration to meet maintenance requirements and averaged 688 ± 34 kg BW and 8.0 ± 0.4 kg/d DMI. Cows were euthanized and samples of duodenum, jejunum, ileum and mammary tissue, rapidly excised, were analyzed for mRNA, protein expression and glucose uptake using qPCR, immunohistochemistry, Western blotting, and U-[14C]-glucose uptake by brush border membrane vesicles (BBMV). Effects of tissue type and lactation were evaluated using ANOVA and Dunnett’s t-tests. In both lactating and dry cows intestinal SGLT1 mRNA, protein, and activity were highest in the duodenum and lowest in the ileum. In lactating vs dry cows, there was a 1.8- (P = 0.02), 3.8- (P = 0.02), and 2.8-fold (P = 0.01) increase in SGLT1 mRNA expression in the duodenum, jejunum, and ileum, respectively. This was matched by increased SGLT1 protein abundance in BBMV by 3.1- (P = 0.02), 6.8- (P = 0.03), and 2.8-fold (P = 0.02) increase in initial rates of Na+-dependent glucose transport into BBMV isolated from duodenum, jejunum, and ileum. We believe this is the first study showing SGLT1 protein being co-localized with GLUT1 on the basolateral membrane of bovine mammary gland acinar cells. There was a 3.6- (P = 0.05) and 9.3-fold (P = 0.01) increase in GLUT1 and SGLT1 expression in mammary tissue of lactating vs dry cows. Greater basolateral membrane expression of GLUT1 and SGLT1 in mammary secretory cells during lactation enhances glucose uptake and is accompanied by increased expression and activity of intestinal SGLT1.

Key Words: SGLT1, GLUT1, mammary gland


Heat stressed dry cows have lower milk yield (MY) in the next lactation. Cooling systems abate the effects of heat stress (HT) during the dry period (DP) and improve performance after calving. The objective of this study was to evaluate the effect of HT during early or late DP on performance. Cows were randomly assigned to treatment based on mature equivalent MY, dried-off 45 d before parturition, and assigned to cooling (shade, fans and soakers; CL) or heat stress (shade; HT). Treatment groups included: HT (HT, n = 20) or cooling (CL, n = 20) during the entire DP, HT first 3 weeks then CL (CLHT, n = 19) or CL first 3 weeks then HT until calving (HTCL, n = 20). During the DP, data were divided into 2 periods: first 3 weeks of the DP (cows were exposed to either HT or CL); and from 3 weeks until calving (after switch). HT increased rectal temperature (RT; HT vs. CL; 39.1 vs 38.8 ± 0.04°C, P < 0.01) and respiration rate (RR; breaths/min; HT vs. CL; 69 vs. 53 ± 1.8 bpm, P < 0.01) during the first 3 weeks of DP. After the switch, cows that were exposed to HT had increased RT versus CL cows (HT, 39.1; HTCL, 38.9; CL, 38.7; CLHT, 39.1 ± 0.05°C, P < 0.01) and increased RR (HT, 64; HTCL, 53; CL, 47; CLHT, 66 ± 2.1 bpm, P < 0.01). During the first 3 weeks, HT reduced DMI (kg/d) versus CL (HT vs. CL; 10.6 vs. 11.8 ± 0.35 kg/d, P = 0.02). However, DMI did not differ after cows were switched (HT, 10.7; HTCL, 11.1; CL, 11.2; CLHT, 10.1 ± 0.55 kg/d, P = 0.45). Heat stress at any time reduced gestation length relative to cooling (HT, 275; HTCL, 274; CL, 277; CLHT, 273 ± 1.2d, P = 0.04). There were no differences in hemocrit, total protein and body weight (BW) change during the DP among treatments, nor did BW or DMI differ after parturition (P > 0.20). Cooling during early or late DP alone only rescued MY in the first 3 weeks of lactation (HT, 26.6; HTCL, 30.7; CL, 32.9; CLHT, 29.7 ± 1.37 kg/d, P = 0.02), whereas CL for the entire period DP increased milk, fat, protein, and lactose yield among treatments (P < 0.05) up to 140 DIM (HT, 37.8; HTCL, 38.3; CL, 42.9; CLHT, 37.8 ± 1.4 kg/d, P = 0.03). Thus, HT at any time during the DP compromises performance of dry cows after calving.

Key Words: milk yield, cooling, switch


A dry period (DP) is necessary for cows to attain maximal milk yield in the next lactation and heat stress during this phase compromises mammary gland involution and redevelopment. The objective of this study was to evaluate the effects of nutritional and housing strategies to overcome the effects of heat stress on mammary gland involution and redevelopment of cows during the DP. Before dry-off, all cows were kept in the same environment and exposed to cooling systems, i.e., shade, fans and soakers. For 60 d before dry-off, cows were divided into 2 groups: control (fed 56 g/d of AB20; CON) and OmniGen-AF (fed 56 g/d of OmniGen-AF; OG). Cows were dried off 45 d before expected calving and, within nutritional treatment, assigned to cooling (shade, fans and soakers; CL) or heat stress (only shade; HT) pens, which resulted in 4 treatment groups: HT (n = 17), CL (n = 16), HT + OG (HTOG, n = 19) and CL + OG (CLOG, n = 14). Mammary biopsies were collected on d 3, 7, 14, and 25 during the DP from a subset of cows (HT, n = 6; CL, n = 7; HTOG, n = 6 and CLOG, n = 5) for histological evaluation of cell apoptosis and alveolar structures. Mammary tissue was placed in 4% paraformaldehyde overnight at 4°C, dehydrated, paraffin embedded, and sectioned at 5 μm. Mammary alveoli and apoptotic cells were visualized by hematoxylin and eosin staining and TUNEL assay, respectively. Alveoli number and positive apoptotic cells were counted using Image J software. Data were analyzed by mixed models using the MIXED procedure of SAS. There was an interaction of heat stress and dietary treatment (P = 0.08), where the apoptotic rate of CLOG cows was higher versus CL, HT, and HTOG cows (2.2; 1.46; 1.52; 1.47 ± 0.2%, P < 0.05, respectively). Relative to cooling, alveolar number was reduced when cows were exposed to HT (176 vs. 144 ± 12; P = 0.06) and increased
when animals received OG versus CON (179 vs. 141 ± 12; P = 0.02). Thus, OG supplementation with CL increased mammary cell apoptotic rate; OG supplementation increased alveoli number and CL increased alveoli number during the DP. OG might improve the capacity of the mammary gland for milk yield after calving.

Key Words: mammary gland, heat stress, OmniGen-AF

402 Effect of heat stress and methionine or arginine supplementation on mTOR signaling in bovine mammary cells. A. A. K. Salama1*, L. Wang2, M. Duque3, and J. J. Loor4, 1Group of Ruminant Research (G2R), Universitat Autònoma de Barcelona, Bellaterra, Spain, 2Department of Animal Science, Southwest University, Rongchang, Chongqing, China, 3Grupo de Investigación Biogénesis and GRICA. Facultad de Ciencias Agrarias, Universidad de Antioquia, Medellin, Colombia, 4Department of Animal Sciences, University of Illinois, Urbana, IL.

Heat stress (HS) affects mammary cells directly and reduces milk component synthesis. On the other hand, dietary essential amino acid (AA) supplementation enhances milk protein and in some instances also fat content. Little is known about the interaction between HS and AA on mammary cell synthetic capacity. To test mechanisms by which mammary activity is affected by HS and AA, MAC-T cells were incubated at thermo-neutral (TN; 37°C) or heat stress (HS; 42°C) conditions. In both conditions, culture media varying in essential AA concentrations were used. These media were: an optimal AA profile served as the control (Con), and treatments were Con plus methionine (Met), and Con plus arginine (Arg). Consequently, there were 6 treatment combinations: TN-Con, TN-Met, TN-Arg, HS-Con, HS-Met and HS-Arg. After incubation, aliquots (20 mg protein) of cell lysates were used for Western blot analyses of mammalian target of rapamycin (mTOR), eukaryotic translation elongation factor 2 (eEF2), serine-threonine protein kinase (AKT), 4E binding protein 1 (4EBP1), ribosomal protein S6 (RPS6), RPS6 kinase 1 (S6K1), and eukaryotic initiation factor 2α (eIF2α). The HS reduced (P < 0.01 to 0.10) total (T) and phosphorylated (P) mTOR, eEF2, AKT, and 4EBP1. However, P:T ratios (P < 0.05) for mTOR (+25%), AKT (+86%), and eIF2α (+45%) increased, while 4EBP1 (−37%) decreased (P < 0.01) under HS conditions. The lower P:T of 4EBP1 and the greater P:T of eIF2α could inhibit translation initiation and might explain the lower milk protein content observed in cows during HS. There was a significant interaction (P < 0.05) between AA supplementation and ambient temperature, and Met addition increased the P:T of 4EBP1 and decreased eIF2α. In conclusion, HS seems to exert its inhibitory effects on milk protein synthesis by decreasing the phosphorylation of 4EBP1 and increasing eIF2α. Methionine supplementation alleviates these effects and might be a good management strategy to improve milk synthesis under heat stress conditions.

Key Words: mammary cells, protein expression, heat stress


As one of the most limiting AAs, methionine (Met) and its peptide form have been investigated decades for the ability to promote milk protein synthesis in vitro. This study aimed to investigate the effects of methionyl-methionine (Met-Met) on prolificacy and milk performance in mice. 1) To study the first pass effect of Met, 48 pregnant mice were randomly divided into 6 groups with intraperitoneal injection of 0, 5, 15, 25, 35 and 45% Met daily (based on 5g /d dry matter intake), from embryonic d 1 to 17. The control group was fed with Met supplementation diet, the other groups were fed with Met free diets. 2) Then 56 pregnant mice were assigned to 7 groups with intraperitoneal injection of 0, 5, 15, 25, 35 and 45% Met-Met replaced of 35% Met daily. At embryonic d 17, all mice were slaughtered to collect mammary gland for the underlying mechanisms of milk protein synthesis. Data were analyzed by the ANOVA procedure of IBM SPSS statistics 20. The results showed that (1) 35% Met supplementation increased total number of fetuses and placental weight compared with 5, 15% Met treatments. However, placental weights were decreased significantly when mice fed free-Met diet; (2) 25% Met-Met supplementation increased total number of fetuses compared with 45% Met-Met supplementation. Additionally, placental weights were increased when mice supplemented with 25% Met-Met compared with 0, 5 and 45% Met-Met. Furthermore, the mRNA abundance of β-casein, mammalian target of rapamycin (mTOR), Janus kinase 2 (JAK2) and signal transducer and activator of transcription 5 (STAT5) were increased in 25% Met-Met compared with the control and 35% free Met supplementation. In conclusion, Met-Met restored prolificacy and promoted milk protein synthesis by activating mTOR and JAK2-STAT5 signaling pathways.

Key Words: Met-Met, fetal development, milk protein synthesis


The peptide bound amino acids were shown to promote milk protein synthesis more efficiently than free amino acids. However, the underlying mechanism remains unknown. The aim of this study was to investigate the effect of methionyl-methionine (Met-Met) on milk protein synthesis and to elucidate the underlying mechanism of Met-Met regulating milk protein synthesis in primary bovine mammary gland epithelial cells (BMECs). The BMECs were treated with different concentrations of Met-Met (0, 20, 40, 80, 120, 160 µg/mL). The protein expression of mammalian target of rapamycin (mTOR), p70 ribosomal S6 kinase 1 (S6K1), eukaryotic initiation factor 4E binding protein 1 (eIF4E), β-casein (β-CN), peptide transporter 2 (PepT2), peptide histidine transporter (PhT1) and cyclin D1 were further verified by Western blot. Data were analyzed by GLM procedure of SAS software (SAS Institute, USA). Compared with the control group (addition of 0 µg/mL Met-Met), the expression of β-CN were significantly increased by supplementation of 80 µg/mL Met-Met, but were significantly decreased when Met-Met was inhibited by rapamycin. Cyclin D1 and the cell viability were also enhanced by supplementation of 80 µg/mL Met-Met and the inhibition of mTOR suppressed Met-Met-promoted cell proliferation. The expression of PepT2 was significantly enhanced by addition of 80 µg/mL Met-Met. However, the expression of PhT1 was not affected by 80 µg/mL Met-Met. The inhibition of PepT2 by siRNA decreased Met-Met stimulated β-CN expression. The addition of 80 µg/mL Met-Met promoted phosphorylation of mTOR, S6K1 and 4E-BP1. The results suggested Met-Met was absorbed by PepT2 and then Met-Met stimulated β-CN expression. The addition of 80 µg/mL Met-Met, however, was not affected by 80 µg/mL Met-Met. The inhibition of PepT2 by siRNA decreased Met-Met stimulated β-CN expression. The addition of 80 µg/mL Met-Met promoted phosphorylation of mTOR, S6K1 and 4E-BP1. The results suggested Met-Met was absorbed by PepT2 and then Met-Met stimulated β-CN expression. The addition of 80 µg/mL Met-Met, however, was not affected by 80 µg/mL Met-Met.
In dairy goats, the reproductive and productive performances depend on rearing management, notably on strategies aiming at the optimization of the growth, body development, onset of puberty and mammary gland development, while ensuring future milk potential and longevity. In the recent years, early weaning has become more used for numerous reasons, including reduction of the costs and flexibility. A high plane of nutrition just after weaning is an interesting way to offset the low weight of early weaned goats. In any case, the impacts of the diet supplied during early life on the growth, reproduction and the development of the mammary parenchyma have not been determined in goat kids. Hence, the objectives of this study are to investigate the effects of different feeding levels applied until 8 mo of age on the growth, reproduction performances and mammary gland development in early weaned goats. Ninety Alpine goats were weaned at 9.7 ± 1.4 kg (30 d old) and subjected until 8 mo of age to 3 feeding levels: Low (L, 365 g DM/d, n = 30), Moderate (M, 730 g DM/d, n = 30) or High (H, 1090 g DM/d, n = 30) concentrate diet. Goats were weight twice a month and morphometric parameters (heart girth, height at withers and crown-rump length) were performed once a month. At 7 mo of age (before puberty), 5 goats of each group were slaughtered and mammary glands were analyzed. At 4 mo of age, the BW of the L group was 23% lower than in the 2 others groups (P < 0.001). Morphometric parameters were consistent with BW observations (P < 0.001). At slaughter, the mammary glands of the L group were twice lighter than those of M and H group (P < 0.001). Proportion of parenchyma, determined by histological analysis, suggested that the mammary glands are less developed in the L goats group. The CK19 expression, analyzed by Western blot as a marker of luminal cells, was consistent with histological results. Finally, the onset of puberty and reproduction performances were not impacted by feeding levels. To conclude, low feeding level from early weaning to 8 mo old negatively impacted the pre-pubertal growth and mammary gland development.

Key Words: growth, mammary gland, feeding level
Evaluation and comparison of dairy cow dry matter intake prediction models recommended by the Intergovernmental Panel on Climate Change. R. A. Jayasooriya*1,1 and E. Kebreab2,1

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The Intergovernmental Panel on Climate Change Tier 2 (IPCC-Tier 2) guidelines provide 2 models: a comprehensive (IPCC-CMP) and a simplified (IPCC-SMP) model to predict DMI as obtaining actual feed intake measurements of livestock is challenging. The IPCC-CMP includes equations to calculate net energy requirements for body functions, which is then connected to DMI using digestible energy utilization efficiency (REM), and energy digestibility (DE). In the IPCC-SMP, DMI is simply a function of BW and DE. These models are yet to be evaluated systematically for prediction accuracy. The objective of the present study was to evaluate the IPCC-Tier 2 models and compare them to extant models such as Cornell Net Carbohydrate and Protein System (CNCS) model and National Research Council-2001 (NRC) model to predict DMI using an independent data set. Two experiments using lactating Holstein cows provided 209 observations of DMI, milk yield, milk fat content, BW and DIM. The average values were 21 kg/d, 32 kg/d, 3.7%, 670 kg, and 188 d, respectively. The overall agreement between predictions and observed values were determined with the square root of mean square prediction error expressed as a percentage of average observed value (RMSPE). Systematic biases of predictions such as mean bias (MB) and slope bias were also estimated and expressed as a percentage of RMSPE. The CNCS relying on fat corrected milk yield and BW more accurately predicted DMI (RMSPE = 14.1%) than NRC (RMSPE = 19.4%), IPCC-SMP (RMSPE = 16.9%), and IPCC-CMP (RMSPE = 23.4%). The CNCS model had minor systematic bias (<10%), whereas IPCC-CMP had a large mean bias (56.3%) for DMI to be over-predicted. The calculated average net energy requirements (e.g., maintenance = 0.47 MJ/kg of metabolic BW, and lactation = 95.2 MJ/d) were in line with literature values indicating that perhaps representations of REM and DE in the IPCC-Tier 2 methodology need to be revised for dairy cows, at least in North America. Overall, the results demonstrated that DMI of dairy cows can be predicted successfully using information such as milk yield, milk fat content, and BW that are routinely available in dairy farms.

Key Words: dairy cow, dry matter intake, prediction model

County-level gridded methane emissions for the contiguous United States. A. N. Hristov*1, M. Harper1, R. Meinen1, R. Day2, J. Lopes1, T. Ott1, A. Venkatesh3, and C. A. Randles3

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Livestock is considered to be the second largest source of anthropogenic methane emissions in the United States. Down-top approaches for methane source attribution have questioned existing bottom-up estimates of methane emissions, such as the U.S. Environmental Protection Agency’s (USEPA) Inventory of Greenhouse Gas Emissions and Sinks, suggesting that livestock emissions are underestimated. This analysis used a spatially-explicit, bottom-up approach, based on animal inventories, feed dry matter intake, and dry matter intake-based emission factors to estimate county-level enteric (cattle) and manure (cattle, swine, and poultry) methane emissions for the contiguous United States. Counties with the largest combined livestock methane emissions included: Tulare, Merced, Stanislaus, and Kings, CA (217, 123, 80, and 78 Gg methane/year, respectively); Gooding, ID (75 Gg/year); Weld, CO (63 Gg/year); Kern, Fresno, and San Joaquin, CA (62, 59, and 49 Gg/year), Maricopa, AZ (47 Gg/year), and Sampson, NC, Yakima, WA, and Sioux, IA (43 to 44 Gg/year). Overall, the bottom-up approach used in this analysis yielded total livestock methane emissions (8,888 Gg/yr) that are comparable to current USEPA estimates (9,117 Gg/yr) and to estimates from the global gridded Emission Database for Global Atmospheric Research (EDGAR) inventory (8,657 Gg/yr), used previously in several top-down studies. However, the spatial distribution of emissions developed in this study differed significantly from that of EDGAR. As an example, methane emissions from livestock in Texas and California (highest contributors to the national total) in this study were 36% lower and 100% higher, respectively, than estimates by EDGAR. The difference for these 2 states between the current analysis and the latest USEPA gridded inventory was 15 and 4%, respectively. The spatial distribution of emissions in gridded inventories (e.g., EDGAR) likely strongly impacts the conclusions of top-down approaches that use them, especially in the source attribution of resulting (posterior) emissions, and hence conclusions from such studies should be interpreted with caution.

Key Words: methane, inventory, livestock

Cow, herd, and farm level productivity, efficiency, and greenhouse gas emission of different strategies for extended lactation. J. O. Lehmann*, L. Mogensen, and T. Kristensen, Department of Agroecology, Aarhus University-Foulum, Tjele, Denmark.

Managing cows for extended lactations has been proposed to balance the genetic milk yield potential of modern dairy cows with the adverse effect of the negative energy balance on reproduction. However, due to the standard course of the lactation curve, extended lactation may decrease daily milk production per cow besides reducing the number of calves, heifers and dry cows per year. In turn, this reduces herd feed use and alters farm feed production requirement. Thus, the effect of an extended lactation strategy may depend on the system level of interest; i.e., cow, herd and farm or even sector. We modeled the effect of different strategies for extended lactation on herd productivity, efficiency and greenhouse gas emission by simulating herd dynamics, production and feed use with a herd model (www.simherd.com). Extended lactation was defined as calving intervals of either 15 or 17 mo and was applied only during first parity, only during second and higher parities or during all parities. The base scenario resembled an average Danish Holstein dairy herd with 10,500 kg energy-corrected milk (ECM) per cow per year and a calving interval of 13 mo. Extended lactation scenarios reduced milk production by 2 to 486 kg ECM and reduced meat production by 5 to 24% per cow per year. Herd feed intake was reduced by 1 to 7% per cow per year, which led to a 1 to 3% increase in milk production per kg dry matter (DM) at herd level. However, excluding young stock feed intake changed the result to a 0 to 2% loss in kg ECM per kg DM. Including meat production showed that MJ milk and meat produced per kg herd DM intake increased 1 to 2%. Furthermore, extended lactation led to a reduction in feed growing area by 1 to 8%. Thus, MJ milk and meat produced per ha increased 1 to 2% across scenarios. Finally, this reduced the farm level emission from 1.06 to 1.02–1.05 kg CO2-equivalents per kg ECM across scenarios. Utilizing the freed growing area to
produce energy crops reduced emissions by 3 to 12%. However, the benefit would be less at sector level because of emissions from beef cattle raised to compensate for the lower meat production.

**Key Words:** extended lactation, efficiency, greenhouse gas emission

412  Evaluating the effect of herd structure and milk production improvement on farm profitability and enteric methane emission. D. Liang1, J. Tricarico2, K. Weigel1, and V. Cabrera1, 1University of Wisconsin-Madison, Madison, WI, 2Innovation Center for US Dairy, Rosemont, IL.

Cow’s performance and herd management strategies such as productivity and herd structure influence dairy enteric CH4 emission and profitability. This study aims to quantify the effects of culling rate (CUR) milk production improvement (MPI, a proxy for genetic and management progress), and the interaction between them on enteric CH4 emission and farm profitability. Hence, a monthly Markov Chain herd structure model was built and used to simulate the herd for 15 calving intervals (CI, with 13-mo length). Enteric CH4 model was built and used to simulate the herd for 15 calving intervals and farm profitability. Hence, a monthly Markov Chain herd structure model was built and used to simulate the herd for 15 calving intervals (CI, with 13-mo length). Enteric CH4 emission and farm profit (milk income over feed cost minus transaction cost) were calculated as the average for one CI. The model was used to study CUR from 25% to 45% with 5% intervals and annual MPI of 1%, 1.5%, and 2%. Every tested scenario started at a herd’s steady state. Daily milk production in 35% CUR herds (27.36 kg per cow per d) was slightly higher than herds with other CUR levels. Herds with 45% CUR had the lowest daily milk production, followed by 25%, 40%, and 30% CUR herds. Higher CUR herds (30–45%) with 1.5% or 2% MPI started to have higher productivity (milk per cow per d) than 25% CUR with the same MPI during the 4th CI due to faster milk production increase. Overall, the lowest emission occurred when 25% CUR + 2% MPI herds and the highest when 45% CUR + 1% MPI herds. With the same MPI, higher CUR herds always had higher emission per kg of FCM. Farm profit was $5.55/cow per d for 25% CUR and $5.50/cow per d for 45% CUR at the beginning. At the end, herds with 25% CR + 2% MPI had the highest profit ($) and herds with 45% CR + 1% MPI had the lowest profit ($). With the same CUR, higher MPI always led to a higher farm profit. With the same MPI, lower CUR led to a higher farm profit. This study confirmed that MPI was the ultimate approach to reduce enteric CH4 emission and higher CUR leads to higher enteric CH4 emission per unit of milk with the same MPI. Milk production improvement had a greater impact on enteric CH4 emission and profitability than CUR.

**Key Words:** Markov-Chain model, culling rate, income over feed cost

413  The potential role of gut hydrogenotrophic acetogens from herbivores for biofuel production. C. L. Yang*, J. X. Liu, and J. K. Wang, Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China.

Large and diverse homoacetogens exist in the gut of herbivores. The aim of this study was to investigate the hydrogenotrophic features of acetogen enrichments (AE) from the gut of cow, sheep, rabbit and horse, and their potential role in converting syngas (H2 and CO2) into biofuel products. Rumen contents of 6 late lactation cows and 4 Hu sheep, cecal contents of 6 New Zealand White rabbits, and feces of 4 Australian horses were sampled respectively. Potential acetogens were enriched from each sample using modified anaerobic AC11.1 medium with 2-bromothiophene-sulfonic acid. Batch-culture experiments using H2 and CO2 (4:1) as substrates were conducted to determine the hydrogen consumption and short-chain fatty acids (SCFAs) production of AE. Incubations were lasted for 3 d at 37°C, the ratio of gases in the headspace and concentration of SCFAs in the culture were determined by gas chromatography every 12 h of incubation. Data for the moles of hydrogen consumption and SCFAs production were analyzed by one-way ANOVA in R 3.2 with individual animal as experimental unit, species as main effect. Turkey multiple range tests were used to do multiple comparisons among different groups. Significance was declared if P < 0.05. The maximum cell densities (OD600) were observed at 48 h of incubation of all the samples studied, acetate was the major end product and no methane was detected, minor propionate, butyrate and isovalerate were also produced. During 48 h of incubation, AE from cows consumed 4.75 moles of H2 to 1 mol of acetate, significantly lower to rabbits, cows and sheep (5.17, 5.53 and 5.23 moles respectively), and AE from horses produced significantly more butyrate (0.70 mM) compared with others. AE from cows and sheep produced significantly higher propionate (0.84 mM and 0.86 mM) compared with rabbits and horses (0.69 mM and 0.71 mM). Production of isovalerate was significantly higher of AE from the sheep (0.42 mM). SCFAs are important precursors for the synthesis of various biofuel products, such as polyvinyl acetates, ethanol and butanol et al. In conclusion, the gut contents of herbivores maybe promising resources to harvest functional acetogens for chemical and biofuel production.

**Key Words:** hydrogenotrophic acetogen, herbivore, biofuel

414  Effects of precision feeding protein to dairy cattle on emissions from fresh slurry. C. Peterson*, E. Schusterman1, E. DePeters1, Y. Zhao1, Y. Pan1, D. Luchini1, and F. Mitloehner1, 1University of California, Davis, Davis, CA, 2Adisseo, Alpharetta, GA.

The aim was to reduce nitrogenous emissions from cow excreta while maintaining milk production levels by feeding diets designed to balance postruminally available methionine (M) and lysine (L). Twenty lactating Holstein cows were blocked by parity and days in milk and assigned to one of 4 dietary treatments using a randomized complete block design with 5 cows per treatment (n = 5). Diets were corn silage based and supplemented with varying amounts of rumen-protected M (Smartamine; RPM) and L (Ajipro-L 2; RPL). Diets included: A) 15% crude protein (CP) with low rumen protected amino acids (RPAA), B) 15% CP with high RPAA, C) 18% CP with low RPAA, and D) 18% CP with high RPAA. Diets were formulated to have similar metabolizable protein content with a 3:1 ratio of L:M for absorption. Milk samples were analyzed for fat, protein, and urea nitrogen (MUN); blood (BUN) and urine (UUN) for urea nitrogen. Urine and feces were collected from each cow on d 0, 14, and 28. Slurry sample emissions were measured over 72 h using flux chambers. Flux chamber emission measurements included methane (CH4), nitrous oxide (N2O), ammonia (NH3), hydrogen sulfide (H2S), and methanol (MeOH). Data were analyzed using the lmer statistical package in R, with an α of 0.05. Dry matter intake (A:24.3, B:22.8, C:25.0, D:25.8, kg) and milk yield (A:35.6, B:33.5, C:40.4, D:38.4, kg) were lower for the low vs high CP groups. High CP diets increased MUN (A:10.7, B:10.8, C:16.5, D:14.5, kg/dL), but not RPAA, affected both total nitrogen (A:0.66, B:0.63, C:0.75, D:0.75, %) and ammonia nitrogen (A:0.29, B:0.28, C:0.35, D:0.40, %) in the slurry. Gas emissions of NH3 showed a day and time (A:284.4, B:279.6, C:302.4, D:363.5, mg/hr/m2) and N2O a day effect (A:0.99, B:1.18, C:1.03, D:1.17, mg/hr/m2). Treatments were similar for CH4 and MeOH, and H2S decreased with increasing CP (A:0.10, B:0.05, C:0.05, D:0.04). Overall, precision feeding of RPAA had an effect on nitrogenous emissions over time (RPAA). Decreasing dietary CP concentration from
A new enzyme (FumD) to degrade fumonisins in rumen fluid: An in vitro study. S. Schaumberger*, S. Masching*, D. Schatzmayr*, I. Dohnal2, and C. Stoiber2, 1Biomin Holding GmbH, Getzersdorf, Lower Austria, Austria, 2Biomin Research Cente, Tulln, Lower Austria, Austria.

Ruminants are less susceptible to mycotoxins than monogastrics (Kurmanov, 1977). Rumen microbes are responsible for the metabolismization of the toxins. The formed metabolites can be non-toxic, but also equally or even more toxic than the parent mycotoxins. Although often no acute symptoms can be observed, a long-term intake of a diet contaminated with fumonisins (FUM) can lead to reduced feed intake and a loss in milk production. A purified enzyme (fumonisin esterase, FumD) was developed which transforms fumonisin B1 (FB1) into hydrolyzed FB1 (HFB1) and 2 tricarballylic acid chains (TCA) (Hartinger and Moll, 2011). Remaining TCA and HFB1 are not toxic any more. Aim of the in vitro batch trial was to demonstrate that FumD is efficient in bio-transforming FUM into non-toxic metabolites in rumen fluid. Rumen content was taken immediately after slaughter and transported to the lab. The rumen content was incubated under anaerobic conditions together with feed for 24 h in batch reactors (100 mL containers), starting at a pH of 6.5. The 15 reactors were divided into 3 treatment groups: group 1: negative control, group 2: FUM (50 mg/kg feed) and group 3: FUM + FumD (100 Units/kg feed). Samples were taken after 10 min, 1 and 24 h of incubation and were analyzed for FB1 and HFB1 content via HPLC-MS/MS. Statistical analysis was performed using SPSS 19.0. Normal distribution was tested and for comparison of groups One-Way ANOVA or non-parametric Kruskal Wallis test was performed. Within the incubation period of 24 h, it was shown that FB1 was slowly released from FUM culture material into the fermentation broth (FB1 13.4 µmol/L). However, there was no hydrolysis of FB1 to HFB1 observed in group 2 (HFB1 0 µmol/L). In group 3 FB1 was completely converted into the non-toxic hydrolyzed form HFB1 within 10 min (7.3 µmol/L). Moreover, between 1 and 24 h, the enzyme completely hydrolyzed slowly released FB1 into HFB1 (HFB1 9.6 µmol/L). Under field conditions the fumonisin contamination of feed used for farm animals is on average less than 5 ppm, which is 10 times lower than the concentration used in this experiment. Under in vitro conditions, FumD was capable of completely degrading FB1 within rumen environment and remained active between 1 and 24 h.

Key Words: fumonisin, degradation, enzyme

There is growing interest in how US agricultural land use would change if US diets changed. These questions can be driven by interest in reducing environmental impacts of food production or interest in the carrying capacity of land. In particular, the role of dairy as a driver for land use decisions is of interest. This study seeks to understand how diet demands for dairy products may affect US land use. FASOM (Forest & Agricultural Sector Optimization Model) was used to assess agricultural land use changes under 3 diet scenarios. In this model, the endogenous variables include commodity prices as well as production, consumption, export, and import of those primary and secondary commodities. The dependent variable is the allocation of land, and the objective function maximizes the net present value for producers and consumers in the US. The 3 diet scenarios included a 1 cup / person / day increase in dairy. One diet scenario had only this change; the others had decreases, either across the diet or just in sugars and sweetened beverages. In addition, a sensitivity analysis of dairy cow yields and population distribution was conducted. In the base case of FASOM, cereals, corn, hay, and soybeans account for the majority of cropland (35, 25, 8.2, and 21%, respectively). Silage production contributes 3% of land use, and fruits and vegetables are about 1.6% of total cropland. Apportioning crop land uses to dairy, we find that dairy land use accounts for slightly less than 9% of the total. With the diet scenarios, there are modest increases in silage and hay production (up to 14% and 5%, respectively, of existing levels) to accommodate increased dairy demand across all scenarios. These increases are driven by the fact that silage and hay provide relatively high yields at an affordable price (as opposed to pasture – lower yield and low price – or corn – higher yield but higher feed price). Across all scenarios, the relative change in the land use and price of major crops, such as corn and wheat is restricted (less than 1% for the dairy increase alone, less than 5% for other scenarios). The model also indicates that absolute changes to pasture and rangeland use in the United States would be less than 1% across all scenarios.

Key Words: land use, diet, economic model
**Ruminant Nutrition IV**

417 Ruminant planktonic, weakly, and tightly feed-adhered bacterial community as affected by two *Trichoderma reesei* enzyme preparations fed to lactating cattle. J. J. Romero*1,2, D. C. Reyes1, Z. X. Ma2, and A. T. Adesogan2, 1Animal and Veterinary Sciences, School of Food and Agriculture, University of Maine, Orono, ME, 2Department of Animal Sciences, Institute of Food and Agriculture, Gainesville, FL.

The objective was to compare effects of 2 *Trichoderma reesei* enzyme preparations (EFE) on the ruminal planktonic (LIOQ) or the weakly (ASO) or tightly (SOL) feed-adhered ruminal bacterial community (fractions; FRC) of lactating dairy cows. The xylanase activities of the moderate (MIX) and high-xylanase (XYL) EFE tested were 11 and 27 mmol of sugar released/min per g, respectively and both improved milk production from a dairy cow diet in a previous study. Three ruminally cannulated dairy cows (159 ± 47 DIM) were assigned to Control (CON), MIX or XYL treatments in an experiment with a 3 × 3 Latin square design with 23-d periods. The MIX and XYL EFE were sprayed on the TMR just before feeding at rates of 3.4 and 1 mL/kg of DM, respectively. Bacterial diversity of rumen samples was determined using the 16S rRNA gene (VI-V3 region) and the Illumina MiSeq platform. The data were analyzed with a model that included fixed effects of EFE, FRC, their interaction and random effects of cow and period. The XYL increased (P < 0.05) relative abundance (RA, %) of *Paraprevotellaceae* (5.06 vs. 3.69 ± 0.81), *Sporochaetaeae* (4.25 vs. 2.46 ± 0.48) and tended (P = 0.11) to increase those of *Prevotellaceae* (3.18 vs. 24.1 ± 2.82) versus the CON. The MIX decreased (P < 0.05) RA of *Ruminococcaceae* (4.6 vs. 9.1 ± 2.23) and unidentified *Clostridiales* (4.48 vs. 6.61 ± 1.43), and tended to increase (P = 0.06) that of *Succinivibrionaceae* (1.91 vs. 0.55 ± 3.43) versus the CON. The SOL had higher RA (P < 0.05) of *Lachnospiraceae* (13.1 vs. 5.9 ± 1.86), *Veillonellaceae* (8.5 vs. 3.2 ± 1.69), unidentified *Clostridiales* (6.96 vs. 3.53 ± 1.43), and *Sporochaetaeae* (3.4 vs. 1.50 ± 0.48) and lower RA for unidentified *Bacteroidales* (3.5 vs. 10.0 ± 1.49) versus LIQ. The XYL and MIX EFE only tended to reduce the RA of *Veillonellaceae* versus that of CON (6.3 and 5.3 vs. 13.9 ± 2.54) in SOL (FRC × EFE; P = 0.07). The MIX EFE had lower phylogenetic diversity versus XYL and CON (48.6 vs. 51.6 and 51.2 ± 1.47; P = 0.03). The weighted UniFrac distance showed only community structure differences between FRC (P < 0.01). Both XYL and MIX modified the bacterial community profile and diversity of the rumen fractions.

**Key Words:** antibiotic, microbiota transplantation, microbiota reconstruction

418 Antibiotically disturbed rumen microbiota can be redressed by microbiota transplantation in dairy cows. S. Ji*1, H. Yan1, Tao Jiang1,2, C. Y. Guo3,4, J. J. Liu1, Z. J. Cao1, Y. J. Wang1, and S. L. Li1, 1State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2College of Animal Science, University of Manitoba, Winnipeg, MB, Canada, 3Department of Animal Sciences, The Ohio State University, Columbus, OH, 4Department of Animal Science, Iowa State University, Ames, IA. 4CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China, 9Human Co-Innovation Center of Safety Animal Production, CICASP, Changsha, Hunan, China.

Antibiotically disturbed gastrointestinal microbiota may cause a series of problems. To assess the efficiency of microbiota transplantation on redressing the disturbed rumen microbiota by antibiotics, 10 cows were treated by injecting antibiotics (mixture of penicillin and streptomycin) at recommended dose for 14 d, and then randomly assigned to 2 groups. One was fed regularly as controls (CON), and another group was drenched with 10L rumen liquid collected from healthy donor fistula cows as microbiota transplantation treatment (MT) for subsequent 3 d. Rumen liquid were collected pre-antibiotics usage, and on d 3, d 14 after antibiotics injection, then on d 4, d 11, and d 18 after stopping antibiotic usages. The dynamic changes of rumen microbiota were monitored with the next generation sequencing technique. We observed that rumen microbiota significantly changed (ANOSIM P < 0.05) after 3 or 14 d of antibiotics usage, with the α diversity (richness from 1193 to 1173 and 1189; diversity from 5.79 to 5.64 and 5.68; evenness from 0.82 to 0.80 and 0.80) and stability (based on bray-currit dissimilarity from 0.67 to 0.60 and 0.65) reduced (P < 0.05). After stopped antibiotics usage, microbiota in rumen changed rapidly in both groups (ANOSIM P < 0.05); however, the difference of microbiota in CON and MT group can be identified (ANOSIM P < 0.05), the microbiota α diversity (richness from 1189 to 1585; diversity from 5.68 to 5.92; evenness from 0.80 to 0.82) and stability (from 0.65 to 0.74) rebound immediately 1 d after transplantation in MT group, but in CON group, α diversity was not increased during the post-antibiotics period, and the stability not increased until d 18 (from 0.65 to 0.78) after stopping antibiotics usage (P < 0.05). We conclude that the disturbance of rumen microbiota by antibiotics hardly recovered naturally even stopped antibiotics usage for 18 d; however, antibiotically disturbed microbiota can be redressed efficiently by microbiota transplantation.

**Key Words:** antibiotic, microbiota transplantation, microbiota reconstruction

419 Heat stress influences the rumen microbiome of mid-lactation dairy cows. D. P. Bu1,6, S. C. Li1,2, L. Wang3, L. Ma1,5, L. H. Baumgard4, and Z. T. Yu1, 1State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada, 3Department of Animal Sciences, The Ohio State University, Columbus, OH, 4Department of Animal Science, Iowa State University, Ames, IA. 4CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China, 9Human Co-Innovation Center of Safety Animal Production, CICASP, Changsha, Hunan, China.

Heat stress (HS) affects rumen fermentation of lactating dairy cows. This study examined the rumen microbiome in response to HS. Four multiparous Holstein dairy cows (101 ± 10 DIM; 38 ± 2 kg of milk/d) were each randomly assigned to 4 environment chambers in a crossover design. Cows were either subjected to HS [HS: 36°C with light and 32°C without light; THI = 87.2 and 81.8, respectively] or kept under thermal neutral conditions [TN: 20°C; THI = 65.5] for 9d of adaptation and then for another 9d of pair-feeding to eliminate confounding effects of dissimilar feed intake. A 30d washout period was allowed between the 2 periods. Rumen fluid was collected twice at 0400 (before feeding) and 1000 (after feeding) on d9 of each period using an oral probe, and DNA was extracted. The V4-V5 hypervariable region of the 16S rRNA gene of bacteria, ITS1 sequences of fungi, and 18S rRNA gene of protozoa were amplified and sequenced using MiSeq. After sequence processing and analysis using QIIME, α and β diversity measurements were estimated. Bacterial community composition (at phylum and genus levels) and predictive metagenome profiles were compared between HS vs. PFTN using the nbimomWaldTest method of DESeq. HS increased total VFA concentration (by 45.3%, P < 0.05) before feeding. Alpha
diversity of bacterial and protozoal communities was not affected by HS. The richness of fungal community was significantly lower in PFTN ($P < 0.05$) than in HS. PCoA analysis based on weighted UniFrac distance revealed difference in bacterial and protozoal communities ($P < 0.05$), while NMDS analysis using Bray-Curtis dissimilarity showed difference in fungal community between HS and PFTN. The phyla Chloroflexi and Actinobacteria were less predominant in HS than in PFTN ($P < 0.05$). Shuttleworthia, Anaeroplasma, and Ruminococcus were the most predominant genera among 99 affected genera in HS cows ($P < 0.05$).

For protozoa, HS increased the relative abundance of Entodinium but decreased that of the Isotricha and Ophryoscolex. For fungi, both the phyla Ascomycota and Glomeromycota were significantly higher in HS cows than in PFTN cows ($P < 0.05$). Collectively, HS can directly affect ruminal microbiome independent of its effect on DMI, and different ruminal microbes respond to HS differently.

**Key Words:** dairy cow, heat stress, rumen microbiome

420  **Identifying the influence of the rumen microbiome on the feed efficiency phenotype in beef cattle.** H. A. Paz*1, K. E. Hales2, J. E. Wells3, L. A. Kuenz4, H. C. Freety2, M. L. Spangler1, and S. C. Fernando1

The objective of the study was to identify the predominant rumen bacterial groups that explained the variation in average daily dry matter intake (ADFI), average daily gain (ADG), and gain-to-feed efficiency (FE) in beef cattle using linear regression models. Data on individual intake and body weight were collected from a cohort of heifers (n = 125) fed a growing diet during 2009 and a cohort of steers (n = 122) fed a finishing diet during 2014. A rumen sample was obtained from each animal via esophageal tubing and bacterial community composition was determined through 16S rRNA gene sequencing of the V4 region using the Illumina MiSeq platform. Statistical analyses were done within cohort due to the variation in sex and diet. To account for the inherent breed differences in ADFI and ADG, breed fractions were fitted as covariates in a linear model for both ADFI and ADG and residuals were regressed. Based on the regression of the residuals for ADG on residuals for ADFI, 4 animals were selected from each quadrant (n = 16) to determine differential features of the microbiome (operational taxonomic units (OTUs)) that explained variation in feed efficiency traits. Linear discriminant analysis effect size was used to identify differential OTUs. The remaining samples from each cohort were used to perform forward stepwise regressions to identify subsets of predictive OTUs for the feed efficiency traits. To evaluate model accuracy, heifer data was used to assess the steer models and in turn the heifer models were assessed using the steer data. Across quadrants, $a$ ($P \geq 0.40$) and $\beta$ ($P \geq 0.10$) diversities were similar for both heifer and steer cohorts. OTUs belonging to the families Bifidobacteriaceae, BS11, Erysipelotrichaceae, Fibrobacteraceae, Lachnospiraceae, Prevotelaceae, Ruminococcaceae, S24–7, Spirochaetaceae, Veillonellaceae, and Verrucomicrobiaceae explained the variation in ADFI, ADG, and FE in the heifer and steer cohorts. This study shows that the rumen microbiome is an important factor that influences feed efficiency in beef cattle and can contribute as much as 20% of the variation.

**Key Words:** beef cattle, rumen microbiome, feed efficiency

421  **Metatranscriptome sequencing reveals insights into the gene expression of the bovine epimural bacterial community during subacute ruminal acidosis.** S. Schmitz-Esser1,2, S. Wetzel3, Q. Zebeli3, M. Wagner3, and E. Mann3, 1Iowa State University, Department of Animal Science, Ames, IA, 2University of Veterinary Medicine Vienna, Institute for Milk Hygiene, Vienna, Austria, 3University of Veterinary Medicine Vienna, Institute of Animal Nutrition and Functional Plant Compounds, Vienna, Austria.

The exploration of the bovine epimural bacterial microbiota (BEBM) in the rumen is relevant for basic and applied research for explaining the effect of subacute ruminal acidosis (SARA) on BEBM community structure and host-microbe interactions. Comparatively little is known about the metabolic function of the BEBM and whether or how it might be affected by a diet-induced SARA challenge. This study aimed to investigate differences in the gene expression of the BEBM during a diet-induced SARA challenge. Three ruminally cannulated Holstein dairy cows were fed forage (50% grass silage and 50% meadow hay) during a baseline period followed by a 5-week SARA challenge induced by a 60% concentrate diet (barley grain (33.0%), wheat (30.0%), corn (15.0%), and rapeseed meal (17.0%)). Cows were adapted to the high-concentrate diet within the first 6 challenge days. Rumen papillae biopsies were taken at the baseline and after the SARA challenge. The metatranscriptome of the BEBM at the baseline and after the SARA challenge was obtained using Illumina HiSeq sequencing. In total, 291 million reads were obtained, of which 35 million remained after quality control and 2.1 million reads were bacterial reads and were assigned to 25 phyla, with Proteobacteria, Firmicutes and Bacteroidetes being the most abundant phyla. Functional assignments revealed carbohydrate and amino acid metabolism as well as translation to be the most transcribed KEGG/KO pathways. We found that urease was among the highest transcribed genes confirming previous reports of ureolytic activity of rumen wall bacteria. Also genes involved in oxygen scavenging such as superoxide dismutase were among the highest expressed genes. The high abundance of transcripts involved in oxygen scavenging provides evidence that the rumen wall is not a strictly anaerobic ecosystem. PCoA revealed clustering of BEBM community structure, which was not reflected in the functional data. A diet-induced SARA challenge largely affects the composition of the BEBM without strong effects on the functional metatranscriptome, indicating that shifts in bacterial community composition are compensated on a functional level.

**Key Words:** metatranscriptome, bovine bacterial epimural microbiota, SARA

422  **Potential role of rumen bacterial communities in shaping milk production and composition of dairy cows.** M. Y. Xue1, H. Z. Sun1, X. H. Wu1, D. M. Wang1, L. L. Guan2, J. K. Wang3, and J. X. Liu1, 1Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, China, 2Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Canada.

This study was conducted to investigate the correlation between milk production and rumen bacteria of dairy cows. A total of 334 Holstein lactating cows fed a same diet with a ratio of forage-to-concentrate at 40:60 were selected to record milk composition and milk yield. Rumen contents of all the cows were collected by an oral stomach tube for analysis of volatile fatty acids (VFA) and 16S rRNA gene sequencing of V3 and V4 regions using Illumina HiSeq. Spearman correlation was analyzed between VFA or milk performance and bacterial communities. Only bacterial genera that averaged >1% abundance in at least 60% of samples were included in the correlation analyses. An unclassified genus from family Succinivibrionaceae was positively correlated with propionate ($R = 0.56, P < 0.01$). An unclassified genus from family Rikenellaceae was negatively correlated with propionate ($R = -0.54, P < 0.01$) and valerate ($R = -0.50, P < 0.01$). Negative correlations also
existed between a genus from *Christensenellaceae* and propionate (R = 0.57, P < 0.01) or valerate (R = 0.53, P < 0.01). Weak correlations (0.3 < |R| < 0.5, P < 0.01) included the positive correlations between *Prevotella*, *Lachnospira* and several VFA, and negative correlations between VFA and bacterial genera like *Ruminococcaceae* NK4A214 group, *Ruminococcus*, *Lachnospiraceae* NK3A20 group, *Acetobacterium*, or *Fibrobacter*. Moderate correlations existed between milk yield, milk composition and bacterial communities. Milk yield was negatively correlated with bacteria from family *Rikenellaceae* (R = 0.22, P < 0.01) and *Ruminococcaceae* (R = 0.32, P < 0.01). Milk fat content was negatively correlated with genus from family *Succinivibrionaceae* (R = 0.24, P < 0.01) and genus *Lachnospira* (R = 0.22, P < 0.01), but positively correlated with *Ruminococcaceae* NK4A214 (R = 0.25, P < 0.01), *Rikenellaceae* RC9 (R = 0.22, P < 0.01), and *Christensenellaceae* R7 group (R = 0.27, P < 0.01). Milk protein content was only positively correlated with *Prevotellaceae*. In summary, our study suggests a connection between physiological parameters of dairy cows and several genera of their rumen communities, which may play a potential role in shaping dairy cows’ milk production and physiological parameters.

**Key Words:** rumen bacterial communities; milk performance; volatile fatty acids


The objectives were to examine effects of adding *E. coli* O157:H7 with or without chemical or microbial additives on the bacterial diversity of alfalfa silage, and to examine associations between the abundance of bacterial taxa and silage fermentation quality indices. Alfalfa forage was harvested at 54% DM, chopped to 19-mm lengths, and ensiled in quadruplicate in lab silos after treatment with the following: 1. distilled water (Control); 2. 1 × 10^6 CFU/g of *E. coli O157:H7* (EC); 3. EC and 1 × 10^6 CFU/g of *L. plantarum* (EC+LP); 4. EC and 1 × 10^6 CFU/g of *L. buchneri* (EC+LB); and 5. EC and 2.2 g/kg of propionic acid (EC+PA). After 100 days of ensiling, silage samples were chemically characterized and analyzed for bacterial composition by sequencing the V3 - V4 region of the 16S rRNA gene using the Illumina MiSeq platform. The experiment had a completely randomized design. Data were analyzed using the GLIMMIX procedure of SAS. Significant differences were declared at P < 0.05. Pearson correlation coefficients were generated between taxa and chemical components using R software version 3.2.2. Significant correlations were declared at P ≤ 0.10. Relative to the Control, adding EC+LP or EC+LB reduced (P < 0.05) the Shannon index, a measure of species diversity, but adding EC alone did not (P > 0.05). Treatment with EC+LP increased (P < 0.05) the abundance of *Lactobacillus*, *Sphingomonas*, and *Pantoea* while that of *Weissella* and *Methylobacterium* was reduced (P < 0.05) in the EC+LB silage compared with the Control. Lactate concentration correlated positively (P = 0.04) with the abundance of *Lactobacillus*. Negative correlations were detected between NH3-N concentration and abundance of *Sphingomonas* and *Pantoea*. Silage pH was negatively correlated (P < 0.10) with abundance of *Lactobacillus* and *Pantoea*. Abundance of some unidentified species belonging to genus *Rhodococcus* and *Pseudomonas* correlated positively (P < 0.05) with lactate or acetate concentrations, whereas those of unidentified species belonging to *Salana*, *Pantoea*, and *Rhodococcus* correlated negatively (P < 0.05) with NH3-N concentrations. Future studies should aim to speculate and determine the functions of the unidentified bacteria detected in this study.

**Key Words:** additive, alfalfa, bacteria

### 424 Urea nitrogen induces changes in rumen microbial and host metabolic profiles in dairy cows. D. Jin1,4, S. G. Zhao1,3, N. Zheng1,2, Y. Beckers4, and J. Q. Wang1,2, 1Ministry of Agriculture-Key Laboratory of Quality & Safety Control for Milk and Dairy Products, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2Ministry of Agriculture-Laboratory of Quality and Safety Risk Assessment for Dairy Products, Beijing, China, 3State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 4University of Liège, Gembloux Agro-Bio Tech, Precision Livestock and Nutrition Unit, Passage des Déportés, Gembloux, Belgium.

Urea has been used in diets of dairy cow as a non-protein nitrogen source. It is rapidly hydrolyzed to ammonia which can be used for microbial protein synthesis, but excess ammonia absorbed into blood may be harmful to the animals. However, the changes that occur in the rumen microbial and host blood metabolites after urea nitrogen uptake have not been fully characterized. The objective of this study was to identify changes in rumen microbial and plasma metabolite profiles in dairy cows induced by urea nitrogen using a metabolomics approach. Six dairy cows (550 ± 50 kg BW and 100 ± 21 d in milk) with rumen fistulas were randomly assigned to 2 groups used in a 2 period crossover trial and each experimental period lasted 21 d. All the cows were fed the same total mixed rations, but were intraruminally supplemented with 180 g urea per cow daily or not during the experimental period. Rumen fluid and blood samples were collected and analyzed using nuclear magnetic resonance spectroscopy and multivariate ANOVA. Differences in rumen and plasma metabolite concentrations in cows from the 2 groups were assessed using orthogonal partial least-squares discriminant analysis and identified by searching against related databases. Levels of valine, aspartate, glutamate, and uracil in the rumen, and urea and pyroglutamate in the plasma, were higher (1.36- to 3.17-fold, P < 0.05) in the urea-supplemented group than in the control group. Metabolic pathway analysis of the affected metabolites revealed that pantothenate and CoA biosynthesis, β-alanine metabolism, valine, leucine, and isoleucine metabolism in the rumen, and urea and glutathione metabolism in the plasma were significantly influenced by urea nitrogen. The levels of aspartate and glutamate in the rumen all correlated strongly (r = 0.73 and r = 0.74, respectively, P < 0.01) with the level of urea in plasma. These findings provided novel information to aid understanding of the metabolic pathways affected by urea nitrogen in dairy cows, and could potentially help to guide efforts directed at improving the efficiency of urea utilization in the rumen.

**Key Words:** urea, NMR spectroscopy, metabolites

### 425 Circulating ceramide concentrations are influenced by saturated fatty acid chain length in mid-lactation dairy cows. J. E. Rico*,1, D. E. Rico2, Z. C. Phipps1, Q. Zeng3, B. A. Corl1, P. Y. Chouinard2, R. Gervais1, and J. W. McFadden1, 1West Virginia University, Morgantown, WV, 2Université Laval, Québec, QC, Canada, 3Virginia Tech, Blacksburg, VA.

Ceramide mediates the development of insulin resistance, and the hepatic synthesis of ceramide is promoted by saturated fatty acids (FA). Feeding palmitic acid to lactating cows increases plasma ceramides, relative to a non-added fat control. Our objective was to evaluate the relationship between saturated FA chain length and circulating ceramide concentrations. Eleven cannulated Holstein cows (150 ± 52 DIM) were administered continuous abomasal infusions (280 g/d) of palmitic acid (PA; 85% C16:0), stearic acid (SA; 98% C18:0), or medium-chain triglycerides (MCT; C8:0/C10:0) for 7 d in a replicated Latin square design. Blood was collected on d 5–7 and liver biopsied on d 7 of each trial and each experimental period lasted 21 d. All the cows were fed the same total mixed rations, but were intraruminally supplemented with 180 g urea per cow daily or not during the experimental period. Rumen fluid and blood samples were collected and analyzed using nuclear magnetic resonance spectroscopy and multivariate ANOVA. Differences in rumen and plasma metabolite concentrations in cows from the 2 groups were assessed using orthogonal partial least-squares discriminant analysis and identified by searching against related databases. Levels of valine, aspartate, glutamate, and uracil in the rumen, and urea and pyroglutamate in the plasma, were higher (1.36- to 3.17-fold, P < 0.05) in the urea-supplemented group than in the control group. Metabolic pathway analysis of the affected metabolites revealed that pantothenate and CoA biosynthesis, β-alanine metabolism, valine, leucine, and isoleucine metabolism in the rumen, and urea and glutathione metabolism in the plasma were significantly influenced by urea nitrogen. The levels of aspartate and glutamate in the rumen all correlated strongly (r = 0.73 and r = 0.74, respectively, P < 0.01) with the level of urea in plasma. These findings provided novel information to aid understanding of the metabolic pathways affected by urea nitrogen in dairy cows, and could potentially help to guide efforts directed at improving the efficiency of urea utilization in the rumen.

**Key Words:** urea, NMR spectroscopy, metabolites
period. Plasma ceramide, monohexosylceramide (GlcCer), and lactosylceramide (LacCer) levels were determined using mass spectrometry. Plasma free FA levels and hepatic gene expression were evaluated. Data were analyzed under a mixed model. Orthogonal contrasts compared PA vs. SA and PA vs. MCT, and correlation analysis performed. As established, PA infusion increased milk fat yield and fat-corrected milk (FCM) feed efficiency relative to SA (6.5 and 9.3%, respectively; P < 0.05). Relative to SA, PA increased plasma free FA by 21% (P < 0.05). Infusing PA increased plasma total ceramide and GlcCer levels relative to SA and MCT (~21%, P < 0.05). For example, PA increased C24:0-ceramide by 28 and 36% relative to SA and MCT, respectively (r = 0.3–0.65; P < 0.05). Although not influenced by infusate, hepatic carnitine acyltransferase I and apolipoprotein-B100 mRNA expression were inversely associated with plasma ceramide and GlcCer levels (r = 0.32–0.72; P < 0.05). We conclude that saturated FA chain length can influence ceramide levels in relation to hepatic gene transcription. The preferential ability of palmitic acid to induce ceramide synthesis is likely due to the selective requirement of serine palmitoyltransferase for palmitoyl-CoA.

Key Words: ceramide, lactation, saturated fatty acid

426 Characterization of bovine lipoprotein ceramide. Z. C. Phipps*, F. Seck, A. N. Davis, J. E. Rico, and J. W. McFadden, West Virginia University, Morgantown, WV.

Lipoprotein ceramide can antagonize insulin signaling. We have previously demonstrated increased circulating ceramide in dairy cows transitioning from gestation to lactation; however, the origin of ceramide required validation. Therefore, our objective was to characterize the ceramide composition of bovine lipoproteins. Basal blood samples were collected from 4 non-pregnant, nonlactating Holstein dairy cows ad libitum fed a diet containing corn silage and grass hay. To fractionate triacylglycerol (TAG)-rich, low density, and large and small high density lipoproteins (VLDL, LDL, and buoyant and dense HDL, respectively) from serum, we employed fast protein liquid chromatography using a size exclusion column (10 × 300 mm; 5 to 650 kDa). Thirty 0.5 mL fractions were continuously collected and analyzed for TAG, phospholipid, total cholesterol, and protein levels using colorimetry. In turn, fractions corresponding to VLDL, LDL, and buoyant and dense HDL were pooled (1.5 mL). Following extraction, ceramide levels within whole serum were quantified using mass spectrometry. Data were analyzed using a mixed model with repeated measures. Results (presented relative to all other pooled fractions) demonstrate that VLDL primarily contained TAG (2.2 mg/dL; 50.9% of total components; P < 0.01). Low density lipoproteins exhibited the greatest concentrations of cholesterol and phospholipid (15.6 and 15.5 mg/dL, respectively; P < 0.01). Buoyant HDL contained elevated levels of cholesterol, phospholipid, and protein (6.8, 6.7, and 8.2 mg/dL, respectively; P < 0.01). In contrast, dense HDL primarily contained protein (5.4 mg/dL; P < 0.01). Our results confirm that LDL are enriched with ceramide (P < 0.01); although, ceramide was compartmentalized to a lesser extent within both HDL subclasses and VLDL. Comparable to whole serum, C16:0-ceramide was the predominant ceramide quantified. Interestingly, the proportion of C24:0-ceramide to total ceramide was elevated in VLDL (P < 0.01). We conclude that bovine LDL are enriched with ceramide, and lipoprotein ceramide profiles mimic levels quantified in whole serum. Future research must determine the biological importance of lipoprotein ceramides.

Key Words: ceramide, dairy cow, lipoprotein

427 Micronutrient supplementation and the peripartal plasma lipidome. Y. Zang*, S. S. Samii1, H. R. Bailey4, W. A. Myers1, A. N. Davis1, E. Grilli2, and J. W. McFadden1, 1West Virginia University, Morgantown, WV, 2University of Bologna, Bologna, Italy.

The development of metabolic disease in dairy cows is associated with increased hepatic lipid deposition caused in part by decreased export of triacylglycerol (TG) as very low-density lipoprotein (VLDL). A component of VLDL includes phosphatidylcholine (PC), synthesized from micronutrients including methionine (Met), choline, and betaine. Therefore, our objective was to evaluate the effects of peripartal micronutrient feeding on the lipidome. Thirty multiparous Holstein cows were provided diets with or without rumen-protected micronutrients (22 g/d Met, 10 g/d choline chloride, and 3 g/d betaine; Mecovit, Vetagro S.p.A.) from −28 d prepartum to d 14 postpartum. Blood was collected routinely, and liver tissue was biopsied at d −28, 5, and 14, relative to parturition. In addition to routine analyses, plasma amino acids were quantified using targeted mass spectrometry. Plasma lipidomics was performed using liquid chromatography and time-of-flight mass spectrometry. Following transformation, data were analyzed using a mixed model with repeated measures. Characteristic changes in metabolic status were detected in cows transitioning from gestation to lactation including increased plasma fatty acids, β-hydroxybutyrate, and liver lipid content, and decreased plasma insulin, glucose, and total TG and cholesterol esters (CE; P < 0.05). Micronutrient feeding decreased circulating fatty acids, selectively increased serum methionine levels 17 to 35%, and lowered serum Lys:Met ratio 13 to 28% (P < 0.05). Although plasma PC levels declined during the peripartum, treatment did not modify PC concentrations. In contrast, micronutrient feeding increased CE 22.0, 32.0, and 34.0 (P < 0.05). Moreover, cows fed micronutrients displayed increased plasma levels of TG 46:0, 48:0, 52:0, 54:0, 56:0, and 58:0 (P < 0.05). Most increases in CE and TG levels in response to micronutrients were observed from −28 to −7 d prepartum (e.g., TG 46:0 increased 132%; P < 0.05). We conclude that the described micronutrient feeding regimen can increase specific CE and TG found within circulation; however, the importance of these unique lipids for VLDL export needs to be determined.

Key Words: hepatic health, lipiddomics, peripartal cow


There is a permanent interest to identify and to understand the lactogenic activity of some plant extracts. In the case of barley, pectins and β-glucans (glucose polysaccharides linked with both β-1,3 and β-1,4 backbone bonds) seems to increase the synthesis of prolactin because of their homology with its receptors. Administration of β-glucans i.v. proved to have lactogenic responses in ewes, although the effect of an oral administration and its degradation in the rumen have not been studied yet. Therefore, a short-term investigation was carried out to assess the potential lactogenic effects of a commercial source of barley β-glucans (Glucagel, Zeus Ibérica, Barcelona, ES) supplemented to dairy ewes. Five Lacaune lactating ewes (66.7 ± 2.6 kg BW, 202 ± 22 DIM and 1.58 ± 0.12 kg/d milk yield) fed with alfalfa hay and concentrate,
were allocated in metabolic cages and submitted consecutively to 2 dietary treatments differing in their β-glucan content (C, control low in β-glucans; BG, β-glucans supplemented at a rate of 1.62 g/kg metabolic BW) during 10 d (C, d1 to 5; BG, d6 to 10). 1H Nuclear Magnetic Resonance Spectroscopy (Bruker Avance-III; 600.13 MHz and 298°C) and Multivariate data analyses, including PCA (principal component analysis) and PLS-DA (partial least square–discriminant analysis) were used to generate an integrated vision of changes of metabolic profile in blood plasma, milk and urine samples, obtained at the end of each experimental period. Blood content of β-glucans was analyzed by chromogenic kinetics (Fungitell, Associates of Cape Cod, East Falmouth, MA) as (1,3) β-D-glucan in the laboratory Fontlab2000 (Santa Eulalia de Ronçana, Barcelona, ES). Although β-glucans content in blood did not change (618 ± 43 pg/mL, on average; P = 0.426), metabolomics showed that ewes fed β-glucans for 5 d had higher β-glucose in plasma (P = 0.019), lactose in milk (P = 0.035) and a higher excretion of sucrose in urine (P = 0.004). Further research and examination in long-term studies are needed to establish the lactogenic properties of β-glucans when administrated orally to ruminants.

Key Words: lactating ewe, β-glucan, metabolomics
Production performance of high-producing Holstein cows consuming diets containing hulled or hull-less barley as the grain source in diets containing different forage to concentrate ratios.

Y. Yang1, G. Ferreira1, C. L. Teets1, B. A. Coral1, W. E. Thomason2, W. Brooks3, and C. A. Griffey2, 1Department of Dairy Science, Blacksburg, VA, 2Department of Crop and Soil Environmental Sciences, Blacksburg, VA.

The objective of this study was to evaluate production performance in high-producing cows consuming diets containing hulled or hull-less barley as the grain source combined with low or high forage concentrations. The experiment was designed as a replicated 4 × 4 Latin square with 21-d periods and a 2 × 2 factorial arrangement of treatments (45 vs. 65% forage and hulled vs. hull-less barley). The cultivars utilized were Thoroughbred and Amaze 10 for the hulled and hull-less grains, respectively. Eight primiparous (610 ± 40 kg BW and 72 ± 14 DIM) and 16 multiparous (650 ± 58 kg BW and 58 ± 16 DIM) Holstein cows were fed once daily (1100 h) by means of a Calan gate system. Treatments consisted of: 1) 45% forage and hulled barley, 2) 65% forage and hulled barley, 3) 45% forage and hull-less barley, 4) 65% forage and hull-less barley. All variables were analyzed using the MIXED procedure of SAS. The statistical model included the effects of square, treatment, square by treatment interaction, period, cow within square, and the random residual error. Milk yield (41.8 kg/d; P < 0.76), milk lactose percentage (4.84%; P < 0.19), milk lactose yield (2.05 kg/d; P < 0.29), and body weight gain (0.64 kg/d; P < 0.79) did not differ among treatments. Dry matter intake tended to be lower for high-forage diets (25.4 vs. 26.8 kg/d; P < 0.07) and was not affected by grain type (P < 0.47). Milk fat percentage (3.91 vs. 3.50%; P < 0.01) and yield (1.61 vs. 1.49 kg/d; P < 0.03) were greater for high-forage than for low-forage diets but were not affected by grain type (P > 0.17). Milk protein percentage (3.13 vs. 3.07%; P < 0.01) and yield (1.33 vs. 1.26 kg/d; P < 0.03) were greater for high-forage than for low-forage diets but were not affected by grain type (P > 0.48). Milk urea nitrogen was reduced when feeding low-forage diets (15.4 vs. 14.1 mg/dL; P < 0.01) and hull-less barley (15.6 vs. 13.9 mg/dL; P < 0.01). Their interaction was not significant.

In conclusion, feeding either hulled or hull-less barley as the energy source in high-forage or low-forage diets resulted in similar production performance in high-producing cows.

Key Words: hull-less (hullless) barley, hulled barley


Fall-grown oat forage (OF) has potential to offer a second crop to augment forage supplies. Our objective was to evaluate lactating dairy cow performance when OF was substituted for corn silage (CS). Lactating Holstein cows (47) were randomly assigned to diets in a randomized complete block design with a 2 wk covariate period in which cows consumed a common diet, followed by an 8-wk period in which 3 experimental diets were fed. Two cows were removed for stealing of feed. Measurements were made in the last week of each period. To evaluate total-tract neutral detergent fiber (NDF) digestibility (TTNDFD), 6 fecal samples were collected from each cow over 3 d to represent every 4 h in a 24-h period; indigestible NDF was used as the internal marker. Experimental diets contained 20% alfalfa silage, 35, 27, or 19% brown midrib CS, and 0, 8, or 16% OF on a dry matter (DM) basis; diet canonical meal was reduced and high moisture corn increased with increasing OF. Diets were formulated to have similar concentrations of crude protein (CP) and NDF. OF and CS respectively contained 12.5 and 7.1% CP, 0.2 and 34.1% starch, 48.7 and 36.4% NDF, and 10.3 and 1.6% water-soluble carbohydrates on a DM basis. Significance was declared at P < 0.05. DM intake and eating time did not differ among diets. Fat- and protein-corrected milk (−0.27 kg/1% diet OF) and milk urea nitrogen declined linearly with increasing OF. TTNDFD and rumination time showed quadratic responses. Oat forage can support high production, however lactation performance was less than with corn silage. Alternate diet formulations with OF should be explored.

Table 1 (abstract 430).

<table>
<thead>
<tr>
<th>Measure1</th>
<th>0% OF2</th>
<th>8% OF</th>
<th>16% OF</th>
<th>SED3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg</td>
<td>29.7</td>
<td>29.4</td>
<td>29.0</td>
<td>0.54</td>
<td>0.44</td>
</tr>
<tr>
<td>3.5% FPCM, kg</td>
<td>50.5</td>
<td>48.5</td>
<td>46.2</td>
<td>1.44</td>
<td>0.02</td>
</tr>
<tr>
<td>MUN, mg/dL</td>
<td>12.6</td>
<td>12.5</td>
<td>11.9</td>
<td>0.26</td>
<td>0.02</td>
</tr>
<tr>
<td>TTNDFD, % of NDF</td>
<td>56.3</td>
<td>54.9</td>
<td>58.0</td>
<td>0.81</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Eating, min/d</td>
<td>202</td>
<td>204</td>
<td>207</td>
<td>16.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Rumination, min/d</td>
<td>441</td>
<td>485</td>
<td>467</td>
<td>18.2</td>
<td>0.06</td>
</tr>
</tbody>
</table>

1FPCM = fat- and protein-corrected milk; TTNDFD = total-tract NDF digestibility.
2OF = oat forage.
3SED = standard error of the difference.

Key Words: forage, oats, dairy cow

Effect of forage changes on the dynamic variation of the rumen fermentation in sheep. X. Xie1, J. K. Wang1, L. L. Guan3, and J. L. Liu1, 1Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, China, 2Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Canada.

A shortage of high quality forage may force smallholder farmers to suddenly change their cow ration to variable forages available locally such as cereal stover. To understand the adaptation and recovery of rumen fermentation in sheep. 

In a 3-periods crossover study, 10 ruminal cannulated sheep (BW = 15.1 ± 0.5 kg) were equally allocated to 2 treatments with same concentration mixture but different forage sequences: (1) alfalfa hay (AH) to corn stover (CS) then back to AH; and (2) CS to AH then back to CS, in quick succession for 21 d. Each forage treatment lasted for 2 wk and rumen contents were collected before morning feeding on d 0, 1, 2, 4, 6, 9, and 14, respectively after first and second transition. Rumen content samples were also collected 3 and 6 h after morning feeding during transition for short-term observation. The ruminal pH, ammonia nitrogen, volatile fatty acids and microbial protein (based on purine method) were analyzed. The data were analyzed according to mixed linear model and visualized using R with ggplot2 packages. The pH value did not change with the transition (P > 0.05). Rumen fermentation variables reversed on d 1 of forage transition between 2 sequences. Microbial protein concentration was increased (P < 0.01) from 6.78 on d 1 to 5.21 mg/dL on d 6 with transition from AH to CS, and from 7.86 on d 1 to 5.97 mg/dL on d 6 with transition from CS to AH in either sequence, along with corresponding increase in ammonia...
and milk production were recorded individually. Ruminal E(hstarch) for 21 d. Thereafter, they were fed 3 successive diets (contain-
with low level of RFC (% DM) (1.4% of soluble sugars, 18.2% of
maize silage, 10.8% alfalfa hay, and 21.5% concentrate, DM basis)
Holstein cows fitted with ruminal cannulas were fed a basal diet (67.7%
(ii) to compare the daytime and 24-h measurement of these 2 parameters.
Dietary starch concentration formulated with
increased proportions of RFC in the diet on the diurnal pattern of
ruminal E(h) and pH of lactating dairy cows fed increasing proportions of readily fermentable
The experiment lasted 37 d. Eight early (averaged 47 DIM) lactating cows were arranged as a 2 × 2 factorial with 2 types of corn silages [conven-
tional (CS) and brown midrib (BMR) corn silage] and 2 dietary starch concentrations (19 and 25% of DM). Diets were formulated to contain 60.7% forage and 39.3% concentrate on DM basis. Dried corn grain was replaced with soyhulls and beet pulp to decrease dietary starch concentration. Cow was the experimental unit. Silage × starch interactions were detected (P ≤ 0.05) for milk yield, energy-corrected milk (ECM), and feed efficiency (ECM/DMI). Milk yield was similar between cows fed BMR-25% starch and CS-19% starch, but was greater than for cows fed other diets. ECM was greatest for cows fed BMR-25% starch compared with the other 3 diets. Feed efficiency was greatest for cows fed CS-19% starch and BMR-25% starch and least for cows fed BMR-19% starch. Milk protein percentage was affected by starch concentration, resulting in greater protein concentration for cows fed 25% starch compared with cows fed the 19% starch diet. There was no effect of diet on DMI, milk fat percentage, milk fat and protein yield, and MUN. Overall, the milk and ECM of lactating dairy cows was superior when feeding BMR-25% starch, but cows fed BMR-19% starch responded similarly to cows fed CS diets at either 19 or 25% starch.


The objectives of the present study were (i) to investigate the changes in ruminal E(h) and pH of lactating cows during a dietary transition from a low to a higher level of readily fermentable carbohydrates (RFC), and (ii) to compare the daytime and 24-h measurement of these 2 parameters. The experiment lasted 37 d. Eight early (averaged 47 DIM) lactating Holstein cows fitted with ruminal cannulas were fed a basal diet (67.7% maize silage, 10.8% alfalfa hay, and 21.5% concentrate, DM basis) with low level of RFC (% DM) (1.4% of soluble sugars, 18.2% of starch) for 21 d. Thereafter, they were fed 3 successive diets (containing 3.5%, 5.6% and 8.6% soluble sugars; 16.4%, 17.7%, 19.4% starch, respectively) at d 22, d 27 and d 32 to manage a progressive transition. Diets were offered ad libitum in equal amounts twice daily. The DMI and milk production were recorded individually. Ruminal E(h) and pH were continuously measured for 3 d of each dietary treatment, by using a ruminal submersible data logger (Dascor, Escondido, CA). The E(h) and pH data were summarized as mean E(h) and pH over daytime (from 1 h before morning feeding to 8 h after) and over 24 h. Dry matter intake (P = 0.361) and milk yield (P = 0.868) did not change during the dietary transition: in average 18.2 kg DM/d and 32.6 kg/d respectively. Increasing proportions of dietary RFC increased significantly E(h) (+ 56 mV) and decreased pH (- 0.32). Compare with mean daytime pH, mean pH over 24 h allows a better distinction between treatments (Table 1). In conclusion, a long-term continuous 24-h measurement shows an effect of increased proportions of RFC in the diet on the diurnal pattern of ruminal E(h) and pH.

Key Words: forage transition, rumen fermentation variables, sheep

433 Impact of dietary starch concentration formulated with two types of corn silage on the performance of dairy cows. J. I. Sanchez-Duarte*1 and K. F. Kalscheur2, 1South Dakota State University, Brookings, SD, 2US Dairy Forage Research Center, USDA, ARS, Madison, WI.

This study explored the effect of feeding different starch concentrations and conventional or brown midrib corn silage on the performance of lactating dairy cows. Forty-eight Holstein cows were assigned to 1 of 4 diets using a randomized complete block design with a 2-wk covariate period followed by 8-wk experimental period. Experimental diets were arranged as a 2 × 2 factorial with 2 types of corn silages [conven-
tional (CS) and brown midrib (BMR) corn silage] and 2 dietary starch concentrations (19 and 25% of DM). Diets were formulated to contain 60.7% forage and 39.3% concentrate on DM basis. Dried corn grain was replaced with soyhulls and beet pulp to decrease dietary starch concentration. Cow was the experimental unit. Silage × starch interactions were detected (P ≤ 0.05) for milk yield, energy-corrected milk (ECM), and feed efficiency (ECM/DMI). Milk yield was similar between cows fed BMR-25% starch and CS-19% starch, but was greater than for cows fed other diets. ECM was greatest for cows fed BMR-25% starch compared with the other 3 diets. Feed efficiency was greatest for cows fed CS-19% starch and BMR-25% starch and least for cows fed BMR-19% starch. Milk protein percentage was affected by starch concentration, resulting in greater protein concentration for cows fed 25% starch compared with cows fed the 19% starch diet. There was no effect of diet on DMI, milk fat percentage, milk fat and protein yield, and MUN. Overall, the milk and ECM of lactating dairy cows was superior when feeding BMR-25% starch, but cows fed BMR-19% starch responded similarly to cows fed CS diets at either 19 or 25% starch.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Basal diet</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime ruminal Eh, mV</td>
<td>−317.7a</td>
<td>−292.7ab</td>
<td>−280.8b</td>
<td>−261.0b</td>
<td>42.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24 h Ruminal Eh, mV</td>
<td>−318.3a</td>
<td>−291.6b</td>
<td>−282.7b</td>
<td>−261.9b</td>
<td>42.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Daytime ruminal pH</td>
<td>6.25a</td>
<td>6.16a</td>
<td>6.13b</td>
<td>5.95b</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24 h Ruminal pH</td>
<td>6.25a</td>
<td>6.14a</td>
<td>6.09b</td>
<td>5.93c</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 1 (abstract 432). Ruminal Eh and pH in lactating dairy cows fed increasing proportions of readily fermentable carbohydrates

<table>
<thead>
<tr>
<th>Item</th>
<th>19%</th>
<th>25%</th>
<th>19%</th>
<th>25%</th>
<th>SEM</th>
<th>P &gt; F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>25.9</td>
<td>26.8</td>
<td>27.0</td>
<td>26.8</td>
<td>0.54</td>
<td>NS</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>44.1a</td>
<td>43.4b</td>
<td>43.4b</td>
<td>45.7a</td>
<td>0.69</td>
<td>1</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>45.1b</td>
<td>44.9b</td>
<td>44.2b</td>
<td>46.8a</td>
<td>0.71</td>
<td>1</td>
</tr>
<tr>
<td>ECM/DMI</td>
<td>1.76a</td>
<td>1.69ab</td>
<td>1.66b</td>
<td>1.76a</td>
<td>0.04</td>
<td>1</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.83</td>
<td>3.89</td>
<td>3.87</td>
<td>3.88</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Fat, kg/d</td>
<td>1.66</td>
<td>1.68</td>
<td>1.63</td>
<td>1.73</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Protein, %</td>
<td>2.91</td>
<td>3.00</td>
<td>2.90</td>
<td>2.98</td>
<td>0.04</td>
<td>S</td>
</tr>
<tr>
<td>Protein, kg/d</td>
<td>1.27</td>
<td>1.27</td>
<td>1.23</td>
<td>1.32</td>
<td>0.02</td>
<td>NS</td>
</tr>
<tr>
<td>MUN, mg/dL</td>
<td>11.1</td>
<td>10.5</td>
<td>11.4</td>
<td>11.0</td>
<td>0.35</td>
<td>NS</td>
</tr>
</tbody>
</table>

aMeans with different superscripts differ (P ≤ 0.05).

b1S = starch effect; I = silage by starch effect; NS = not significant.

Key Words: redox potential, rumen, dietary transition

434 Effects of replacing corn with different levels of starch degradability with beef pulp as a source of soluble fiber on fermentation in continuous culture. L. E. Koch*, B. M. Koch, R.

Table 1 (abstract 433).

Key Words: BMR corn silage, milk yield, starch concentration
Evaluating starch degradability (ShD) in combination with starch level can be used as a better predictor for a diet to induce milk fat depression (MFD). Starch with high rates of degradability may lead to decreased ruminal pH and changes in rumen biohydrogenation (BH) and accumulation of certain fatty acid isomers known as milk fat inhibitors (MFI). Substituting starch from corn with beet pulp (BP) as a source of soluble fiber (SF) can yield different fermentation patterns, yet still provide similar energy to the animal without compromising performance or exacerbating production of MFI. We hypothesized that replacing starch with a source high in SF, such as BP, will improve fermentation and flow of BH intermediates when added to a diet with high MFD potential. Treatments included 2 levels of ShD, high (HDS) and low (LDS), and 4 combinations of BP replacing corn, low (LSF; 0% beet pulp), medium low (MLSF; 13% BP), medium high (MHSF; 26% BP), and high (HSF; 39% BP). Diets were formulated to replace a portion of the starch with SF from BP and contained a basal level of soybean oil. Fermenters were randomly assigned to treatments in a 2x4 factorial design and ran for 4, 10 d periods. Data were analyzed using the MIXED procedure of SAS with repeated measures in a model including ShD and sugar as fixed and fermenter and period as random. Preplanned contrasts were utilized to compare N vs all, S vs L, and, S and L vs C. Dry matter, OM, and ADF apparent digestibility (AD) were unaffected by ShD or sugar, but NDF AD differed with S and L vs C (P = 0.02). Outflow of the saturated FA C12, 20, 22, and 24 were all reduced by HDS (P < 0.01). Outflow of trans-11 18:1 tended to be greater with LDS, and trans-12 18:1 was greater with HDS (P = 0.08 and P < 0.01). Other FA outflows are listed in Table 1. Methane output decreased with sugar addition (P = 0.03) and S vs L differed (P < 0.01). Ammonia was greater with sugar addition (P < 0.01) and all contrasts were significant. These results suggest that ShD with sugar can alter production of FA isomers, AD, and ammonia.

Key Words: lipid, biohydrogenation, sugar

<table>
<thead>
<tr>
<th>Item, mg/d</th>
<th>Sugar Addition</th>
<th>SEM</th>
<th>Contrasts, P-value</th>
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<tbody>
<tr>
<td>18:1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDS</td>
<td>361.2 378.6 343.5 319.6</td>
<td>29.54</td>
<td>0.12 0.13 0.39</td>
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<tr>
<td>HDS</td>
<td>351.9 368.4 369.9 358.9</td>
<td>52.82</td>
<td>0.25 0.84 0.10</td>
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<tr>
<td>18:2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LDS</td>
<td>573.3 655.8 576.5 536.2</td>
<td>6.51</td>
<td>0.37 0.87 &lt;0.01</td>
</tr>
<tr>
<td>HDS</td>
<td>653.8 736.8 720.8 686.6</td>
<td>1.42</td>
<td>0.54 0.74 0.90</td>
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<tr>
<td>18:3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDS</td>
<td>89.3 96.2 84.9 79.3</td>
<td>1.80</td>
<td>0.31 0.15 0.07</td>
</tr>
<tr>
<td>HDS</td>
<td>69.7 96.6 92.2 89.3</td>
<td>2.06</td>
<td>0.09 0.13 0.57</td>
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<tr>
<td>18:1 trans 12</td>
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<tr>
<td>LDS</td>
<td>9.2 8.1 9.3 8.2</td>
<td>1.0 1.0 1.0 1.0</td>
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<tr>
<td>HDS</td>
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<td>1.4 1.4 1.4 1.4</td>
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<tr>
<td>LDS</td>
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<td>0.31 0.15 0.07</td>
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<tr>
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<tr>
<td>LDS</td>
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<td>1.4 1.4 1.4 1.4</td>
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<tr>
<td>HDS</td>
<td>12.4 15.5 12.4 13.0</td>
<td>1.80</td>
<td>0.31 0.15 0.07</td>
</tr>
</tbody>
</table>

N. Klopp, S. M. Hussein, V. R. Trutwin, and G. J. Lascano, Clemson University, Clemson, SC.
m ent with 24 Holstein heifers (6.6 ± 0.7 mo and 218 ± 27 kg of BW) was conducted. Heifers were blocked by age. Treatments diets were (1) 10% cold-pressed CRM (glucosinolates at 2.06 g/kg of diet DM), and (2) 10% DDGS on a DM basis. The remainder of the diets consisted of grass hay, ground corn, soybean meal and mineral mix, and diets were formulated to be isonitrogenous and isocaloric. Heifers were individually fed using a Calan gate feeding system, and the rations were limit-fed at 2.65% of BW on DM basis. Jugular blood samples were collected 3–4 h post-feeding on 2 d during wk 0, 4, 8, 12, and 16 for metabolite and metabolic hormone analyses. Data were analyzed using MIXED procedures with repeated measures in SAS 9.4, and wk 0 results were used as covariate terms for each parameter. Significance was declared at \( P < 0.05 \). There were no interactions of treatment by wk for any of the metabolites and metabolic hormones measured. Glucose (84.0, and 82.3 mg/ dl; \( SEM = 1.25 \)) for CRM and DDGS, respectively, plasma urea nitrogen (19.8, and 19.77 mg/dL; \( SEM = 0.48 \)), IGF-1 (117.1, and 105.0 ng/mL; \( SEM = 4.9 \)), triiodothyronine (140.2, and 154.7 ng/dL; \( SEM = 5.3 \)), and thyroxine (6.3, and 6.6 μg/dL; \( SEM = 0.23 \)) concentrations were similar (\( P > 0.05 \)) among treatments. Cholesterol concentration was greater (\( P < 0.05 \)) in the CRM heifers than DDGS heifers (89.9, and 78.2 mg/dL; \( SEM = 2.6 \)). These results demonstrate that growing heifers can be limit-fed diets with 10% CRM without negative effects on the thyroid hormones and metabolic status.

**Key Words:** canarita meal, glucosinolates, dairy heifer

### 437 Milk production and composition of dairy cows fed hydroponic barley sprouts. R. D. Lawrence*, J. L. Anderson, S. I. Martinez Monteaqudo, and L. Metzger, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Our objective was to determine the effects on milk production and rumen fermentation of feeding cows hydroponically grown barley sprouts (HydroGreen Inc., Renner, SD). Twenty mid-lactation Holsteins (DIM 205 ± 47.4) were used in a 6-wk randomized complete block design study. Treatments included: 1) control diet with ground corn and soybean meal as major concentrate ingredients (CON) and 2) 8% (DM basis) as hydroponic barley sprouts replacing some corn and soybean meal (HYD). Both diets were individually fed as total mixed rations among treatments. Milk fat % tended (\( P > 0.10 \)) to be higher when cows fed the positive control and 0.5% LMA diets. Yields of solids-corrected milk and fat did not differ (39.43 ± 2.3 and 1.55 ± 0.10, kg/d, \( SEM = 0.28 \)) among treatments. Treatment by wk interactions (\( P < 0.01 \)) amount of time spent under pH 5.8 compared with the other treatments. The cows receiving 1.0% LMA in their diet consumed less (\( P < 0.001 \)) DM (26.1 vs 27.6 and 27.4, kg/d) than cows fed the positive control and 0.5% LMA diets. Yields of solids-corrected milk and fat did not differ (39.43 ± 2.3 and 1.55 ± 0.10, kg/d, \( P > 0.10 \)) among treatments. Milk fat % tended (\( P > 0.10 \)) to be higher when cows received the positive control diet compared with the 0.5% LMA diet (3.90 vs 3.76 ± 0.13). These results reveal that the 0.5% LMA diet does not differ from positive control diet containing sodium bicarbonate in rumen buffering ability, and may be a suitable replacement for sodium bicarbonate in rations for high producing dairy cows.

**Key Words:** layer manure ash, buffering capacity, rumen pH

### 438 Efficacy of layer manure ash (LMA) byproduct in lactating dairy cow diets as a replacement for the buffering capacity of sodium bicarbonate. M. D. Miller*1, C. S. Ballard1, H. M. Dann1, J. Noland2, D. Axe1, L. M. Klieber1, K. W. Cotanch1, and R. J. Grant1, 1William H. Miner Agricultural Research Institute, Chazy, NY, 2EnergyWorks Biopower LLC, Annapolis, MD, 3Axe Agri-Services, Richmond, VA.

The objective of this study was to evaluate the efficacy of layer manure ash (LMA) byproduct in lactating dairy cow diets as a replacement for buffering capacity of sodium bicarbonate. Layer manure ash is derived from egg-layer manure through a controlled thermochemical reaction. Rumen pH, feed intake, milk yield and composition were measured in 16 ruminally cannulated Holstein cows averaging 183 (SD = 64) DIM. A replicated 4 × 4 Latin square design with 2 wk adaption periods and 1-wk collection periods was used to evaluate cows fed a common diet with the following treatments: 1) 0.86% calcium carbonate (Negative Control), 2) 0.86% sodium bicarbonate + 0.86% calcium carbonate (Positive Control), 3) 0.5% LMA + 0.43% calcium carbonate (0.5% LMA), and 4) 1.0% LMA (1.0% LMA). Starch and neutral detergent fiber content of the base diet were 24 and 30% of DM. Data were analyzed using MIXED procedures in SAS with cow as experimental unit. Daily rumen pH was not different across all treatment diets (6.01 ± 0.04, \( P > 0.10 \)), but the negative control diet had the lowest (\( P < 0.05 \)) minimum rumen pH over a 24-h period with greatest variability (5.96 ± 0.28 SD). Area under the curve (AUC) < 5.8 as a measure for sub-acute rumen acidiorms tended to be more severe (1.32 (0.70 to 2.51 95% CI), pH units x h/d, \( P = 0.08 \)) for cows fed the negative control diet and had the most (\( P < 0.001 \)) amount of time spent under pH 5.8 compared with the other treatments. The cows receiving 1.0% LMA in their diet consumed less (\( P < 0.001 \)) DM (26.1 vs 27.6 and 27.4, kg/d) than cows fed the positive control and 0.5% LMA diets. Yields of solids-corrected milk and fat did not differ (39.43 ± 2.3 and 1.55 ± 0.10, kg/d, \( P > 0.10 \)) among treatments. Milk fat % tended (\( P < 0.10 \)) to be higher when cows received the positive control diet compared with the 0.5% LMA diet (3.90 vs 3.76 ± 0.13).These results reveal that the 0.5% LMA diet does not differ from positive control diet containing sodium bicarbonate in rumen buffering ability, and may be a suitable replacement for sodium bicarbonate in rations for high producing dairy cows.

**Key Words:** layer manure ash, buffering capacity, rumen pH

### 439 Growth performance of dairy calves fed microbiobally enhanced soy protein in starter pellets with pasteurized milk. N. D. Seneviratne*1, J. L. Anderson1, and W. R. Gibbons2, 1Dairy and Food Science Department, South Dakota State University, Brookings, SD, 2Department of Biology and Microbiology, South Dakota State University, Brookings, SD.

Our objective was to investigate feeding microbiobally (fungal)-enhanced soy protein (MSP) in dairy calf starter pellets on growth performance, health, and nutrient utilization. Thirty-eight Holstein calves (2 d old, 25 females, 13 males) in individual hutches were used in a 12-wk randomized complete block design study. Treatments were 2 starter pellets including: a control (CON) versus 8% MSP (DM basis). Calves were fed 2.83 L of pasteurized milk 2×/d during wk 1 to 5 and 1×/d during wk 6. Pellets and water were fed ad libitum. Fecal scores (0 = firm, 3 =
watery) and respiratory scores (healthy ≤3, sick ≥5) calculated from the sum of scores for rectal temperature, cough, ocular, and nasal discharge were recorded daily. Body weights (BW) and frame growth were measured 2 d and jugular blood samples were taken 1 d every 2 wk at 3 h post morning feeding. Fecal grab samples were collected in wk 12 for analysis of total-tract digestibility (TTD). Results were analyzed using MIXED procedures with repeated measures in SAS 9.4. Significant differences were declared at $P < 0.05$ and tendencies were $0.05 \leq P < 0.10$. Total DMI (1,522 and 1,470 g/d; SEM = 48.62) was greater ($P = 0.02$) in CON than MSP. Calf BW (75.4 and 75.0 kg; SEM = 2.39), ADG (0.77 and 0.75 kg/d; SEM = 0.05), and withers height (89.1 and 90.1 cm; SEM = 0.86) were similar. Gain:feed (0.62 and 0.60 kg/kg; SEM = 0.03) was similar; however there was an interaction of treatment by wk ($P < 0.01$). Plasma urea nitrogen (12.6 and 11.1 mg/dL; SEM = 0.39) was less ($P < 0.01$), but β- hydroxy butyrate (31.1 and 34.5 mg/dL; SEM = 1.28) was greater ($P = 0.04$) in calves fed MSP. Glucose (124.3 and 123.6 mg/dL; SEM = 2.59) and triglycerides (31.5 and 30.1 mg/dL; SEM = 1.19) were similar. Calves fed MSP had greater ($P < 0.05$) CP, NDF and ADF and tendency ($P = 0.06$) for greater DM TTD. Fecal scores were similar with an interaction of treatment by wk ($P < 0.01$). Body temperature and respiratory scores were similar ($P > 0.05$). Results demonstrated that feeding calves MSP improved TTD, fecal consistency, and maintained growth performance.

Key Words: microbially enhanced soy protein, dairy calf, growth performance

440 Dry period plane of energy and periparturient disease status: Effects on feed intake, energy balance, milk production, and milk composition. A. Pineda*, F. C. Cardoso, and J. K. Drackley, University of Illinois, Urbana, IL.

The aim of the study was to assess the effects of energy intake during the dry period on cows that suffered non-disease (ND) or disease (DD; displacement of abomasum, retained placenta, metritis, or milk fever) postpartum. Twenty-nine multiparous Holstein cows dried-off 50 d before calving were blocked by parity, body weight (BW), and body condition score (BCS), and then randomly assigned to 1 of 2 dietary treatments: controlled energy (CE; NE\textsubscript{L} = 1.39 Mcal/kg; n = 17) or higher-energy (HE; NE\textsubscript{L} = 1.58 Mcal/kg; n = 12) to supply 100 or ~150% of energy (NE\textsubscript{L}) requirements at ad libitum intake, respectively. After calving all cows were fed the same lactation diet. Cows were individually fed and remained in the study until 28 d after calving. At dry-off, BW was similar ($P = 0.15$) among treatments but DD cows postpartum had greater ($P = 0.01$) initial BW than ND (820 vs. 735 kg). Prepartum intakes of NE\textsubscript{L} (16.5 and 20.2 Mcal/d) and energy balance (EB) were greater ($P < 0.05$) in HE than CE. Significant interaction of diet and time ($P = 0.03$) showed greater decrease in EB 2 wk prior calving in HE than CE. Postpartum, cows fed HE tended ($P = 0.08$) to lose more BW, had greater BCS ($P = 0.05$), but lost more BCS (0.53 and 0.21; $P = 0.02$) than CE. Cows DD postpartum lost more BW ($P = 0.03$), had lower BCS ($P = 0.01$), and lost more BCS ($P = 0.05$) than ND. Cows fed CE tended ($P = 0.10$) to have greater intakes of dry matter (DM) and NE\textsubscript{L} postpartum than HE. Cows ND had greater ($P < 0.01$) DM and NE\textsubscript{L} intakes than DD. Cows that were DD but fed CE (n = 6) had greater ($P < 0.05$) intakes of DM and NE\textsubscript{L} than DD cows fed HE (n = 5). Postpartum EB was greater ($P = 0.04$) in ND than DD cows. Dietary treatment had no effects ($P > 0.20$) on milk yield or milk components. Cows ND had greater ($P < 0.05$) milk yield, milk protein concentration and yield, and lactose concentration and yield than DD. High-BW cows were more likely to suffer DD and performed poorly. Cows fed CE diet prepartum had lesser decrease in EB 2 wk before calving followed by lower BW and BCS losses and greater intakes of DM and NE\textsubscript{L} postpartum. Among DD cows, those previously fed CE showed benefits in DM and NE\textsubscript{L} intakes compared with those fed HE.

Key Words: dairy cow, dry period, energy intake
Milk Protein and Enzymes Symposium:
Protein Interactions—Aggregations and Interfaces

441 Milk proteins: Aggregation and interactions at interfaces and within dairy networks. S. Gras*, The University of Melbourne, Melbourne, VIC, Australia.

Milk protein aggregation is central to the manufacture of many dairy products but studies of individual milk proteins reveal that these proteins can participate in several different aggregation pathways; with different physiochemical environments leading to diverse final protein structures that display varied properties. This talk will focus on the aggregation of kappa casein and the associated casein macro peptide generated by cleavage with rennet. Drawing on new and existing data it will contrast the structures formed in dairy products with other aggregates, known as amyloid fibrils and compare the methods used to characterize and image gels and networks formed from these proteins. A better understanding of the landscape of protein folding and misfolding for dairy proteins will not only open up opportunities for new products and textures but also help to ensure that these proteins promote health on digestion.

Key Words: protein, aggregation

442 Effect of aggregation and interfaces on the digestion of dairy proteins. A. Mackie1*, N. Rigby1, and A. Macierzanka2, 1University of Leeds, Leeds, United Kingdom, 2Gdansk University of Technology, Gdansk, Poland.

It is becoming clear that the role of food structure is important in digestion, nutrient absorption and health. Over the last 10 years, I have studied the effects of processing on rates and patterns of protein digestion both in vitro and in vivo. Protein digestion is governed by accessibility of the enzyme to the substrate and the removal of the hydrolysis products. Thus in principle, more structure leads to slower digestion. Using this idea, we will show that the thermal processing of whey proteins to form aggregates of different sizes can have a profound effect on the rate at which the protein can be digested in simulated gastric and intestinal phase [Macierzanka et al., Food Chemistry, 2012, 134:2156–2163]. The unfolding of proteins induced by absorption to an oil/water interface during emulsification can increase the susceptibility to digestion [Macierzanka et al., Soft Matter, 2009, 5:538–550]. In my final examples, I will show how enzymatic cross linking of proteins at interfaces using transglutaminase can alter digestion kinetics, both in vitro and in vivo [Juvonen et al., Br. J. Nutr., 2015, 114:418–429]. This latter work showed that we could successfully simulate the behavior in the GI tract, and also that the cross-linking of the protein that stabilizes the emulsions was only able to alter the digestion of proteins and had no effect on the hydrolysis of the lipid it was stabilizing.

Key Words: digestion, structure, protein

443 The role of soluble aggregates on the processing functionality of milk and milk concentrates. Milena Corredig1*, 1Gay Lea Foods Cooperative, Research and Development, Guelph, ON, Canada, 2University of Guelph, Food Science Department, Guelph, ON, Canada.

Much is known about the changes in the physical and chemical properties of casein micelles during processing in skim milk. Membrane technologies have become increasingly widespread as a means to prepare concentrated casein suspensions as ingredients, and the interactions occurring among the milk proteins as a function of their volume fraction is yet to be fully understood, especially during processing. Of particular interest is the soluble fraction, known to affect the final processing functionality of the milk matrix. In untreated skim milk, the soluble phase is mostly constituted of whey proteins. Heating induces the formation of soluble aggregates containing caseins and whey proteins, and these aggregates strongly affect, for example, the texture of acidified milk products. While we know how to control the processing history of skim milk to modulate the properties of dairy products, this is much less understood in concentrated milk systems. Extensive work is needed to characterize the type and concentration of soluble aggregates in casein suspensions depending on casein volume fraction and processing conditions. As the volume fraction increases, there are profound changes in the composition of the serum phase. This paper will discuss the importance of soluble protein complexes on rheological properties and texture formation of dairy matrices. Small changes in composition may strongly affect the physical chemical properties of the concentrates used as ingredients. Understanding how to control compositional changes in milk concentrates will unravel the development of a new generation of functional ingredients from milk.

Key Words: aggregation, milk processing functionality, milk concentrates


The drive to produce new dried high protein dried ingredients for the infant formula and healthy aging sectors can often result in unforeseen problems during production. A critical factor in high protein ingredients is uncontrolled protein aggregation which may manifest as unwanted biofouling, pipeline/filter blockage, precipitation/sedimentation and overall poor visual appearance on reconstitution. The complexity of composition combined with multiple process effects (mixing, heating, pressure, shear forces etc.) together with the dynamic nature of continuous production is highly challenging and will require new tools to capture data in real time and that is spatially resolved. This presentation will briefly review existing imaging tools and will then describe new approaches to characterizing hydration of milk powders including milk protein concentrates. “Traditional” imaging tools, such as optical, confocal and electron microscopy are very useful but new approaches such as x-ray microtomography, atomic force microscopy, Raman microscopy and particularly high speed video can further our understanding of how powders are formed during the spray drying pipeline as well as the characterization of wetting, moisture uptake and dispersion of single powder particles. A new confocal microscopy technique employing fluorescent tracer dyes will be described that characterizes hydration rates of individual powder particles. Results show that these techniques (new and old) can be used to characterize powder hydration at the micrometre scale and in real time. Data from these analyses can be used to reverse-engineer powders with desired re-hydration properties.

Key Words: powder, protein, microscopy
First stage (0–6 mo) infant milk formulae (IMF) are designed to approximate the protein, carbohydrate, fatty acid and micronutrient composition of human milk. Protein profile of the formulation is a key consideration and whey protein is typically added to bovine skim milk to more closely mimic the profile in breast milk. The whey to casein ratio is increased from 20:80 in bovine milk to 60:40 in IMF. Consequently, IMF can exhibit lower in-process heat stability compared with bovine milk, partially due to the heat labile nature of whey and, in particular, β-Lactoglobulin (β-Lg). Recent research carried out at Teagasc demonstrated the effect of temperature, pH and concentration on aggregate formation from heat treated whey proteins. Techniques such as nuclear magnetic resonance (NMR), light scattering (LS) and SDS-PAGE were used to determine heat induced changes in matrices of varying complexity, from simple β-Lactoglobulin (β-Lg) solutions to model IMFs. NMR showed structural changes in β-Lg solutions were minimal at 62°C, however at 85°C, increasing protein concentration from 1 to 12% resulted in a more stable structure in some reactive regions. LS showed that aggregate size decreased significantly (P < 0.05) from 100 to 59nm on increasing the concentration during heating (85°C × 30 s) from 1 to 12%, while heat stability increased at pH values greater than 6.9. In more complex model 1st stage formulations, heat-induced changes in whey protein structure were not only a function of concentration but were also influenced by the presence of casein, lactose and fat. A greater amount of unresolved high molecular weight aggregates (SDS-PAGE) were present when skim milk and whey protein were heated in-combination compared with when heated separately, indicating that the extent of aggregation is influenced by the presence of casein. Higher protein aggregation resulted in increased concentrate viscosity, reduced atomisation efficiency and increased finished powder particle size. The research highlights the role that aggregation of whey protein and subsequent interaction with casein has on in-process stability, which impacts on drying parameters and finished powder functionality.

Key Words: protein aggregation, infant formula, whey protein
Ruminant Nutrition VI

446 Ethyl-cellulose rumen-protected methionine enhances animal performance during the periparturient period and early lactation in dairy cows. F. Batistel1, J. M. Arroyo1,2, A. Bellingeri1, L. Wang3, B. Saremi4, C. Parys4, E. Trevisi3, F. C. Cardoso1, and J. J. Loor1, 1University of Illinois at Urbana-Champaign, Urbana, IL, 2Universidad de la Republica, San José, Uruguay, 3Southwest University, Rongchang, China, 4Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany, 5Università Cattolica del Sacro Cuore, Piacenza, Italy.

The aim of this study was to evaluate the impact of feeding ethyl-cellulose rumen-protected methionine on the performance and liver function of dairy cows during the periparturient period and early lactation. Sixty multiparous Holstein were used in a block design and assigned to either a control diet or Met-supplemented (Mepron, Evonik Nutrition & Care GmbH, Germany) diet. Mepron was supplied from −28 to 60 d relative to parturition at a rate of 0.09% and 0.10% of DM during the prepartum and postpartum period, respectively. That rate ensured that the ratio of Lys to Met in the MP was close to 2.8:1. Blood samples from 15 cows per treatment were collected at −30, −14, 1, 7, 21, 30 and 60 d relative to parturition. The statistical model included the random effect of block and fixed effect of treatment, time and interactions. Cows fed Met had intakes of dry matter that were 1.2 kg/d greater during the periparturient period. Compared with control, during the fresh period (1 to 30 DIM) feeding Met increased DMI by 1.65 kg/d, milk yield by 4.1 kg/d, fat yield by 0.17 kg/d, milk protein yield by 0.2 kg/d, 3.5% FCM by 4.3 kg/d and ECM by 4.4 kg/d. Although Met supplementation increased milk protein content by 0.16% units compared with control during the fresh period, no differences were observed for milk fat, lactose, and MUN concentration. During the high production period (31 to 60 DIM), compared with control cows, feeding Met increased DMI by 1.45 kg/d and milk yield by 4.4 kg/d. Met also increased fat yield by 0.19 kg/d, milk protein yield by 0.17 kg/d, 3.5% FCM by 4.7 kg/d and ECM by 4.8 kg/d. Among the biomarkers analyzed, Met led to overall lower (P = 0.01; 15.1 vs 22.9 U/L) γ-glutamyl transferase. For cholesterol, a treatment × time (P = 0.07; 4.0 vs 3.5 mmol/L) was observed due to a greater increase over time in Met-fed cows. Aspartate aminotransferase, alkaline phosphatase and bilirubin were not affected by Met. Liver tissue triacylglycerol concentration also was not affected by Met. In conclusion, ethyl-cellulose rumen-protected methionine supplementation improved dairy cow performance during the peripartum and through the peak of lactation.

Key Words: biomarkers, methionine, transition period


In vitro experiments have demonstrated independent, additive casein synthesis responses to supplies of Ile, Leu, Met, and Thr. We hypothesized that lactating cattle would respond in a similar manner. Forty-eight Holstein cows were fed a diet containing 75% of NRC (2001) predicted, metabolizable protein (MP) requirements (LoMP, 13.5% CP) in a randomized block design with replicated 4 × 4 Latin squares within each block. Each of the 4 ruminally protected (RP) amino acids (AA) represented a block. Period length was 12 d. Treatments within each block were LoMP and LoMP plus RPIle, RPLeu, RPMet, or RPThr at doses of 0, 50, 100, and 150% of the difference between absorbed AA supplied by the LoMP and MP sufficient diets. Intestinal availability of each RPAA was assessed by abomasal dosing of the RPAA after 8 h of ruminal incubation. The RPAA doses were 0, 8, 15, and 25.5 g of absorbed Ile/cow/d; 0, 14, 28, and 42 g of absorbed Leu/cow/d; 0, 3, 6, and 9 g of absorbed Met/cow/d; and 0, 8, 16, and 24 g of absorbed Thr/ cow/d. DMI increased linearly with increasing dose of Ile (P = 0.02), and tended to increase quadratically with respect to Met and Thr. Leu had no effect on DMI. Milk yield (kg/d) increased quadratically (P < 0.05) in response to Ile, Met, and Thr, and decreased quadratically in response to Leu. Milk protein yield (kg/d) tended to increase quadratically (P = 0.11) in response to Met and linearly (P = 0.12) in response to Thr, and decreased quadratically in response to Leu. Ile had no effect. Body weight (kg/d) decreased quadratically (P < 0.001) with Met dose, and tended to increase linearly (P = 0.11) with Leu dose, suggesting that changes in milk protein yield for animals supplemented with Leu and assigned to either a control or Met-supplemented (Mepron, Evonik Nutrition & Care GmbH, Germany) diet. Mepron was supplied from −28 to 60 d relative to parturition at a rate of 0.09% and 0.10% of DM during the prepartum and postpartum period, respectively. That rate ensured that the ratio of Lys to Met in the MP was close to 2.8:1. Blood samples from 15 clinically-healthy cows per treatment were collected at −30, −14, 1, 7, 21, 30 and 60 d relative to parturition and analyzed for biomarkers of energy balance, inflammation and oxidative stress. Neutrophil and monocyte function was measured at −10, 1, 7, 21 and 30 d relative to parturition. The statistical model included the random effect of block and fixed effect of treatment, time and interactions. The indicators of energy balance β-hydroxybutyrate, glucose and fatty acids were not affected by supply of Met. Among inflammation biomarkers measured, Met led to greater (P = 0.01; 38.1 vs 37.1 g/L) albumin (negative acute-phase protein) while ceruloplasmin (positive acute-phase protein), and myeloperoxidase were not affected by Met supply. The lower concentrations of reactive oxygen metabolites (P = 0.07; 15.2 vs 16.8 mg of H2O2/100 mL) and greater paraoxonase (P = 0.01; 38.1 vs 49.7%) and oxidative burst (P = 0.06; 53.1 vs 44.6%). However, monocyte function was not affected by Met. Overall, the results indicate that increasing Met supply by feeding ethyl-cellulose rumen-protected methionine is an effective approach to help mitigate oxidative stress as well as enhance neutrophil function during the peripartum period through the peak of lactation.

Key Words: biomarkers, methionine, transition period

447 Effect of ethyl-cellulose rumen-protected methionine supplementation on inflammation, oxidative stress and neutrophil function during the periparturient period and early lactation in dairy cows. F. Batistel1, J. M. Arroyo1,2, C. I. M. Garces3, E. Trevisi3, B. Saremi4, C. Parys4, M. A. Ballou5, and J. J. Loor1, 1University of Illinois at Urbana-Champaign, Urbana, IL, 2Universidad de la Republica, San José, Uruguay, 3Università Cattolica del Sacro Cuore, Piacenza, Italy, 4Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany, 5Texas Tech University, Lubbock, TX.

We hypothesized that increasing the intestinal supply of Met could help alleviate inflammation and oxidative stress, and enhance neutrophil and monocyte function during the periparturient period and early lactation. Sixty multiparous Holstein cows were used in a block design
may be driven by non-mammary tissue use. Conversely, DMI and BW responses for animals supplemented with Met and Thr do not explain the trend for increased milk protein yield, suggesting that Met and Thr stimulated milk protein synthesis. Revising dairy requirement models to include animal responses to individual AA may improve milk production predictions leading to increased N efficiency, and reduced N excretion from lactating dairy animals.

**Key Words:** amino acid, requirement, lactation

### 449 Lactational performance of ruminally protected methionine and lysine prototypes.

A. Myers1, K. Estes1, H. Choi1, R. White1, B. Barton2, C. Zimmerman3, and M. Hanigan*1, 1Virginia Tech, Blacksburg, VA, 2Balchem Corp., New Hampton, NY, 3Balchem Corp., Walkersville, MD.

Methionine (Met) and lysine (Lys) are often limiting amino acids in lactating cow diets. The objective of this work was to assess a lipid encapsulated Lys (RP-Lys) and 3 lipid encapsulated Met (RP-Met) prototypes (P1, P2, and P3) to determine animal performance responses. Twenty Holstein cows were randomly assigned to 2 trials (n = 10 each) in a replicated Latin square design with 14 d periods. Both trials were analyzed using a linear mixed effect model, however, the Lys trial was analyzed using a dose response technique. The base diet was predicted to be deficient in metabolizable Met (−14.8 g/d) and Lys (−16.1 g/d). In the Met trial, the base diet was supplemented with RP-Lys to meet the lysine requirement. The treatments included no added RP-Met (NC), Smartamine (SM), and P1, P2, or P3 at 148% of the Met content of SM. In the Lys trial, the base diet was supplemented with RP-Met to meet the methionine requirement. Treatments included no added RP-Lys (NC), AjiProL (AL), or the RP-Lys prototype at 55%, 78%, or 102% of the Lys in AL. Performance results are listed in Table 1. Milk protein percent significantly increased when diets were supplemented with P2 or P3 compared with NC, but none were different from SM. Overall, P2 had the greatest numerical production response among the 3 Met prototypes suggesting it had the greatest efficacy when supplemented into these rations. There was a significant linear increase for milk protein percent for the RP-Lys prototype compared with AL when fed at a range of 55–102% of lysine content, indicating that it could support comparable performance.

**Key Words:** methionine, lysine, performance

### Table 1 (abstract 449). Performance results when supplementing RP-Met or RP-Lys Prototypes

<table>
<thead>
<tr>
<th></th>
<th>DMI, kg/d</th>
<th>Milk, kg/d</th>
<th>Milk fat, %</th>
<th>Milk protein, %</th>
<th>Milk protein, kg/d</th>
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<td><strong>Met Trial</strong></td>
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<tr>
<td>SM</td>
<td>28.1</td>
<td>45.8</td>
<td>3.50</td>
<td>3.11b</td>
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<tr>
<td>NC</td>
<td>28.3</td>
<td>45.2</td>
<td>3.67</td>
<td>3.02a</td>
<td>1.35</td>
</tr>
<tr>
<td>NC+P1</td>
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<td>45.1</td>
<td>3.77</td>
<td>3.07b</td>
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</tr>
<tr>
<td>NC+P2</td>
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<td>45.5</td>
<td>3.64</td>
<td>3.12b</td>
<td>1.42</td>
</tr>
<tr>
<td>NC+P3</td>
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<td>45.3</td>
<td>3.51</td>
<td>3.12b</td>
<td>1.41</td>
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<td>AL</td>
<td>29.2</td>
<td>50.4</td>
<td>3.38</td>
<td>3.05</td>
<td>1.53</td>
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<td>3.52</td>
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<td>78%</td>
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<td>50.2</td>
<td>3.41</td>
<td>3.09</td>
<td>1.55</td>
</tr>
<tr>
<td>102%</td>
<td>28.8</td>
<td>50.2</td>
<td>3.37</td>
<td>3.11</td>
<td>1.54</td>
</tr>
</tbody>
</table>

*Values with differing superscripts differ (P < 0.05).

1Significant linear effect of Lys dose on milk protein %.

450 Effects of abomasal infusions of amino acids or glucose on energy and protein metabolism during an induced negative energy balance. I. Ansia*1, Y. Ohta2, T. Fujieda2, and J. K. Drackley1, 1University of Illinois, Urbana, IL, 2Ajinomoto Co. Inc., Tokyo, Japan.

The aim of the study was to assess the effects of 5 supplements during a short-term period of negative energy balance (NEB) induced by feed restriction (FR). Seven multiparous Holstein cows (93 ± 15 DIM) were randomly assigned to 7 treatments in a 7 × 4 incomplete Latin square design with 5-d periods. Daily DMI was restricted to provide 60% of net energy requirements except in one treatment that was fed for ad libitum (AL) DMI. Treatments were 4-h abomasal infusions (0.4 mol/kg BW) initiated at feeding time (0900 h) of: glucose (GLC), monosodium glutamate (MSG), lysine (LYS), glutamine (GLN), valine (VAL), and water (CON and AL) as control. Effects of infusions were compared using the MIXED procedure of SAS. Milk yield was lower (P = 0.05) than AL for all except MSG and GLN, with MSG the only treatment with no decrease (MSG × d; P = 0.39). Milk protein yield during GLN only tended (P = 0.07) to differ from AL. Lactose yield was not lower than AL for VAL, MSG, and GLN. Concentrations of NEFA did not differ from AL for MSG and GLN. Treatments MSG and VAL had no linear increase of BHB across periods. Plasma glucose tended to decrease (P = 0.10) during GLN, but increased continuously after d 2 with MSG. The LYS treatment increased plasma concentrations of Lys (LYS vs., CON; P < 0.01), Arg (LYS × h; P < 0.01), α-AAA (LYS × h; P < 0.01) and 3-methylhistidine (LYS vs. CON; P < 0.01), suggesting that both catabolic and anabolic processes were induced. Treatment VAL increased concentrations of Val (VAL vs CON; P < 0.01) and resulted in the lowest plasma concentrations of urea N and AA involved in the urea cycle (Arg, Orn, Cit). The MSG treatment increased concentration of Glu (MSG × d; P < 0.06) across periods. Moreover, MSG was the only treatment that did not decrease Orn, Asp, and Trp, induced the biggest increase of Arg (18%), and increased (MSG x d; P = 0.01) linearly (P < 0.01) serum albumin concentration during FR. Enhancing metabolic
451 Branched-chain amino acids direct other essential amino acids to extra-mammary tissues in lactating dairy cows. R. V. Curtis⁴, J. J. M. Kim⁴, L. E. Wright⁴, J. Doelman², and J. P. Cant⁴, ¹University of Guelph, Guelph, ON, Canada, ²Nutreco Nederland BV, Boxmeer, the Netherlands.

Infusing glucose into cows increases milk protein yield in some experiments but not others. Invariably, glucose infusion decreases plasma concentrations of the branched-chain amino acids (BCAA) Val, Ile, and Leu. The objective of this study was to evaluate the response to replenishment of BCAA during postpartum glucose infusion. Twelve cows (80 ± 22 DIM) were assigned, in a replicated 4 x 4 Latin square design, to 96-h continuous jugular infusions of saline, 1 kg/d glucose, or 1 kg/d glucose + 75 g/d or 150 g/d BCAA. All cows were given ad libitum access to a TMR of 12.6% crude protein and 1.52 Mcal/kg NE₃ on a dry basis. Infusion of glucose alone did not affect DMI, milk yield, or protein yield (P > 0.19), but increased lactose yield 98 g/d (P < 0.01). Addition of BCAA to glucose infusions caused a decrease in milk protein yield (P < 0.01), and tended to decrease DMI, milk yield, and lactose yield (P < 0.05). Concentrations of non-branched-chain essential amino acids (non-BEAA) in plasma decreased 19% (P < 0.01) during glucose infusion and BCAA concentrations decreased 30% (P < 0.01). Mammary blood flow was 30% higher (P < 0.01) during glucose infusion and net mammary uptakes of essential AA remained unchanged compared with saline (P > 0.24). Concentrations of BCAA in plasma returned to 6% higher (P < 0.34) than levels on the saline control at the BCAA infusion rate of 75 g/d, and were 49% higher than control levels (P < 0.01) at 150 g/d of infusion. Addition of BCAA to glucose infusions caused a linear decrease in non-BEAA concentrations in plasma (P < 0.01), as well as their mammary uptakes (P < 0.04). Plasma urea concentration was unaffected by BCAA infusion (P = 0.88), indicating that catabolism of non-BEAA was not stimulated. Evidence that neither mammary utilization nor whole-body catabolism of non-BEAA accounted for their disappearance from plasma leads us to conclude that BCAA caused a partitioning of non-BEAA to extra-mammary tissues for protein deposition. It was estimated that 60 g/d BCAA was sufficient to counteract the decrease in plasma BCAA concentrations induced by 1 kg/d i.v. glucose.

Key Words: glucose infusion, amino acid infusion, mammary uptakes

452 Impact of choline on the inflammatory response of innate and adaptive immune cells. M. Garcia¹, J. Shaffer², L. Mamadova¹, B. Barton², and B. J. Bradford¹, ¹Kansas State University, Manhattan, KS, ²Balchem Corporation, New Hampton, NY.

Research supports the beneficial effect of choline on metabolic health and productive performance of transition dairy cows. However, research evaluating the impact of choline on immunity and disease incidence is limited. The objective was to assess the impact of choline on the inflammatory response of stimulated and non-stimulated immune cells (neutrophils [PMN] and mononuclear cells [PBMC]) from 16 Holstein cows during the transition period (7.9 ± 1.7 DIM, n = 8) and mid-lactation (123.6 ± 3.7 DIM, n = 8). Blood immune cells were isolated using density gradient media and were incubated at 37°C and 5% CO₂. First, cells were incubated for 2 (PMN) or 24 h (PBMC) with 1 of 3 supplemental levels of choline (0, 5, or 10 µM). Then PMN were primed or not with LPS (1 µg/mL) for 30 min, followed by a 50-min labeled E. coli phagocytic and oxidative burst assay (indicated negative controls); PBMC were challenged or not with concanavalin-A (10 µg/mL) for 48 h, followed by a 24-h proliferation assay. Data were transformed to attain normality and analyzed as a randomized block design. Phagocytosis tended to be attenuated by choline if PMN were primed with LPS (79.4 vs. 76.4 ± 4.3% of cells, P = 0.06). Regardless of LPS priming, oxidative burst was attenuated by choline supplementation (61.4 vs. 58.8 ± 7.9%, P = 0.05). The proliferation of PBMC from cows in mid-lactation, but not that of transition cows, was attenuated (P < 0.01) with choline supplementation. These findings suggest that choline can conditionally regulate the inflammatory response of immune cells. Evaluating the expression pattern of genes involved in choline metabolism and inflammation may uncover potential mechanisms of choline action on immune cells.

Key Words: choline, immune cells, transition cow

453 Supplementation of rumen-protected choline (RPC) to periparturient dairy cows improved cow and calf performance. M. G. Zenobi¹, R. Gardinal¹, B. A. Barton², J. E. P. Santos¹, and C. R. Staples¹, ¹University of Florida, Gainesville, FL, ²Balchem Corp., New Hampton, NY.

Choline is a vitamin-like nutrient and a methyl donor involved in many physiological processes. Objective was to evaluate the effect of RPC supplementation (0 or 60 g/d of ReaShure, Balchem Corp., New Hampton, NY) during the periparturient period to multiparous Holstein cows consuming prepartum energy in either maintenance or excess amounts on performance. The RPC was top-dressed on TMR daily from −21 to 21 d postpartum (PP). Cows were fed prepartum high energy (1.63 Mcal NEL/kg DM; 58% corn silage) or controlled energy (1.40 Mcal NEL/kg DM; 37.5% wheat straw) diets in ad libitum amounts with or without RPC (n = 21–25 per diet). After calving, cows were fed a common diet (1.68 Mcal NEL/kg DM; 37.5% wheat straw) and assigned to one of 3 supplemental levels of choline (0, 5, or 10 µM). Incidence of subclinical hypocalcemia (<8.5 mg of Ca/dL) from 2 to 12 mo of age. Pregnancy at 40 wk PP (69.8 vs. 62.5%). Supplementing RPC for 6 wk first timed AI tended to be improved (41.3 vs. 23.6%; P < 0.10) to produce more milk during the first 15 wk PP (43.5 vs. 41.3 kg/d) and throughout the first 40 wk (37.1 vs. 35.0 kg/d). Cows consuming RPC were in a more negative energy balance at 2 and 3 wk PP without greater mean concentration of plasma NEFA or BHBA (0, 1, 2, 3, and 5 wk PP) or of liver triacylglycerol (1, 2, and 3 wk PP). Incidence of subclinical hypocalcemia (<8.5 mg of Ca/dL plasma) was reduced from 52.1 to 31.6% during the first 7 d PP by RPC supplementation. Rectal temperature, taken at 4, 7, and 12 d PP, increased linearly by 0.18°C for control cows but decreased linearly by 0.29°C for cows fed RPC. Yield of colostrum was not changed (9.9 vs. 8.5 kg) but concentration of IgG was improved by RPC (78.9 vs. 58.3 g/L for RPC and controls, respectively). Heifers born from dams fed RPC had better ADG (0.89 vs. 0.85 kg/d) from 2 to 12 mo of age. Pregnancy at first timed AI tended to be improved (41.3 vs. 23.6%; P < 0.10) but did not differ by 40 wk PP (69.8 vs. 62.5%). Supplementing RPC for 6 wk during transition had long-term benefits to Holstein cows and calves.

Key Words: choline, transition
Objective was to evaluate the effect of RPC supplementation (0 or 60 g/d of ReaShure, Balchem Corp., New Hampton, NY) and excess or maintenance intake of energy on indicators of immune function of early lactating multiparous Holstein cows. Cows (n = 93) were fed prepartum high energy (HE; 1.63 Mcal NEL/kg DM; 58% corn silage) or controlled energy (CE; 1.40 Mcal NEL/kg DM; 37.5% wheat straw) diets in ad libitum amounts with or without RPC. The RPC was top-dressed on TMR daily from −21 d to 21 d postpartum (PP). Whole blood was stimulated with LPS in vitro at 3 and 14 d PP. Relative transcript abundance (RTA) of inducible nitrous oxide synthase (iNOS) and select cytokines from WBC was measured by RT PCR using 3 housekeeping genes after 6 h of exposure with or without LPS. The neo-antigen hen-egg lysozyme (HEL) was injected s.c. at −4, −2, and 0 wk PP. HEL-specific IgG in plasma was measured at −4, −2, 0, 1, 2, 3, 4, and 7 wk PP. Acute phase proteins were measured in plasma at 0, 1, 2, 3, and 5 wk PP. Data were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS. Significance was declared at \( P < 0.05 \).

The RTA of cytokines and iNOS were increased by LPS at both days PP. The RTA of the proinflammatory cytokine, IL-6 (−LPS), tended to be less in WBC of RPC-fed cows (1.56 vs. 0.88). In contrast, the RTA of IL-8, IL-1β, and iNOS from −LPS cells at least 50% greater at both days PP for cows fed RPC. The RTA of IL-8 and IL-10 after LPS stimulation of WBC from RPC-fed cows decreased from 3 to 14 d PP whereas that of cows not fed RPC remained constant (RPC by day PP interaction). Feeding HE prepartum resulted in greater RTA of IL-8 and TNFα across days PP. Mean plasma concentration of haptoglobin was unchanged but fibrinogen was lower at wk 1 PP of cows fed RPC. Responses of plasma IgG to lysozyme injections were not affected by treatments. Collectively, feeding CE diets prepartum or supplementing RPC to periparturient cows produced changes in WBC cytokine production during the first 3 wk of lactation that are potentially important for regulating innate and adaptive immune responses.

Key Words: choline, immunity

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**Table 1 (abstract 455).** Production performance and plasma concentrations of EAA

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>( P ) (Contrast)</th>
<th>FPSBM vs. FPCM</th>
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<tr>
<td></td>
<td>FPSB</td>
<td>FPSBM</td>
<td>FPCM</td>
<td>FPCM</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>28.1</td>
<td>27.8</td>
<td>28.6</td>
<td>29.1</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
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<td>40.4</td>
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</tr>
<tr>
<td>Milk fat, %</td>
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<td>3.52</td>
<td>3.53</td>
<td>3.55</td>
</tr>
<tr>
<td>Milk protein, %</td>
<td>3.32</td>
<td>3.44</td>
<td>3.32</td>
<td>3.38</td>
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<tr>
<td>Milk protein, kg/d</td>
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<td>1.31</td>
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<tr>
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<td>Plasma Lys, μM</td>
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<tr>
<td>Plasma Met, μM</td>
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<td>Plasma His, μM</td>
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J. Dairy Sci. Vol. 100, Suppl. 2
The objective of this experiment was to investigate the effect of trace mineral (TM) source on Cu, Mn, and Zn rumen solubility and in vitro release from the insoluble portion of rumen digesta following a bolus dose of trace minerals in cattle. Eight cross-bred steers (BW 718.9 ± 64.9 kg), fitted with ruminal cannulas, received TM treatments (n = 4/treatment) as a bolus-dose directly into the rumen. Treatments consisted of (1) sulfate TM (STM): 20 mg Cu/kg DM from CuSO₄; 40 mg Mn/kg DM from MnSO₄; 60 mg Zn/kg DM from ZnSO₄, and (2) Hydroxychloride TM (HTM): 20 mg Cu/kg DM from tribasic CuCl; 40 mg Mn/kg DM from Mn hydroxychloride; 60 mg Zn/kg DM from Zn hydroxychloride. Rumen samples were obtained at 2 h intervals starting at −4, and ending at 24 h post dosing. Once collected, samples were centrifuged and supernatant and pellet fractions were analyzed for TM. For dialysis, pellets from the 0, 6, and 12 h sampling times were dialyzed against one of the following chelating buffers: 1) 0.01M ethylenediaminetetraacetate in 0.05 M Tris (Tris-EDTA; strong chelator) or 2) 0.01M L-histidine hydrochloride in 0.05M Tris (weak chelator). Data were analyzed as a randomized block design with repeated measures. A treatment x time interaction (P < 0.05) existed for rumen soluble Cu and Zn concentrations. Rumen soluble Cu concentrations were lesser (P < 0.05) from 2 to 12 h post dosing (mean difference = 0.18 vs. 0.44 ± 0.06 mg Cu/L) and rumen soluble Zn concentrations were lesser (P < 0.04) from 2 to 10 h post dosing (mean difference = 0.75 vs. 1.29 ± 0.13 mg Zn/L) for steers receiving HTM compared with steers receiving STM. Dialysis against Tris-EDTA resulted in a greater (P < 0.05) percent release of Cu (78.2 ± 20.3 %) and Zn (89.3 ± 41.2 %) from samples obtained 12 h post dosing and a greater (P < 0.04) percent release of Zn from samples obtained at 6 (89.3 vs. 41.2 %) and 12 (77.8 vs. 31.3 ± 18.3 %) h post dosing in HTM compared with STM steers. These data indicate that rumen solubility of Cu and Zn is reduced for HTM compared with STM sources and that Cu and Zn are less tightly bound in the insoluble rumen fraction of steers fed HTM compared with STM sources.

Key Words: trace mineral, solubility, rumen

Concerns have been raised in the past about the Dairy Herd Improvement Association recording frequency, because the interval between samples, about 4 weeks, may not capture the peak production for cows with shorter lactation (less than 10 mo), which have led to the conclusion that these cows have an atypical lactation curve shape. This may be due to sampling frequency rather than biological differences of cows or the influence of biotics and abiotics variables. Geometric morphometrics is a methodology that is used to measure biological shapes and curves, which has already proved its application in biology, medicine, and engineering. This methodology can be used for quantifying, testing, and visualizing shape variation and its covariation with biotic and abiotic variables. We propose to apply this method to determine the variation and covariation of lactation curves for milk, fat, and protein production. Daily records of milk production and fat and protein concentration collected by the Afimilk recording system (Afimilk, Kibbutz Afikim, Israel) from January 2014 to January 2017 from 47 large kibbutz (communal) herds distributed throughout Israel, will be analyzed. Lactation data will be plotted into an orbital graph to depict a closed curve. Currently methods to predict future lactation prediction of individual cows are based chiefly on production on the last available test day. Using geometric morphometrics it should be possible to derive more accurate estimates of future production, which can be used both to improve management decisions and genetic evaluations.

Key Words: geometric morphometrics, milk prediction, daily fat recording


Milk color and processing traits are important factors informing the potential and ease to manufacture milk into different dairy products. The objective of the present study was to estimate (co)variance components of milk lightness (L*), redness-greenness (a*), yellowness-blueness (b*), rennet coagulation time (RCT), curd-firming time (k20), curd firmness (a30 and a60), heat coagulation time (HCT), casein micelle size (CMS), and pH, measured by mid-infrared spectroscopy on 136,807 test-day records from 9,824 Irish dairy cows between 5 and 305 d-in-milk (DIM) from parities ≤ 10. Cow breed was defined as the proportion of Holstein, Friesian, Jersey, Norwegian Red, Montbéliarde, and “Other.” Random regression models using Legendre polynomials were performed to describe the change of both additive genetic and within-lactation permanent environmental variances across different DIM. Heritability estimates averaged across all DIM for milk color were 0.31 (L*), 0.11 (a*), and 0.42 (b*); average heritability estimates for processing traits ranged from 0.31 (pH) to 0.49 (k20), except for HCT (0.17). Within-trait genetic correlations approached unity between adjacent DIM, and were <0.40 at the peripheries of lactation. Eigenvalues and associated eigenfunctions of the additive genetic variance of all traits revealed that at least 80% of the total variation was associated with the height of the lactation profile. Average genetic correlations between color traits across all DIM were the weakest between a* and b* (−0.19); fat concentration was strongly genetically associated with b* (0.91), while milk yield was negatively genetically correlated with all color traits. On average, RCT was strongly genetically correlated with both a30 (0.68) and pH (0.75); genetic correlations between HCT and the other processing traits were, on average, weak ranging from −0.02 (with pH) to 0.28 (with RCT). Milk yield was genetically correlated with both RCT (0.31) and a30 (−0.49). Breeding for milk color and processing traits is possible but with some negative impact on genetic gain for milk yield.

Key Words: milk quality, breeding, infrared spectrometry


Increasing consumer concerns on dairy product nutritional quality have stressed the importance of some features of milk such as the fatty acid (FA) profile. In this study, genetic parameters of milk FA profile of dairy sheep were investigated to evaluate the feasibility of breeding for improving the nutritional quality of sheep milk. Individual milk samples of 989 Sarda ewes farmed in 47 flocks located in the 4 provinces of the Island of Sardinia, Italy, were analyzed for FA composition by gas- chromatography. Genetic parameters of 15 FA (expressed as g/100 g of FA methyl ester) were estimated with an animal model, including fixed effects of lambing type, lambing month, altitude of flock, lactation stage, province and parity, and the random effects of flock-test date (FTD), and animal additive genetic. A generally high contribution of FTD to the phenotypic variance was observed (on average 51%). Heritability (h2) estimates ranged from 0.03 for C18:3 n-3 to 0.48 for C16:0. Saturated and unsaturated C18 FA showed moderate to low values of h2 (from 0.22 to 0.03, for the same FA). On the other hand they exhibited a large contribution of FTD, ranging from 0.46 for C18:0 to 0.82 for C18:3 n-3, respectively. The high heritability estimate of C16:0 reflects the probable genetic control of its milk content, being partly synthesized de novo in mammary gland. Genetic correlations were negative among C4:0 and short and medium chain SFA. C16:0 showed a negative correlation with most of the investigated FA, and a positive correlation with C4:0, C14:0, C14:0c9 and C16:1c9. The high heritability of C16:0, considered harmful for human health, and its negative genetic correlations with unsaturated C18 FA could be used in genetic strategies to improve the nutritional properties of milk.

Key Words: fatty acids, genetic parameters, sheep milk

461 Genomic predictions for crossbreds from all-breed data. M. E. Tooker*, P. M. VanRaden, and G. C. Fok. Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD.

Genomic predictions of transmitting ability (GPTas) for crossbred animals were computed from marker effects of 5 dairy breeds weighted by each breed’s genomic contribution to the crossbreds. Estimates of genomic breed composition are labeled breed base representation (BBR)
and are reported since May 2016 for all 1.6 million genotyped dairy animals. Animals with > 94% of any breed were rounded to 100%, and contributions of other breeds were set to 0%. All-breed scale GPTAs were first computed for each pure breed for traits milk, fat, protein, productive life, somatic cell score, daughter pregnancy rate, cow conception rate, livability, and net merit. These estimates included foreign information from multi-trait across-country evaluation (MACE) and foreign dams converted from within-breed to the all-breed base. Then, marker effects for each breed were blended by BBR to compute evaluations for crossbreds (≤94% purebred) for those same traits. Conformation traits do not have an all-breed scale, so only the Jersey marker effects were applied to the crossbreds, and results seemed reasonable. Calving traits are not predicted for crossbreds, and instead a common mean was used for all crossbreds as is the current practice for breeds other than Holstein and Brown Swiss. All-breed GPTAs were then converted to within-breed GPTAs. Correlations of GPTAs for purebreds computed on the all-breed vs. current within-breed scales were 0.97 to 0.99 for most traits and breeds. Crossbred GPTAs were then computed for 44,023 crossbreds, 20,367 of which had no previous GPTAs because of breed check edits. The new GPTAs were for 1,822 Jersey × Holstein crossbreds with >40% of both breeds (F1 crosses), 75 Brown Swiss × Holstein F1, 7,237 Holstein backcrosses with >67% and <94% Holstein, 7,820 Jersey backcrosses, 313 Brown Swiss backcrosses, 1,763 other crossbreeds of various mixtures, and 1,337 purebreds that had previously failed breed checks. Additional automation and redesign of many downstream programs is required for the new all-breed system to be used in weekly, monthly, and full releases. The new system is expected to provide accurate predictions for crosses among the 5 dairy breeds evaluated.

Key Words: crossbreeding, genomic prediction, breed composition

462 Genetic trends from single-step GBLUP and traditional BLUP for production traits in US Holstein. Y. Masuda*1, I. Misztal1, P. M. VanRaden2, and T. J. Lawlor3, 1University of Georgia, Athens, GA, 2USDA, AGIL, Beltsville MD, 3Holstein Association USA Inc., Brattleboro, VT.

The objective of this study was to compare genetic trends from a single-step genomic BLUP (ssGBLUP) and the traditional BLUP (tradBLUP) models for milk production traits in US Holstein. We used 764,029 genotyped animals in this study. Phenotypes were 305-d milk, fat, and protein yield from 21,527,040 cows recorded between January, 1990 and August, 2015. The pedigree file included 29,651,623 animals limited to 3 generations back from recorded or genotyped animals. We applied a 3-trait repeatability model with the same genetic parameters used in the US official genetic evaluation. Unknown parent groups were incorporated into the inverse of a relationship matrix \((H^{-1})\) in ssGBLUP and A\(^{-1}\) in tradBLUP with the QP-transformation. In ssGBLUP, 18,359 genotyped animals were randomly chosen as core animals to calculate the inverse of genomic relationship matrix with the APY algorithm. Computations with tradBLUP took 6.5 h and 1.4 GB of memory, and computations with ssGBLUP took 13 h and 115 GB of memory. Estimated breeding values were adjusted to a genetic base on recorded cows born in 2000 in each model and converted to GPTA in ssGBLUP and PTA in tradBLUP. For genotyped sires with at least 50 daughters with phenotype(s) born between 2000 and 2010, the genetic trend of GPTA was always greater than PTA in all traits. The difference in 2 genetic trends was almost constant for the sires born up to 2008 (on average, 11 kg in milk, 0.5 kg in fat, and 0.3 kg in protein yield) and the difference was greater in the last 2 years. The difference between the GPTA means for the bulls born in 2010 was 35 kg for milk, 2.2 kg for fat, and 1.2 kg for protein yield. For genotyped cows with phenotype(s), the GPTA trend was identical to or slightly greater than the PTA trend up to 2006. Two trends started to diverge obviously in 2007 and the GPTA trend kept rising while the PTA trend remained at the same level. The single-step method provides very similar genetic trends to the traditional evaluations except for the last few years. The recent lower PTA trend can be due to a downward bias caused with genomic pre-selection of young animals.

Key Words: genomic evaluation, genetic trend, PTA

463 A Genetic Diversity Index method to improve imputation accuracies of rare variants. A. M. Butty*1, F. Miglior1,2, P. Stothard1, F. S. Schenkel1, B. Gredler4, M. Sargolzaei3, and C. F. Baes1, 1Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, 2Canadian Dairy Network, Guelph, ON, Canada, 3Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, 4Qualitas AG, Zug, ZG, Switzerland, 5Semex Alliance, Guelph, ON, Canada.

Different methods to select animals for sequencing have been developed, which rely on pedigree-based relationship matrices, genomic relationships matrices, or on haplotype frequencies. Relationship-based methods select representative key animals of a population whereas haplotype frequency methods aim for better coverage of rare variants. Good average accuracies of imputation from SNP chip to whole-genome sequence (WGS) for common haplotypes were reached with the relationship-based methods. Imputation of rare variants, however, still needs to be improved, which can possibly be accomplished with a newly developed Genetic Diversity Index (GDI). This algorithm optimizes the count of unique haplotypes present in a group of animals composed of already sequenced individuals and a fixed number of sequencing candidates. Optimization is run iteratively, exchanging one candidate at a time and computing the GDI of the new group. Use of the simulated annealing algorithm defines whether the last individual added to the group should be kept. Simulated annealing has the advantage of searching for a global optimum in a situation where multiple local optima are present. The previously mentioned key ancestor and haplotype-based methods for selecting sequencing candidate were assessed and compared with the GDI algorithm using simulated cattle WGS data. Average squared correlation coefficients were used to assess imputation accuracy. A preliminary study showed that the accuracy was 1.5% higher when using GDI to enlarge the reference population than the second-best method. Application of the different methods of selection in North American Holstein data showed that the GDI algorithm selected animals carrying a higher percentage of rare haplotypes than other methods examined. Principal component analysis of the population showed that the animals selected with all tested methods were similarly distributed over the pool of candidates. When representative animals of a population are already sequenced and good overall imputation accuracies are reached, sequencing of genetically diverse animals improved the accuracy of the imputation of rare variants to the WGS density level.

Key Words: sequencing, simulation, imputation

464 Determination of quantitative trait variants by concordance via application of the a posteriori granddaughter design to the US Holstein population. J. I. Weller*1,2, D. M. Bickhart2, G. R. Wiggins3, M. E. Tooker2, J. R. O’Connell4, J. Jiang5, and P. M. VanRaden2, 1Agricultural Research Organization, The Volcani Center, Rishon LeZion, Israel, 2Agricultural Research Service, Beltsville, MD, 3Council on Dairy Cattle Breeding, Bowie, MD, 4University of
Experimental designs that exploit family information can provide substantial predictive power in quantitative trait variant discovery projects. The a posteriori granddaughter design was applied to the US Holstein dairy cattle population. Twenty-nine trait-by-chromosomal segment effects were found with probabilities < 10^{-20} that a segregating quantitative variant was detected by chance. Polymorphism genotypes for 79 grandsires and 16,236 sires were determined by imputation for 3,148,506 polymorphisms across the entire genome; 444 Holstein bulls had complete genome sequence, including 38 of the grandsires. Concordance between quantitative trait locus genotype and polymorphism was determined for all 29 effects. Complete concordance was obtained only for daughter pregnancy rate on chromosome 18 and protein percentage on chromosome 20. For each quantitative trait locus, effects of the 20 polymorphisms with the highest concordance scores for the analyzed trait were computed by stepwise regression. The effects for stature on chromosome 7, daughter pregnancy rate on chromosome 18, and protein percentage on chromosome 20 met the following 3 criteria: complete or nearly complete concordance, significance of the polymorphism effect after correction for all other polymorphisms, and a marker coefficient of determination that was > 50% of the total multiple-regression coefficients of determination for the 20 polymorphisms with highest concordance. An intronic variant SNP on chromosome 5 at position 93,945,738 explained 7% of the variance for fat percentage and 85% of the total variance explained by the multiple-marker regression. Variants identified in this study are likely to provide improved predictive power for genomic evaluation of dairy cattle.

Key Words: genomic selection, granddaughter design, quantitative trait variant


Methods for SNP selection can improve prediction accuracy over genomic BLUP, but in practice, the improvement is trait and population specific. This study investigates the importance of SNP selection in populations with 2000 to 25,000 genotyped animals. Populations were simulated with effective population sizes (Ne) of 20 or 100, and assuming that 10.50, or 500 QTL were affecting a trait with heritability of 0.3. Pedigree information was available for 6 generations; phenotypes were recorded for the 4 middle generations. Animals from the last 3 generations were genotyped for 45,000 SNP. Single-step genomic BLUP (ssGBLUP) and weighted ssGBLUP (WssGBLUP) were used to estimate genome EBV (GEBV). For WssGBLUP, 2 iterations of weights were calculated and were used to derive SNP variances and to construct a weighted genomic relationship matrix (G). Improved prediction accuracies are expected in WssGBLUP because more weight is placed on important SNP. Prediction accuracies were calculated for 1000 genotyped animals in the last generation. Reference populations included 2000, 5000 and 25,000 genotyped animals. The latter genotyped set was used to assess the dimensionality of genomic information (number of effective SNP or effective chromosome segments - Ne). This was calculated as the number of the largest eigenvalues explaining 98% of the variation in the genomic relationship matrix with and without the weights. For the data sets with Ne = 20 and 10 QTL, the accuracy gain from WssGBLUP was 12, 9, and 4 points for 2000, 5000, and 25,000 genotyped animals, respectively. With Ne = 100, this gain was 8, 10, and 7 points, respectively. For both Ne of 20 and 100, the gain assuming 50 QTL was halved, and no gain was observed assuming 500 QTL. The number of effective SNP was about 4-fold less in weighted G (~1512) than in unweighted G (~5790), explaining the greater gain in accuracy with fewer genotyped animals. The impact of SNP selection decreases with increasing size of the reference population and number of QTL. In large populations, the detection of chromosome segments is more difficult, requiring more genotyped animals.

Key Words: accuracy, variable selection, weighted ssGBLUP


The objective was to determine the effect of using core animals from different generations in single-step genomic BLUP with the Algorithm for Proven and Young (APY). Effective population size and number of independent chromosome segments (ICS) are limited in livestock populations indicating limited dimensionality of genomic information. The APY takes advantage of this dimensionality and assumes that breeding values (BV) for noncore animals are functions of the BV for core animals. The core animals represent the same information as the ICS. Simulations comprised a moderately heritable trait for 95,010 animals and 50,000 genotypes for animals across 5 generations. Genotypes consisted of 25,500 SNP distributed across 15 chromosomes. Core animals were defined based on individual generations, equal representation across generations, and at random. For a sufficiently large core size, core definitions had the same accuracies (r² = 0.90 ± 0.01) and biases (β = 1.02 ± 0.01) for young animals, even if the core animals had imperfect genotypes because of imputation. Using the youngest generations as core caused an increase in the number of rounds to convergence indicating some numerical instability with these core definitions. When 80% of genotyped animals had unknown parents, accuracy and bias were significantly better (P ≤ 0.05) for random and across-generation core definitions (r² = 0.71 ± 0.01; β = 0.75 ± 0.01) than for single generation core definitions (r² = 0.61 ± 0.01; β = 0.53 ± 0.01). This difference could result from improved relationship estimates between animals in different generations, because all generations were represented in the core partition that was directly inverted in APY. Thus, any subset of genotyped animals can be used to approximate the ICS when pedigrees are complete, but core animals should represent all generations when pedigrees are incomplete.

Key Words: APY, genomic selection, single-step genomic BLUP


The purpose of this study was determining, by simulation, whether (single-step) GBLUP is useful for genomic analyses when causative Quantitative Trait Nucleotides (QTNs) are known. Simulations included 180k animals in 11 generations. Simulated population mimicked a cattle population with weak selection intensity (Ne ~200). Phenotypes were available for animals in generations 6–10. Genotypes were available for 24k parents and 5k young animals in generation 11, and included 60k regular SNPs in 10 chromosomes, with genetic variance fully accounted for by 100 or 1,000 biallelic QTN, with effects sampled from a gamma distribution with shape parameter equal 0.4. LD (r²) between SNPs

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Impact of pedigree truncation on accuracy and convergence of ssGBLUP in a population with long pedigree when only a fraction of animals are phenotyped. I. Pocrnic*1, D. A. L. Lourenco1, H. L. Bradford1, C. Y. Chen2, and I. Misztal1, 1Department of Animal and Dairy Science, University of Georgia, Athens, GA, 2Genus PIC, Hendersonville, TN.

In a genomic evaluation, it is desirable to have low computing cost while retaining high accuracy of evaluation for young animals. When the population is large but only few animals have phenotypes, especially for low heritability traits, the convergence rate of BLUP or single-step genomic BLUP (ssGBLUP) can be very slow. While eliminating old pedigrees can seriously affect (G)EBV for old animals, usually only younger animals are candidates for selection. This study investigates the effect of pedigree truncation on convergence rate and accuracy of prediction for young animals. The data consisted of 216k, 221k, 722k, and 579k phenotypes on 4 traits (T1, T2, T3, T4) from a purebred pig line. Heritabilities were <0.1 for T1 and T2, and >0.2 for T3 to T4. A total of 2.4 million animals born from 1971 to 2016 were included in the complete pedigree. Genotypes were available for 33,502 animals and consisted of 60,003 SNP. A bivariate animal model was fit for T1–2, and T3–4, separately. Computations were done by BLUP or ssGBLUP, and were conducted with complete pedigree or different levels of pedigree depth (Pn), where n = 1, 2, 3, 4, 5. Pedigree depth n was defined as n ancestral generations from the animals with phenotypes. The number of pedigree animals for T1–2 (T3–4) varied from 226k (760k) for P1 to 228k (767k) for P5. Genomic relationship matrix was inverted either by the APY inverse or higher order Taylor expansion using the APY inverse. The rank of weighted GRM is between the rank of unweighted GRM and that computed with causative SNP only. Single-step GBLUP can account for causative SNP when variances of causative QTN are known.

Key Words: genomic relationship matrix, genomic prediction, causative variant


Single-step genomic best linear unbiased Predictor (ssGBLUP) has become increasingly popular for whole-genome prediction (WGP) modeling as it utilizes any available pedigree and phenotypes on both genotyped and non-genotyped individuals. The WGP accuracy of ssGBLUP has been demonstrated to be greater than or equivalent to popular Bayesian regression models. However, these assessments have not typically included phenotypes of non-genotyped individuals in the Bayesian regression analyses, making the interpretation of these comparisons difficult. Increasingly, ssGBLUP has been used for genome-wide association (GWA) studies, although there is no clear guidance on how to determine statistical significance in these analyses. We address this issue and additionally propose a GWA based on a Bayesian single-step stochastic search and variable selection (ssSSVS) model that allows for phenotypes on non-genotyped animals. Our study was based on a dairy consortium data set including 3,186 Holstein cows from 6 US research stations based on the 60671 USDA-ARS bovine SNP panel. In a replicated simulation study using these same genotypes, a different number of causal variants (n_c = 30, 300, or 3,000) were randomly assigned to the markers, masking 20% of cows as non-genotyped, for a trait having a heritability of 0.25. We determined that ssSSVS had greater (P < 0.05) WGP accuracy than ssGBLUP with n_c = 30 or n_c = 300. Moreover, ssSSVS always performed better (P < 0.05) than ssGBLUP for GWA measured as partial area under a receiver-operating characteristic (ROC) curve (pAUC) up to a false positive rate of 5%. In a 10-fold within-station cross-validation study using phenotypes from the dairy consortium, we determined that ssSSVS had greater (P < 0.05) WGP accuracies in milk fat compared with ssGBLUP for genotyped individuals, although no such differences were detected for body weight. No differences between ssSSVS and ssGBLUP for prediction accuracies for non-genotyped individuals were determined for either trait. Overall, ssSSVS is a promising method for both WGP and GWA, particularly for genetic architectures characterized by a few genes with large effects.

Key Words: Bayesian variable selection, genome wide association, whole genome prediction

470 SSGP: SNP-set based genomic prediction to incorporate biological information. J. Jiang*1, J. O’Connell2, P. VanRaden3, and L. Ma4, 1Department of Animal and Avian Sciences, University of Maryland, College Park, MD, 2University of Maryland School of Medicine, Baltimore, MD, 3Animal Genomics and Improvement Laboratory, ARS-USDA, Beltsville, MD.

Genomic prediction has emerged as an effective approach in plant and animal breeding and in precision medicine. Including biological information into the genomic model can be of great advantage. Due to the statistical and computational challenges in large genomics studies,
however, a fast and flexible method to incorporate such external information is still lacking. Here, we proposed a linear mixed model that can incorporate biological information in a flexible way and developed a fast variational Bayes-based software package named SSGP. In our model, whole genome markers can be split into groups in a user-defined manner, and each group of markers is given a common effect variance. Since previous functional genomics studies have accumulated much evidence on which genes, genomic regions or pathways are more/less important for a trait of interest, we can divide genome-wide SNPs into several groups based on their levels of importance and then use the predefined SNP sets in SSGP. Additionally, each marker has a pre-specified weight for which the rule can be flexibly assigned, e.g., based on minor allele frequency or LD pattern. The model was implemented with the parameter expanded variational Bayesian method. For testing purpose, we analyzed a large cattle data set consisting of ~24k bulls (20k in training set and 4k in validation set) and ~760k whole-genome SNP markers. By simply grouping markers based on proximity (markers were divided into continuous, non-overlapping chunks, each containing 1k SNPs) and considering only additive effects, SSGP already performed better than Bayes A in all 5 milk traits analyzed, with an increase of up to 8 percent points in prediction accuracy. Meantime, it took only ~5h for each trait with 20 threads. We also analyzed many simulation data sets and the WTCCC heterogeneous stock mice data set for which the results of many existing methods had been reported. Generally, SSGP could achieve similar prediction performance compared with the best approaches reported, though only proximity was used for grouping SNPs. Collectively, the method and software show great potential to increase accuracy in genomic prediction, particularly in the future when more useful biological information is becoming available.

Key Words: genomic prediction, SNP set, biological information
Animal Behavior and Well-Being Symposium:
Allowing for Natural Behavior in Dairy Cattle


Animal welfare is a multi-dimensional and value-laden concept, focusing on 3 broad questions: (1) is the animal functioning well, (2) is the animal feeling well, and (3) is the animal able to live a reasonably natural life? However, even experts disagree about which attributes to consider and how these should be weighted in welfare assessments. The natural living aspect of animal welfare is least clear academically, and is likely the animal welfare issue that causes the farm animal industries the most difficulty. The “unnaturalness” of modern housing conditions is, however, one of the greatest sources of public concern, and is one area where common practices on dairy farms are not in step with public values. For instance, in the United States, less than 5% of the US lactating dairy herd is able to graze routinely, in direct contrast to what the public want and expect for dairy cows. Another interesting example concerns the early separation of the cow and calf. In this case the majority of the public see early separation as unnatural and thus a threat to the welfare of both cow and calf, whereas most working within the industry believe that later separation creates greater problems. The public is also concerned about restriction of movement, suggesting that highly restrictive housing practices such as tie-stalls and calf hutches will be considered important threats to welfare. In this presentation, we describe examples from our most recent work focusing on the natural living aspect of welfare, how these relate to public concerns, and how resolving these concerns about natural living may address and aggravate other welfare issues. We conclude that failure to address deeply held public values around naturalness may threaten the long-term sustainability of the dairy industry.

Key Words: tie stall, public, grazing

472 Housing and management that promotes natural behavior in dairy calves. J. F. Johnsen*, Norwegian Veterinary Institute, Department of Health Surveillance, Oslo, Norway.

In feral cattle, the cow isolates at birth and hides the calf during the first days. The cow seeks the calf for nursing 5–10 times/day. After some days the cow-calf pair joins the herd. Once calf age approaches 2 weeks, the calf will spend more time with its peers while seeking the cow for nursing and friendly interactions. The cow weans the calf around 7–10 mo of age during which the cow keeps close contact to the calf but gradually denies the calf to nurse. The concept of Animal Welfare, in which natural behavior is an important element, has been updated. Rather than emphasizing on survival, animals should now have “lives worth living” and a balance between negative and positive subjective experiences is emphasized. In parallel, many new solutions have been developed for modern dairy farms enabling calves to perform important elements of natural behavior which will be reviewed in this presentation. New knowledge on calf housing in terms of the importance of social group complexity now exists; from isolation in single pens through pair housing, housing in social groups and housing with the dam. Research on pair and group housing of dairy calves has revealed many important assets both for the calf and the farmer. Also, feeding management that closer resembles that of natural feeding of the calf has many benefits. New solutions also exist for housing the calf together with its dam. Flexibility in the cow-calf bond and weaning and separation methods resembling that of natural weaning allows for many alternatives for more contact between the cow and the calf.

Key Words: cow-calf suckling, milk feeding, social housing

473 Designing the maternity pen to allow for maternal behavior in dairy cattle. K. L. Proudfoot*, P. D. Krawczel, and M. A. G. von Keyserlingk1, 1The University of British Columbia, Vancouver, BC, Canada, 2The Ohio State University, Columbus, OH, 3The University of Tennessee, Knoxville, TN.

In ungulates, the survival of offspring depends on the quality of the mother-young bond. During parturition, many wild ungulate species separate from the herd and find a naturally covered area to avoid predators and help facilitate the bond between dam and calf. Domesticated ungulates, such as Holstein dairy cows, are often provided protection from predation indoors, but indoor facilities provide little opportunity for cows to separate from the herd during parturition. Dairy cows are usually kept in group pens, or are moved from the group into an individual “maternity” pen to give birth. These pens are often in high-traffic areas and are dynamic in social structure – cows enter the pen before calving and are moved out after calving. Due to the high variation in management strategies at calving, there has been increased interest in understanding the cow’s perspective of her environment at calving, including her innate maternal behaviors. The objective of this presentation is to review the current literature on the maternal behavior of Holstein dairy cows kept indoors and outdoors at calving, with a main focus on maternal isolation behavior. Researchers have determined that cattle kept on range segregated from the herd and calved in covered areas including tall grass and trees. When indoor-housed cows were provided a choice between calving on pasture or a covered barn, their decision was dependent on parity; heifers calving for the first time were more likely to seek natural outdoor areas for seclusion whereas mature cows were more likely to calve indoors. When housed completely indoors, individually-housed cows maintained the motivation to calve in secluded areas if available. However, the motivation to hide in an indoor setting was hindered by social competition over the secluded space. Although data are limited, findings from this early research is promising; despite generations of domestication, dairy cows appear to have strong maternal instincts including the desire to separate from herdmates and seek seclusion at calving. This type of research is an example of how an understanding of natural behaviors can be useful in drawing inferences about the welfare of domesticated species.

Key Words: natural behavior; animal welfare

474 What to build next: Alternatives to freestall housing that promote natural behavior. J. M. Bewley*, University of Kentucky, Lexington, KY.

Freestall or cubicle housing systems are the primary housing system for lactating dairy cattle in many parts of the world. Early freestall barns lacked in features that maximized cow comfort. Today, modern freestall barns are generally cow-centered with a large focus on cow comfort. Among freestall barns, the sand freestall barn is often promoted as the ideal freestall system. However, after decades of housing cows in freestalls, limitations remain. Lameness levels remain high in many
freestall barns. Manure handling is challenging. Further, as the science of animal behavior grows and consumers are placing more pressure on dairy farms, new questions arise around natural behavior within freestalls. Should freestall barns be the housing of the future? Are there other housing options that might promote more natural behavior and remove some of the limitations of freestall barns? What alternative systems hold promise? The term “freewalk housing” has been used to describe housing systems that provide animals the opportunity to walk more freely within the barn, often in combination with grazing access. Compost bedded pack barns and cow gardens are 2 examples of freewalk housing. A compost bedded pack barn is a lactating dairy cow housing system consisting of a large, open resting area, usually bedded with sawdust or dry, fine wood shavings. Bedding material is composted in place, along with manure, when mechanically stirred on a regular basis. Producers report reduced incidence of lameness and improved hoof health resulting from greater lying times and a softer, drier surface for standing. The cow garden system has a multi-layer, semi-permeable floor. Manure is removed by robotic scrapers. And trees and plants are planted within the barn. Questions remain about the suitability of these facilities. In evaluation of alternative systems, a systems approach must be applied including animal behavior, economics, and environmental sustainability.

Key Words: housing, behavior
**Dairy Foods Symposium: Biology LAB Symposium: Recent Developments in Lactic Acid Bacteria**

**475 Advances in nonstarter microbiology related to gassy defect in cheese.** C. Oberg*, Webers State University, Ogden, UT.

Gas formation or gassy defect in aging cheese continues to be a concern in the dairy industry. This defect is manifest by slits or cracks in the cheese body and/or “blown wrappers” in cheeses aged longer than 3 mo, and tends to be sporadic and recurrent. These slits, cracks, and voids are not usually evident until cheese is graded unless cheese packaging becomes loose. Over the years, a long list of microbial culprits has been assembled, but with limited evidence concerning their continuing role in causing gassy defect. In Cheddar cheese, nonstarter lactic acid bacteria (NSLAB) populate the cheese in the latter stages of ripening, and are sometimes associated with undesirable flavors and textures, including gas production. Because enumeration and identification of NSLAB has generally been restricted to species forming easily observed colonies within 2 d at 30 or 37°C on MRS, slow growing lactobacilli species have been overlooked in past attempts to identify the cause of gas and textural defects in aging cheese. A new *Lactobacillus* species, *Lactobacillus wasatchensis*, was recently isolated using prolonged low temperature incubation from a sample of gas-blown Cheddar cheese. Genomic analysis, metabolic characterization, cheese trials, and survey data strongly indicates this organism is a primary cause of late gas defect. Recent studies have also shown several other *Lactobacillus* species to produce gas in culture studies and experimental cheese batches, especially under specific conditions. The presence of *Lb. wasatchensis*, and other lactobacilli, in commercial Cheddar cheese could mean the possibility of cheese becoming blown or split during aging, resulting in downgraded cheese. Strategies for detecting and enumerating *Lb. wasatchensis*, and other gas-producing LAB, along with best practices to decrease late gassy defect in aging cheese will be discussed.

**Key Words:** gassy defect, cheese, non-starter lactic acid bacteria

**476 Lactococcal lantibiotics and bioengineering thereof.** P.D. Cotter*1,2, 1Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland, 2APC Microbiome Institute, Cork, Ireland.

The traditional approaches to finding “new” bacteriocins have relied on conventional microbiology-based techniques. This has yielded several bacteriocins, including class I bacteriocins (or lantibiotics) from *Lactococcus*. Of these, nisin has been most extensively characterized. However, in recent years the potential applications of these natural food preservatives has been even further expanded through the use of (bio)engineering. The latest developments with respect to lactococcal lantibiotics and bioengineering thereof will be described during the course of this presentation.

**Key Words:** *Lactococcus*, lantibiotic, bioengineering

**477 Less is more: Improving starter cultures to bring out the best in yogurt.** E. Johansen*, Chr. Hansen A/S, Horsholm, Denmark.

Consumers have a strong preference for foods with a minimum of artificial ingredients. This poses a challenge to the dairy industry because commercial yogurt varieties often contain ingredients which enhance the organoleptic properties of the yogurt. For example sugar or artificial sweeteners may be added to increase sweetness and thickening agents may be used to create the desired texture. In addition, some consumers avoid yogurt due to its fat content or the presence of lactose which can lead to discomfort in individuals with lactose intolerance. Research in our laboratories is directed toward addressing these perceived shortcomings by removing these additives/components through the use of starter cultures specifically developed to create the desired consumer experience. This concept that “Less is more” will be illustrated with several specific examples from our laboratories.

**Key Words:** yogurt, starter culture, clean label

**478 CRISPR-Cas: Research and application of natural systems in dairy starter cultures.** D. Romero*, DuPont Nutrition & Health, Madison, WI.

CRISPR (clustered regularly interspaced short palindromic repeats) loci are composed of short DNA repeats interspersed by short unique DNA sequences (spacers). CRISPRs are often allied with a set of proteins (Cas - CRISPR associated) that together form a microbial immune system. Since its first description in *Escherichia coli* in 1987, CRISPR-Cas has been found in most archaea and about half of bacteria. Such is the case with dairy starter bacteria *Streptococcus thermophilus*, where up to 4 unique CRISPR-Cas loci have been in a single strain and where the biological activity of the immunity was first described. As with earlier applications, the diversity and uniqueness of the CRISPR spacer content was extremely useful for strain typing. Upon closer investigation, it was observed that the spacer sequences were homologous to extrachromosomal elements; namely phage DNA. Subsequently, a direct correlation was established showing that a spacer with sequence identity to a given phage can direct Cas proteins to interfere with that phage’s ability to propagate within the host – phage resistance. The ensuing studies of the interference mechanism of action and enzymology gave rise to what is commonly known today as CRISPR-Cas9 genome editing; a powerful tool for targeted genomic modifications and the object of much attention in the scientific community. In its natural state, the native *S. thermophilus* CRISPR-Cas system has proven to be a highly effective defense against virulent phage; a persistent issue in industrial dairy fermentations. Exposing *S. thermophilus* to a virulent phage naturally induces incorporation of a new spacer into the CRISPR array, conferring resistance to the virulent phage. The adaptation process is such that the level of phage resistance increases by incorporation of additional unique spacer sequences to a given phage, expands the range of inhibition by spacer addition from unrelated phages, or both following an iterative process of phage challenge and strain selection. When performed under controlled conditions, secondary mutations are virtually non-existent, therefore preserving the physiological properties of highly functional unique strains.

**Key Words:** CRISPR, starter culture

**479 Mining and exploiting CRISPR-Cas systems in lactic acid bacteria.** A. Briner* and R. Barrangou, North Carolina State University.

Dairy starter cultures have long been engaged in an evolutionary arms-race against nucleic acid-based intruders like phages and plasmids. While dairy microorganisms have many forms of protection against foreign
invaders, their adaptive immune systems, or CRISPR-Cas systems, arguably hold the most potential for developing better starter cultures both through natural immune function and via exploitation of the native Cas machinery for biotechnological purposes. Since the discovery of CRISPR-Cas as a bacterial immune system in 2007, we have begun to understand that CRISPR-Cas systems preferentially occur in many dairy-related organisms, like *Streptococcus thermophilus* and *Lactobacillus casei*, and probiotics, like *Lactobacillus gasseri* and *Lactobacillus rhamnosus*, likely due to the overwhelming phage burden found in fermentation environments. After a decade of investigating the genetics, biology, and application of CRISPR-Cas machinery, these systems are now used to enhance phage resistance, increase resolution in strain typing, vaccinate strains against specific undesirable sequences, select rare variants through genetic screening, perform large-scale genetic remodeling, selectively kill microbes for next-generation antibiotics, and genetically engineer strains. In this talk, we will highlight the major historical milestones in CRISPR research, discuss the various applications in dairy bacteria, and show how this technology is revolutionizing the food industry.

**Key Words:** CRISPR, starter cultures, lactic acid bacteria
**Dairy Foods IV: Dairy Ingredients**

480  **Formation of surface composition on spray-dried milk powder.** M. Foerster¹, T. Gengenbach², M. W. Woo³, and C. Selomulya*⁴, ¹Monash University, Clayton, VIC, Australia, ²CSIRO, Clayton, VIC, Australia.

Milk is often spray-dried for better preservation, easier handling, and a reduced bulk volume for transportation. An unwanted dominance of fat typically occurs on particle surface, leading to lower solubility, oxidative stability and increase stickiness. Here we used 2 convective drying methods, single droplet drying and spray drying, to understand the mechanisms of component segregation in drying milk droplets. Modified single droplet drying allowed tracking of surface composition at discrete drying times, in addition to changes in temperature, diameter and mass. The fat was observed to accumulate at the surface during droplet generation (surface covered by about 97% wt/wt fat as measured by x-ray photoelectron spectroscopy, for a bulk fat content of 44% wt/wt). The hydrophobic fat shell resulted in less droplet shrinkage and greater resistance to water evaporation compared with a protein-rich shell. Comparison of surface compositions of spray-dried milk particles and their atomized droplets indicated that the atomization stage strongly influenced final surface composition. For instance, milk emulsions with a dry matter bulk composition of 32, 24 and 44% wt/wt in lactose, protein and fat, respectively, resulted in atomized droplets featuring a surface composition with approximately 5, 12 and 83% wt/wt in lactose, protein and fat, which was relatively unchanged after drying (0, 11 and 89% wt/wt in lactose, protein and fat). When the emulsions were modified by addition of λ-carrageenan before spray drying, rheological analysis revealed that the presence of a dispersed fat phase significantly reduced the extensional viscosity (from 26 to 12 mPa·s for a fat content from 0.3 to 31% wt/wt in dry matter), which increased by stabilization with λ-carrageenan (31 mPa·s for 0.3% wt/wt carrageenan). Milk emulsions were least stable against disintegration along the oil/water interface of fat globules under extensional stress and hence preferably broke up during atomization, leading to the observed fat coverage as soon as individual droplets were formed. A numerical model incorporating drying and shrinkage kinetics confirmed that the initial surface composition after droplet generation is not induced by diffusive component segregation, but by the droplet generation process itself.

**Key Words:** α-lactalbumin-enriched whey protein, flowability, wettability

481  **Influence of composition and microstructure on flowability and wettability of α-lactalbumin enriched whey protein ingredients.** G. Barone*¹, J. O'Regan², and J. O'Mahony¹, ¹School of Food and Nutritional Sciences, University College Cork, Cork, Cork, Ireland, ²Nestlé R&D Center, Wyeth Nutritional Ireland, Askeaton, Co. Limerick, Ireland.

α-Lactalbumin enriched ingredients are being used increasingly in the formulation of nutritional products (e.g., infant formulae). Technologies used to produce such ingredients include selective precipitation, membrane filtration and ion exchange chromatography. The aim of this study was to determine the effects of differences in macrochemical composition and microstructure of 3 commercially available α-lactalbumin-enriched whey protein ingredients (LAC 1, LAC 2 and LAC 3) on powder flowability and rehydration properties. The protein content was 78.8, 78.2, and 92.5% wt/wt for LAC 1, LAC 2 and LAC 3, respectively. The lipid content was higher in LAC 2 (9.32% wt/wt) than in LAC 1 (0.84% wt/wt) or LAC 3 (0.40% wt/wt). The LAC 1 powder had the largest particles, with a D[4,3] of 159 μm and the lowest specific surface area (SSA); the particles in this powder were observed to have crater-like dents and vacuoles using scanning electron microscopy. The powder particles in LAC 3 were smaller, with a D[4,3] of 36.4 μm, had the highest SSA and were observed to have shell-like, partially fragmented particles. Particles of LAC 2 with a D[4,3] of 72.7 μm were more spherical with smooth surfaces and a SSA similar to LAC 1. The analysis demonstrates that the lipid content influenced powder flow properties, as LAC 2 was very cohesive, with a flow index (i) of 3.29, (where a low value indicates poor flowability). In contrast, LAC 1 and LAC 3 (both with lower fat content) were easy flowing and free flowing, with i values of 6.48 and 11.3, respectively. However, LAC 3 had poor rehydration properties, with contact angle (θ) > 89°, whereas LAC 1 and LAC 1 had similar 0° of ~70°. This study clearly shows that flowability was influenced more strongly by composition than the microstructure or particle size of the powders, while the total protein content significantly influenced the rehydration properties of the powders.

**Key Words:** α-lactalbumin-enriched whey protein, flowability, wettability

482  **Characterization of dairy mix powders with maltodextrin and inulin produced by spray drying.** C. Raimundo da Silva¹, R. Stephani², E. Martins¹, P. Schuck¹, A. Fernandes de Carvalho*¹, and I. T. Perrone¹, ¹Federal University of Viçosa, Viçosa, MG, Brazil, ²Federal University of Juiz de Fora, Juiz de Fora, MG Brazil, ³UMR STLO-INRA, Agrocampus-Ouest, Rennes, France.

Products made from the mixing of milk, whey and other food substances have been gaining space in the dairy market due to their nutritional characteristics, competitive price and capacity to supply specific necessities of some consumers. By contrast, dairy mixes containing high content of whey are subject to stickiness and caking during and after the drying process which is a drawback for the dairy industries. This work aimed to evaluate the use of drying aids (maltodextrin or inulin) on the physicochemical and techno-functional properties of dairy mixes with increasing concentrations of whey. Mixtures of whole milk and crystallized-lactose concentrated whey (50% of crystallization) were added of maltodextrin or inulin, and dried in a pilot single stage spray dryer. The dairy mixes were evaluated as to their centesimal compositions, powder particle agglomeration profiles, particle size during the hydration process, theoretical glass transition temperatures, objective color and the spectroscopic profile. Higher whey concentrations reduced the proportion of protein and fat in the mixes as well as reduced the mean hydrodynamic diameter of particles during the rehydration of powders in water. Otherwise, the increase in the whey concentration was accompanied by increasing of the carbohydrate concentration and the browning saturation index. Furthermore, the higher whey content promoted greater particle agglomeration during drying and induced changes in the Raman spectrum profile with respect to the spectral contribution of lactose. The addition of maltodextrin and inulin reduced the powder agglomeration and the browning of products. It was also observed that the drying aids increased both the average hydrodynamic diameter of particles during rehydration in water and the theoretical glass transition temperature. The addition of maltodextrin and inulin favorably contribute to the conservation and drying of powders; however, such...
addition increases the hydrodynamic particle size during rehydration, which can be an advantage in the technological point of view.

**Key Words:** spray drying, glass transition, size distribution of particles

### Effect of milk protein composition on in vitro digestion of a model infant formula

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Objective of this work was to study the effect of protein composition, and in particular, the presence of whey proteins (WP) or β-casein (β-cas), on the digestion behavior of a model infant formula. Three model formulas were prepared with the same concentration of protein, fat and lactose, as well as same caloric content. One formula contained only WP and 2 others contained a casein to WP ratio of 40:60, but differing by 24% in β-cas amount. The formula was obtained by mixing milk protein isolates and permeates prepared with a 80 KDa molecular weight cut-off membrane, and with addition of whey protein. An in vitro gastric digestion was performed to better understand the physico-chemical properties of the gastric curd, and it was then followed by an intestinal digestion for studying physiological responses. There were no differences in the properties of the gastric digestate, within a treatment, at both 60 or 120 min of digestion. All curds showed a shear-thinning behavior, with a significantly higher viscosity and a higher modulus for curds obtained from casein/WP formula, compared with the curds from WP formula. Electrophoretic analysis of digestate samples after the gastric stage showed caseins extensively hydrolysed to peptides, while β-lg and α-la still largely intact after 60 or 120 min of digestion. Confocal microscopy showed structures with a higher density throughout the matrix for digestates obtained from cas/WP formula, compared with the WP formula digestate. The effect of in vitro intestinal digestate on secretion of pro-inflammatory cytokines was also studied on LPS stimulated human macrophages treated with the basolateral fraction of Caco-2 cells. Cas/WP formulas resulted in lower TNF-α secretion than WP formula. Moreover, high β-cas digestate showed lower secretion of IL-6, compared with WP and low β-cas formula. There was not a significant difference in secretion of IL-1β, between the 3 formulas. The results bring significant advances to our understanding of the effect of milk protein composition on the digestion of dairy matrices and physical properties of the gastric digestate, as well as their role on physiological responses.

**Key Words:** milk proteins, in vitro digestion, infant formula

### Hydrogenation of lactose for the production of nutritive sweeteners

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Due to its chemical structure, low cost and large-scale production, lactose is a promising source for the production of nutritive sweeteners or sugar alcohols. Lactose is converted into lactitol through a set of chemical reactions known as catalytic hydrogenation. Although lactitol is the predominant product, there is a considerable formation of lactulitol, lactobionic acid, sorbitol, and galactitol. These subproducts are formed through a combination of isomerization, hydrolysis, oxidation, and hydrogenolysis. Reaction products are strongly dependent on the reaction conditions and catalyst type. This presentation will summarize our laboratory efforts and elsewhere in understanding reaction kinetics of catalytic hydrogenation of lactose. We studied the effects of temperature (90–150°C) and pressure (0–150 bar) on lactitol selectivity using 5% Ru/C as a catalyst system. Experimentation utilized a laboratory scale high-pressure reactor with custom fabricated catalyst basket. Such configuration allows the catalytic hydrogenation of lactose to be controlled by intrinsic kinetics rather than diffusion and mass transfer. Preliminary data followed the Langmuir–Hinselwood–Hougen–Watson kinetics under the assumption of surface reaction as rate-determining step. We have developed a mathematical model to describe the influence of temperature and pressure on the reaction rate based on the Arrhenius and Eyring theory. The activation energy for lactitol formation was 73.54 ± 6.71 kJ mol⁻¹, while the activation volume was 57.25 ± 11.2 cm³ mol⁻¹. Research in this area is not as advanced as enzymatic catalysis, and there are opportunities for further studies in the field of reaction optimization, detailed characterization of products and their properties, and system scale up.

**Key Words:** lactose hydrogenation, lactitol, kinetics

### Enzyme-triggered microcapsules to selectively color Cheddar cheese and obtain white whey powder

R. Ravanfar* and A. Abbaspourrad, Cornell University, Ithaca, NY.

The yellow color of Cheddar cheese whey is due to the presence of annatto, which partitions into the whey during the Cheddar cheese making process. Currently, bleaching of the color using strong oxidizing agents such as hydrogen peroxide or benzoyl peroxide brings up the challenges such as health issues for asthmatic people, generation of off-flavors as a result of lipid oxidation, and loss of nutritive value due to whey protein oxidation. As the global market for whey protein is projected to reach $13.5 billion by 2020, providing an appropriate method to obtain high quality whey from the colored Cheddar cheese process to avoid challenges associated with current bleaching processes is of significant importance. The objective of this study is to selectively add colorant to Cheddar cheese and prevent partitioning of color into whey. In this work, we designed a controlled-release platform to selectively deliver and release annatto within Cheddar cheese matrix. We encapsulated annatto within a protein-polysaccharide complex shell using emulsification followed by spray drying steps (42% encapsulation efficiency). The engineered shell material disintegrates by the enzymes secreted during the ripening period of Cheddar cheese and releases the encapsulated annatto into the Cheddar cheese matrix. The Hunter CIE Lab color analysis shows an increase in the yellowness ($b^*$ value) of the cheese during the ripening period of 1 mo. Exploiting this method, we produced colored Cheddar cheese and white whey powder. In this work, we designed a controlled-release platform to selectively deliver and release annatto within Cheddar cheese matrix. We encapsulated annatto within a protein-polysaccharide complex shell using emulsification followed by spray drying steps (42% encapsulation efficiency). 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Cancer patients receiving chemotherapy often experience metallic taste and immunity dysfunction, which negatively impacts their nutritional status. However, there has been no effective treatment for this frequent side-effect. In this study, dietary supplementation through lactoferrin (LF) was developed as a treatment to reduce metallic taste disorder and improve immune system for cancer patients. Sixteen patients with self-reported metallic taste disorder since undergoing chemotherapy and 10 healthy subjects were recruited in this study. All participants took LF supplements, 3 tablets per day (250 mg/tablet), for 30 d. Saliva samples were collected at baseline, 30 d after LF supplements daily intake, and 30 d after LF supplementation ended. Taste function of cancer patients was assessed by a self-perceived taste abnormality questionnaire. Salivary proteome and minerals were analyzed at each time point of LF supplementation. LF supplementation for 30 d significantly decreased \( P < 0.05 \) taste abnormality score and salivary Fe in cancer patients. Production of metallic taste was related with the loss of salivary immune proteins. LF supplementation led to an overall increase of immune defense proteins in saliva of all human subjects, including α-amylase, zn-α-2-GP, prolactin-inducible protein (PIP), and low-abundance proteins (pH 5.5–8.5, MW 25–75 kDa) such as immunoglobulin heavy chain, annexin A1, carbonic anhydrase VI precursor (CAVI), proteinase inhibitor, and α-amylase. For 60% of cancer patients, a post-LF supplementation effect further increased the intensity of α-amylase. LF treatment effectively reduced metallic taste disorder induced by chemotherapy and increased production of salivary defense proteins in human subjects.

**Key Words:** lactoferrin, metallic taste, immunity
Calves exposed to heat stress during fetal development have impaired postnatal growth, immune function and performance. We hypothesized that in utero heat stress induces differential DNA methylation patterns within the liver and mammary gland that may influence postnatal performance. Bulls and heifers were born to dams housed in shaded barns either with fans and soakers (in utero CL) or without cooling devices (in utero HT), during late gestation (dry period, ~46 d). Bull calves were euthanized at birth and liver samples were collected. Mammary glands (MG) of heifers were biopsied during their first lactation at 21 d in milk. DNA methylation was analyzed (n = 5 bulls, n = 3 heifers per treatment) by double restriction enzyme reduced representation bisulfite sequencing. DNA fragments were bisulfite treated and libraries were sequenced with Illumina HiSeq 4000. Reads were mapped to the bovine genome assembly UMD3.1.1 using Bismark software. Differentially methylated cytosines (DMCs) and regions between CL and HT were identified via logistic regression (25% methylation difference, q-value < 0.05). We obtained on average 249M and 345M cytosines in CG-enriched regions in liver and mammary gland, respectively. On average, 76% of methylated cytosines were in the CG context. In utero heat stress impacted the epigenome of both liver and MG; in bull calf liver, DMCs were located within genes involved in functions such as cell growth and differentiation (e.g., PTPRU, EIF2F), RNA transport, and mRNA splicing (e.g., U6, SNRPG). Except for PTPRU, there were more methylated cytosines in these genes in the in utero HT bull calves. In the MG of heifers, DMCs were associated with genes involved in signal transduction and cell cycle regulation (e.g., MPZL1, MELK), apoptosis and cell proliferation (e.g., PANX1, RNF122), and formation of cell tight junctions (e.g., CLDN23). Except for MELK and CLDN23, more methylated cytosines occurred in the genes of in utero HT heifers. Our findings point to a possible effect of alternate methylation patterns in calves exposed in utero to heat stress, which may explain the impaired postnatal performance of HT cattle.

**Key Words:** methylation, calf, RRBS

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Heat stress (HS) during pregnancy is a significant risk factor for perinatal mortality, birth complications and early involution through the description of the major pathways and biological function of expressed genes. Holstein cows (n = 6), were housed in free stall shaded barns with fans and soakers during the entire dry period (~46 d). Mammary gland biopsies were collected at −3, 3, 7, and 14 d relative to dry-off. Total RNA extraction, amplification, library preparation, and sequencing were performed following Illumina HiSeq 3000 protocol. Sequencing reads were mapped to the bovine reference genome (bosTau7) using TopHat. The resulting alignments were used to reconstruct transcript models using Cufflinks. Ingenuity Pathway Analysis was used to determine top canonical pathways and biological functions at each time point. The p-value was calculated using Fisher’s t-test from the ratio of genes in the data set to the genes in the Ingenuity Knowledge base for a specific function or pathway. Overall, we identified 63, 112, 78 and 90 canonical pathways for d −3, 3, 7 and 14, respectively (–log P-value > 10). The top 10 pathways across time points were similar but the level of significance differs (i.e., protein ubiquitination, EIF2, mTOR, elf4, p70S6K, integrin, Rho family GTPases, glucocorticoids receptor, PI3K-AKT, B-cell receptor and PTEN signaling; –log P-value > 15). The top 10 biological functions across time points were similar but the level of significance also differs (i.e., cell death, cell proliferation, necrosis, apoptosis, expression and transcription of RNA, response to viral infection, organization of cytoplasm, metabolism of proteins, cell movement; –log P-value > 54). Notably, cell proliferation and cell death functions involve 260 and 269 more molecules on d3 than d-3, the organization of cytoplasm 47 more molecules on d7 than d3, and the function cell movement 17 more molecules at d14 relative to d7. Top biological functions reiterate the importance of cell death and proliferation during mammary involution, and the top canonical pathways reveal novel cellular signaling molecules that may play important roles in early mammary gland involution.

**Key Words:** mammary gland, involution, transcriptome

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**480 Effects of xanthosine on gene expression of mammary epithelial cells using RNA sequencing of goat milk fat globules.** S. Choudhary1, R. K. Choudhary1, R. Verma1, R. S. Sethi2, C. S. Mukhopadhyay1, D. Bickhart2, W. Li*2, and A. V. Capuco3, 1School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab, India, 2Cell Wall Biology and Utilization Research, USDA-ARS, Madison, WI, 3Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD.

Although intramammary xanthosine (XS) treatment was reported to increase the mammary stem cell population and milk yield in bovine and caprine, underlying molecular mechanisms remain unclear. The goal of this study was to evaluate effects XS treatment on the mammary transcriptome in early lactation dairy goats. Primiparous Beetle goats were used. Five d after kidding, one gland was infused with XS (TRT) twice daily for 3 d, and the other gland served as control (CON). Milk from TRT and CON glands was collected 10 d after the last treatment, followed by RNA extraction from milk fat globules (MFGs). MFGs primarily contains transcripts of mammary epithelial cells (MEC) origin. We characterized XS-induced transcriptome changes in MECs of 2 goats, using RNA sequencing technology. Processed reads were aligned to the goat genome using STAR. Transcript raw read counts were obtained using HTseq. Differential transcript analysis was performed using cuffdiff and DESeq2. Differentially expressed genes (DEGs; q-value < 0.002) were used to identify Gene Ontology terms (PANTHER) and gene networks (STRING db). Response to XS treatment was based on identification of DEGs between TRT and CON glands of each goat. Pathways impacted by these DEGs include cell communication, cell cycle and cell proliferation. A predominant portion of the DEGs showed downregulation by XS treatment. Functional prediction of these genes include (i) regulation of inflammatory and immune response (TLR4, IL8, CDKN1A (p21), S100A8, S100A12, VIM) and (ii) anti-proliferative signals by dual specificity phosphatases (DUSP). Remaining DEGs reflected upregulation by treatment. Predicted function of these genes
include (i) mRNA processing and splicing; (ii) and signaling pathways. Notably, upregulation of RHOA highlighted its potential role in modulating response to XS through: (i) linking plasma membrane receptors, (ii) involvement of focal adhesion and (iii) regulating cellular response to stimulus. This study documents XS-induced transcriptome changes in milk producing cells of goats and suggests XS treatment is likely associated with molecular mechanisms of enhanced cell proliferation in milk producing cells in goats.

**Key Words:** MEC, RNAseq, xanthosine

491 The effect of dietary grape marc on the bovine milk proteome. R. A. Scuderi*1, D. B. Ebenstein1, Y. W. Lam2,3, J. Kraft1, and S. L. Greenwood1, 1Department of Animal and Veterinary Sciences, College of Agricultural and Life Sciences, The University of Vermont, Burlington, VT, 2Vermont Genetics Network Proteomics Facility, The University of Vermont, Burlington, VT, 3Department of Biology, The University of Vermont, Burlington, VT.

Grape marc (GM) is a byproduct from grape pressing and contains condensed tannins. The objective of this experiment was to determine whether feeding GM to lactating dairy cows alters the milk proteome through changes in nitrogen (N) partitioning. Ten lactating Holstein cows were blocked by days in milk (141 ± 37 d) in a complete randomized block design. Cows were fed a TMR top-dressed with either 1.5 kg DM/cow/d GM (GM group) or 2.0 kg DM/cow/d of a 50:50 beet pulp:soy hulls mix (control group) for 28 d. DMI was recorded daily, blood samples were collected after AM and PM milking once weekly for plasma, and 24-h urine and fecal samples were collected on d 28. Milk samples were collected at AM and PM milkings thrice weekly for component analysis including milk urea N (MUN). Milk samples collected during the last week of the trial were composited within cow according to milk yield and subsequently skimmed. One aliquot of each skim milk sample was analyzed for high-abundance proteins using HPLC. A second aliquot was depleted of casein and fractionated. The whey fraction was enriched by ProteoMiner treatment before labeling with isobaric tandem mass tags and LC-MS-based peptide identification and quantification. Product ion spectra were searched on Proteome Discoverer 1.4 SEQUEST and Mascot search engines. Plasma urea nitrogen (PUN) was measured using a commercial kit. Urine and fecal samples were analyzed for N content. All results were analyzed using PROC MIXED of SAS (9.3) including repeated measures for DMI, milk yield and MUN. DMI (P = 0.61), milk yield (P = 0.16) and N partitioning were not affected by treatment (MUN, P = 0.61; urine urea, P = 0.34; fecal N, P = 0.41; PUN, P = 0.62). There was no difference in the high-abundance protein profiles across treatments. Of the 82 low-abundance proteins identified (peptide counts > 2), 16 were affected by treatment. GM did not affect known bioactive proteins including lactotransferrin (P = 0.23), osteopontin (P = 0.12), and lactoperoxidase (P = 0.14). These results indicate that low-level dietary supplementation with GM does not affect N partitioning or known key bioactive milk proteins.

**Key Words:** nitrogen partitioning, condensed tannins, milk proteins

492 RNAseq analysis of sow mammary gland reveals strong transcriptomic regulation of colostrogenesis. V. Palombo*1, J. J. Loor2, M. Vailati Riboni2, U. Krogh3, and P. K. Theil3, 1Università degli Studi del Molise, Campobasso, Italy, 2University of Illinois at Urbana-Champaign, Urbana, IL, 3Aarhus University, Tjele, Denmark.

Colostrum and milk are essential sources of antibodies and nutrients for the neonate, playing a key role in their survival and growth. Colostrogenesis is particularly crucial during the final stages of gestation. Characterizing the transcriptome profile and the metabolic and signaling pathways during this period could provide a more detailed understanding of the molecular mechanisms controlling colostrogenesis. To provide a comprehensive transcriptome profiling of the mammary gland around farrowing, mammary tissue was collected from 3 s-parity sows on days −14, −10, −6, −2, and +1 relative to parturition. Extracted total RNA was sequenced using the Illumina platform, and mapped to the S. scrofa genome assembly (v10.2). Statistical analysis was conducted using limma/voom R-package, with time as fixed effect and animal as random effect. Dynamic Impact Approach and gene network analysis with Ingenuity Pathways Analysis were performed to uncover the most-impacted pathways and to identify transcription regulators (TR) and their networks. Data at −14d were used as a baseline to determine the longitudinal transcriptional response (upregulation ↑; downregulation ↓) close to farrowing. A total of 0, 23 (3 ↓; 20 ↑), 1193 (521 ↓; 672 ↑) and 4982 (2352 ↓; 2630 ↑) differentially expressed genes (False Discovery Rate P ≤ 0.05) were detected in the comparison −10vs-14d, −6vs-14d, −2vs-14d, +1vs-14d, respectively. Collectively, our results confirm that the sow mammary gland transcriptome changes dramatically very close to parturition (−2d) where we detected a marked upregulation of metabolic pathways, notably ‘fatty acid biosynthesis’ and ‘galactose metabolism’. Furthermore, the substantial upregulation of CSN1S2 and LALBA, along with activation of XBP1 (a key TR of the endoplasmic reticulum stress response) indicate that the swine mammary gland starts to shift into stage II lactogenesis at −2d before parturition. Furthermore, at +1d the TR IRF7, TP53, NUPR1 and NFIC2 were predicted to be activated. Overall, preliminary evaluation indicates a strong transcriptional component in the control of colostrogenesis.

**Key Words:** mammary gland, sow, transcriptomics


The initiation and maintenance of lactation are complex phenomena governed by biochemical and endocrine processes in the mammary gland (MG). Despite extensive studies at the genetic, physiological and morphological levels, only limited data are available concerning the gene alterations of lactating and non-lactating bovine MG. To determine how the gene expression profile vary during lactation compared with the dry period, we used RNA-sequencing based transcriptomics to identify how genes isolated from lactating and non-lactating bovine MG tissue samples differed. A total of 918 differentially expressed genes (DEG, including 674 up-regulated and 244 down-regulated) were identified in lactating group versus nonlactating group. These up-regulated DEG included genes associated with various macromolecular metabolic processes, and appear to promote the increased metabolic activity related to milk synthesis and secretion. Functional analysis indicated that these DEG were primarily involved in initiation, maintenance, and involution of lactation, and included genes related to glycolysis/gluconeogenesis, the TCA cycle, oxidative phosphorylation, aminoacyl-tRNA biosynthesis, protein folding/synthesis/transport, fatty acid biosynthesis and cell growth. The results also suggested that non-lactating cows may have had depressed milk protein synthesis because these animals had decreased capacity for protein synthesis, enhanced proteolysis, inefficient energy generation and reduced cell growth. The 45 identified DEG associated with the process of protein synthesis were further validated by RT-PCR.
949 Characterization of the non-genetic causes of variation of bovine milk calcium concentrations on French farms. P. Gaingnon*1,2, M. Gele3, C. Hurtaud4, and A. Boudon1, 1PEGASE, INRA, Agrocampus Ouest, Saint-Gilles, France, 2CML, 18 avenue F. Roosevelt, Saint-Malo, France, 3Institut de l’élevage, Angers, France.

Calcium concentration (CaC) in bovine milk has often been described as independent of feeding strategy and mainly dependent on cow genetics and lactation stage. However, isolated experiments showed that variations in milk CaC could be linked to the diet of cows. Our objective was to identify and quantify non-genetic factors of variation in CaC in milk samples collected from a thousand French dairy farms with contrasting feeding strategies and cow breeds. This study was based on the PhénoFinlaït program that consisted of a survey performed between 2009 and 2010 in 924 dairy farms located in the major French milk production areas. The breeds used in the investigated farms were Holstein, Normande and Montbeliarde. Each farm was visited on average 4 to 6 times during the year. Each time, information about cow diets and production were gathered and individual milk samples were collected to extract their mid-infrared (MIR) spectra. More than 200,000 MIR spectra were measured. Nearly 10,000 milk samples were also frozen and stored in a bank for further analyses. We estimated CaC in milk samples from their MIR spectra using a predictive equation. This equation was established from 300 milk samples extracted from the bank and chosen to represent the diversity of investigated dairy systems. From the composition of the cow diets collected at each survey, we characterized 7 feeding strategies using multiple factorial analyses across 3 periods: winter, early and late summer. For each breed, the variations in milk CaC were quantified by ANOVA with a model including the effects of feeding strategy and mainly dependent on cow genetics < 0.05). The difference in CaC can be up to 100 mg/kg between 2 extreme diets at a given month, which is as important as the drop in CaC observed at the beginning of lactation. This study reinforces the idea that the diet of cows has an influence on milk CaC.

Key Words: milk fat globule size, size, fusion

This work provided some gene-associated insights to facilitate further investigation of the mechanisms underlying lactation in dairy cows.

Key Words: lactation, bovine mammary gland, transcriptomics

949 Understanding the regulatory mechanisms of milk production using integrative transcriptomic and proteomic analyses: Reducing inefficient utilization of crop by-products as forage in dairy industry. W. Dai1, Q. Wang1, F. Zhao2, J. Liu1, and H. Liu1, 1Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China, 2Laboratory of Lactation and Metabolic Physiology, Department of Animal Science, University of Vermont, Burlington, VT.

Milk from dairy cows is an essential nutrient for the young and human as well. Forage plays a vital role in dairy husbandry via affecting milk quality and quantity. However, the differences in mammary metabolism of dairy cows fed different forages remain elucidated. In this study, we utilized transcriptomic RNA-seq and iTRAQ proteomic techniques to investigate and integrate the differences of molecular pathways and biological processes in the mammary gland of dairy cows fed differing forages. Bovine mammary tissues were obtained from 6 healthy multiparous lactating dairy cows fed with corn stover (CS, low-quality; n = 6) and alfalfa hay (AH, high-quality; n = 6), respectively. A total of 1631 differentially expressed transcripts (DETs; 1046 upregulated and 585 downregulated) and 346 differentially expressed proteins (DEPs; 138 increased and 208 decreased) were detected in the mammary glands between the CS- and AH-fed animals. Expression patterns of 33 DEPs (18 increased and 15 decreased) were consistent with the expression of their mRNAs. The gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) analyses of the DETs and DEPs indicated that the decreased energy metabolism, increased fatty-acid oxidation, attenuated protein synthesis, enhanced protein degradation, and the lower mammary cell growth may be the prime factors contributing to the lower milk production in the CS-fed cows compared with the AH-fed cows. Moreover, 19 milk-synthesis-related genes were quantitated by real-time RT-PCR to examine the transcriptional profile and validate the proteins identified by LC-MS/MS between CS-fed and AH-fed bovine mammary gland. Four DEPs were further verified by Western blot analysis. These results provide the biological understanding of insights into mammary metabolism alterations affected by differing foraged and will be beneficial in developing highly efficient strategies for utilization of low-quality forages.

Key Words: dairy cow, mammary gland, forage

946 Milk fat globule size is regulated by phosphatidylethanolamine-dependent fusion: In vitro model. N. Argov-Argaman*1, B.-C. Cohen1, and A. Shamay2, 1Hebrew University, Rehovot, Israel, 2The Volcani Center, The Ministry of Agriculture, Rehovot, Israel.

Milk fat is secreted in a unique structure, termed milk fat globule (MFG) which consists of a triglyceride core covered with 3 layers of phospholipids (MFG membrane; MFGM). MFG are secreted in a wide range of sizes; from the nanometer length scale to over 15 µm, and their size is tightly associated with their lipid composition. Particularly, higher MFGM content is found in small compared with large globules. MFG size is determined by the size of its precursors — the intracellular lipid droplets (LD) which are produced and secreted by the mammary epithelial cells (MEC). Fusion is one of the suggested mechanisms controlling LD size. Nevertheless, what controls the extent of fusion and how dominant this mechanism is in controlling LD size is still illusive, especially in mammalian cells. We hypothesized that LD fusion is controlled by the stability of their membrane, which is modulated by the content and mass ratio between 2 main phospholipids - phosphatidylethanolamine (PE) and phosphatidylcholine (PC). We used primary MEC culture, treated with oleic or palmitic acid, to study the role of membrane stability in determining LD size. Results show that 22% of MEC treated with oleic acid had large LD (>2.5 µm) compared with only 4% of the cells treated with palmitic acid. The increased LD size in the oleic acid treatment was associated with 63% increase in PE, and 7 fold increase in LD fusion. Adding NaN3+NaF to the oleic acid treatment decreased PE content by 19%, concomitantly with 8 fold decrease in the number of large LD. Interestingly, the addition of NaN3+NaF to oleic acid treatment did not change the cellular triglyceride content. In contrast, adding 3-deazadenosine to palmitic acid treatment tended to increase PE content by 29%, and consequently increased the number of large LD by 3 fold, relative to cells treated with palmitic acid alone. Our findings have uncovered a defining role for LD fusion in determining their size in MEC, which is independent of triglycerides content of the cells. Understanding the mechanisms controlling LD size in mammalian cells is of great importance, especially in MEC due to the effect of LD size on milk composition.

Key Words: calcium, milk, feeding strategies
Once-daily milking during early lactation decreases production but does not affect dry matter intake of primiparous dairy cows fed pasture and total mixed ration. A. Capelesso1,2, G. Kozloski2, A. Mendoza3, N. E. Amaro1, A. F. Bica1, J. L. Repetto1, and C. Cajarville*, 1Universidad de la República, Facultad de Veterinaria, Uruguay, 2Universidade Federal de Santa Maria, Brazil, 3Instituto Nacional de Investigación Agropecuaria, Uruguay.

To investigate the immediate and long-term effect of once-daily milking during early lactation on milk production and DMI in a pasture-based dairy system, 20 primiparous Holstein dairy cows were assigned to 2 treatments (n = 10), in a randomized complete block design, from d 3 postpartum and for 8 consecutive wk of lactation (treatment period): once-daily milking (1x) or twice daily (2×). After this period all cows were milked 2× until wk 43 of lactation (all lactation). Cows were fed by grazing oats (Avena sativa) and supplementation with a TMR (30:70, DM basis). Individual milk yields were recorded every day and milk composition was determined in 2 consecutive milkings each wk. The TMR intake was determined by weighing the amounts offered and refused. Pasture intake was estimated as the difference between pre and post-grazing pasture mass, using a rising plate-meter previously calibrated. Data were analyzed using the SAS MIXED procedure, and the treatment and treatment × wk effects were tested. Total DMI (18.9 ± 0.56 kg DM/d), pasture and TMR intake were not different (P < 0.10) between treatments. Milk yield was lower in cows milked 1× during the treatment period (15.3 vs. 24.9 ± 0.53 kg/d) and a carryover effect was observed until 21 wk of lactation, with interactions treatment × wk, for both periods (P < 0.001). Milk yield for the entire lactation was lower in cows milked 1× during the first 8 wk of lactation tended to have a lower total milk solids yield by 10.4% until wk 43 (P = 0.052). In conclusion, once-daily milking during the first 8 wk of lactation in primiparous dairy cows did not affect DMI, but had immediate and carryover negative effects on milk and milk solid yields.

Key Words: grazing, milking frequency, milk loss

Prepartum feeding of a negative dietary cation-anion difference (−DCAD) ration has proved to be an effective method of preventing hypocalcemia. However, the optimum Ca concentration to feed with −DCAD rations has not been determined. We conducted an experiment in multiparous nonlactating, non-pregnant Holstein cows fed −DCAD rations (15.1 mEq/100g DM) for 21 d with low (LC;0.45% Ca;n = 5), medium (MC;1.13% Ca;n = 6) and high (HC;2.02% Ca;n = 6) concentrations of dietary Ca and then subjected to a controlled induction of hypocalcemia to determine the ability of cows to respond to the challenge based on dietary Ca. On d 22, 23, and 24 hypocalcemia was induced using an intravenous infusion of 5% ethylene glycol tetraacetic acid (EGTA). During infusion, blood samples were collected every 15 min until 60% of preinfusion iCa concentrations were achieved. Samples were collected post-infusion at 0, 2.5, 5, 10, 15, 30, and every 30 min thereafter until 90% of preinfusion iCa was reached. HC cows maintained higher concentrations of iCa during infusion period and had a longer average time of EGTA infusion to 60% iCa (414.2 ± 38.9min vs. 242 ± 28.0min MC and 282 ± 24.8min LC; P = 0.03). More EGTA was required to reach 60% iCa in HC cows (16.6 ± 1.5g vs. 10.3 ± 1.3 g in MC and 12.3 ± 1.2 g in LC; P = 0.01). Using a regression analysis and forced linearity to compare slopes of lines during EGTA challenge, MC cows reached 60% iCa faster than HC and LC cows in the first 145 min of infusion (P < 0.0001). Total serum Ca decreased in all cows during infusion but did not differ between treatment (P = 0.59). Total serum protein tended to be elevated in HC cows compared with MC and LC cows during EGTA challenge (P = 0.10). Serotonin concentrations were elevated in MC cows (7,076 ± 914.5 ng/mL) compared with HC (5,530.39 ± 806.1ng/mL) during EGTA challenge (P = 0.05), suggesting an interdependent relationship between iCa and serotonin. Cows fed HC had increased resistance to induction of hypocalcemia as measured by iCa, indicating potential metabolic benefits of feeding a higher Ca diet when using a −DCAD ration.

Key Words: calcium, DCAD, hypocalcemia
Dairy cows are frequently immune challenged, and obvious infections include metritis and mastitis. An often-unrecognized source is compromised gastrointestinal tract (GIT) integrity; a consequence of stressors including dietary changes, hind-gut acidosis, systemic inflammation, heat stress, psychological stress, and feed restriction. Immunoactivation begins when immune cells recognize invading pathogens, eliciting inflammatory cytokine responses culminating in an acute phase response characterized by fever, leukocytosis, and hepatic acute phase protein synthesis. Paradoxically, endotoxia (a catabolic condition) either causes insulin (a potent anabolic hormone) secretion or markedly enhances glucose stimulated insulin secretion. We recently demonstrated an in vivo lipopolysaccharide (LPS)-activated immune system consumes >1 kg of glucose within 12 h; a finding consistent with activated immune cells requiring glucose primarily for fuel and as a biosynthetic precursor. Despite increased glucose requirements, anorexia accompanies immunomodulation, which decreases diet-derived glucose precursors. Inflammation decreases milk synthesis and this presumably represents a strategy to spare glucose for the immune system. To further ensure an adequate fuel supply for the immune system, hepatic glucose output increases via both glycolgenolysis and gluconeogenesis. Simultaneously, peripheral insulin resistance develops leading to decreased glucose uptake by skeletal muscle and adipose tissue. These metabolic adaptations are indicative of altered homeostatic partitioning toward a new dominant physiological state of immunoactivation. GIT-derived endotoxin is also likely a key contributor to infertility as LPS and/or LPS-induced hyperinsulinemia markedly disrupts follicular, ovarian and uterine physiology. Additionally, gut-derived LPS also negatively affects the mammary epithelial barrier and causes hypocalcemia. Thus, leaky gut may be a common denominator that explains why transition to milk is associated with preterm birth. We have performed 2 studies that suggest that bacteria may play a role in the development of the fetus and preparation for birth and extrauterine life. To identify the major pattern of gene expression in the developing fetal sheep brain in the latter half of gestation, we used microarray technology to model gene expression in cerebral cortex, hypothalamus, hippocampus, and medullary brainstem at 80, 100, 120, 130, and 145 d gestation (term = 147 d) and 1 and 7 d postnatally. The differentially expressed genes (DEG) were analyzed using Supervised Weighted Gene Co-expression Network Analysis. The gene ontology analysis revealed that genes expressed by immune cells of the hematopoietic lineage (from the hematopoietic stem cells to more differentiated cells, for example macrophage, dendritic cells and T cells) are being transcribed at an increasing rate toward the last stage of gestation and transition to the extra-uterine life. We proposed that this gene expression pattern might be stimulated by the presence of bacteria in the fetal brain. qPCR experiments revealed an increasing abundance of bacterial 16S rRNA in the fetal brain in the latter half of gestation, approximating the rise in gene expression of the immune system within the brain. In other experiments, we discovered that maternal stress (ventilatory hypoxia) causes migration of bacteria into the fetal circulation and stimulates inflammatory pathways in fetal brain and other tissues. Bacterial populations appearing in fetal brain after maternal stress are relatively non-diverse: Staphylococcus simulans and other Staphylococcus species predominate. Together, our studies suggest that, in normal fetuses, there is a fetal microbiome. We propose that the presence of small numbers of bacteria may help direct fetal immune development and that the migration of bacteria into the fetus can be stimulated in conditions of maternal stress.

**Key Words:** endometritis, metabolism, infertility

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**Mechanisms linking metabolic stress with innate immunity and endometrial health.** I. M. Sheldon*, Swansea University Medical School, Swansea, United Kingdom.

Bacteria infect the endometrium lining the uterus of cattle after parturition, and clearance of these microbes depends on robust host tissue defenses. Animals under metabolic stress are at increased risk of postpartum uterine disease, which often leads to infertility. One hypothesis is that metabolic stress impairs host tissue defenses. Innate immunity is a key component of endometrial defense against bacteria. Innate immunity is predicated on host cell receptors that recognize pathogen-associated molecular patterns, and activated cells release inflammatory mediators. Cellular metabolism and innate immunity are highly integrated systems in tissues, and stressing one system might affect the other. Indeed, endometrial responses to the pathogen-associated molecular pattern, lipopolysaccharide, increases endometrial glucose consumption and induces aerobic glycolysis. Conversely, depriving endometrial tissues of their main energy substrates, glucose or glutamine, impairs their innate immune response to pathogen-associated molecular patterns. Furthermore, endometrial inflammatory responses to lipopolysaccharide are reduced by small molecules that modulate the intracellular sensor of energy, AMP-activated protein kinase. Metabolic stress also impacts lipid metabolism in cattle, and manipulating the mevalonate pathway, which precedes cholesterol synthesis, modulates inflammatory responses to pathogen-associated molecular patterns. However, other potential regulators of endometrial function, including mammalian target of rapamycin, insulin-like growth factor-1, and ovarian steroid hormones have limited impact on immunity. In conclusion, metabolic stress perturbs inflammatory responses to pathogen-associated molecular patterns in endometrial tissue, and fundamental regulators of cellular metabolism have the greatest impact on innate immunity.

**Key Words:** inflammation, leaky gut, glucose

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**Consequences of leaky gut on the immune system, metabolism, physiology and animal performance.** L. H. Baumgard1, S. K. Kvidera1, E. A. Horst1, M. J. Dickson1, E.J. Mayorga1, M. Al-Qaisi1, S. Lei1, J. A. Ydstie1, C. S. Shouse1, K. L. Bidne1, J. T. Seibert1, A. F. Keating1, J. W. Ross1, J. T. Selsby1, R. P. Rhoads2, 1Iowa State University, Ames, IA, 2Virginia Tech University, Blacksburg, VA.

Dairy cows are frequently immune challenged, and obvious infections include metritis and mastitis. An often-unrecognized source is compromised gastrointestinal tract (GIT) integrity; a consequence of stressors including dietary changes, hind-gut acidosis, systemic inflammation, heat stress, psychological stress, and feed restriction. Immunoactivation begins when immune cells recognize invading pathogens, eliciting inflammatory cytokine responses culminating in an acute phase response characterized by fever, leukocytosis, and hepatic acute phase protein synthesis. Paradoxically, endotoxia (a catabolic condition) either causes insulin (a potent anabolic hormone) secretion or markedly enhances glucose stimulated insulin secretion. We recently demonstrated an in vivo lipopolysaccharide (LPS)-activated immune system consumes >1 kg of glucose within 12 h; a finding consistent with activated immune cells requiring glucose primarily for fuel and as a biosynthetic precursor. Despite increased glucose requirements, anorexia accompanies immunomodulation, which decreases diet-derived glucose precursors. Inflammation decreases milk synthesis and this presumably represents a strategy to spare glucose for the immune system. To further ensure an adequate fuel supply for the immune system, hepatic glucose output increases via both glycolgenolysis and gluconeogenesis. Simultaneously, peripheral insulin resistance develops leading to decreased glucose uptake by skeletal muscle and adipose tissue. These metabolic adaptations are indicative of altered homeostatic partitioning toward a new dominant physiological state of immunoactivation. GIT-derived endotoxin is also likely a key contributor to infertility as LPS and/or LPS-induced hyperinsulinemia markedly disrupts follicular, ovarian and uterine physiology. Additionally, gut-derived LPS also negatively affects the mammary epithelial barrier and causes hypocalcemia. Thus, leaky gut may be a common denominator that explains why transition to milk is associated with preterm birth. We have performed 2 studies that suggest that bacteria may play a role in the development of the fetus and preparation for birth and extrauterine life. To identify the major pattern of gene expression in the developing fetal sheep brain in the latter half of gestation, we used microarray technology to model gene expression in cerebral cortex, hypothalamus, hippocampus, and medullary brainstem at 80, 100, 120, 130, and 145 d gestation (term = 147 d) and 1 and 7 d postnatally. The differentially expressed genes (DEG) were analyzed using Supervised Weighted Gene Co-expression Network Analysis. The gene ontology analysis revealed that genes expressed by immune cells of the hematopoietic lineage (from the hematopoietic stem cells to more differentiated cells, for example macrophage, dendritic cells and T cells) are being transcribed at an increasing rate toward the last stage of gestation and transition to the extra-uterine life. We proposed that this gene expression pattern might be stimulated by the presence of bacteria in the fetal brain. qPCR experiments revealed an increasing abundance of bacterial 16S rRNA in the fetal brain in the latter half of gestation, approximating the rise in gene expression of the immune system within the brain. In other experiments, we discovered that maternal stress (ventilatory hypoxia) causes migration of bacteria into the fetal circulation and stimulates inflammatory pathways in fetal brain and other tissues. Bacterial populations appearing in fetal brain after maternal stress are relatively non-diverse: Staphylococcus simulans and other Staphylococcus species predominate. Together, our studies suggest that, in normal fetuses, there is a fetal microbiome. We propose that the presence of small numbers of bacteria may help direct fetal immune development and that the migration of bacteria into the fetus can be stimulated in conditions of maternal stress.

**Key Words:** endometritis, metabolism, infertility
501 Effectors of immunometabolic adaptations to lactation: implications on physiology and performance. J. J. Loor*, F. Batis-
tel, M. Vailati-Riboni, and Z. Zhou, University of Illinois, Urbana-
Champaign, IL.

Immunometabolism represents the interface between immunology and metabolism, and is an emerging field of investigation in livestock biosciences. In human medicine, immunometabolism recognizes the link between obesity and the immune system, explicitly acknowledging that obesity-induced inflammation promotes onset of chronic disorders. More importantly, at the core of this concept is the recognition of “multilevel interactions between metabolic and immune systems,” implying “cross-talk” or “communication” among key cells and organs, which are orchestrated by unique mechanisms and their effectors. Such mechanisms correlate closely with health or disease status. The field of immunometabolism as it pertains to periparturient dairy cows is in its infancy. Classic studies before the 21st century defined the key met-
abolic, endocrine, and immune adaptations characterizing the transition into lactation. The advent of high-throughput technologies in the past decade further allowed the exploration of interrelationships among these systems. As a result of discoveries from that research, ongoing focus is on the bi-directional communication between immune and metabolic cells with signaling molecules derived intrinsically or as a result of intermediary metabolism or immune responses in tissues such as liver, adipose, and skeletal muscle. Both, macronutrients and micronutrients can be important effectors in the regulation of the immunometabolic response of the cow with effects often broad in nature. These responses are of great importance during the adaptation phase to lactation, as they seem to be key determinants of feed intake and milk production, repro-
duction, and health status. Management of total dietary energy supply and certain essential nutrients are examples of promising tools that can help regulate and enhance immunometabolic adaptations during the periparturient period and early lactation. A better understanding of the multilevel interactions among the various components of the metabolic and immune systems during the periparturient period has already led to identification of pathogenic mechanisms that underlie certain com-
plications afflicting cows. Future research in this area should lead to promising therapeutic approaches.

Key Words: immunometabolism, nutrients, peripartum

502 Lipids as regulators of conceptus development: implications for nutritional regulation of reproduction. E. S. Ribeiro*, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.

Pregnancy losses are substantial in dairy cattle and threaten reproductive efficiency. Approximately 60% of the fertilized oocytes are lost during development and fail to generate a live calf. Whereas early embryonic losses are more frequent, fetal mortality occurring later in development are more costly. The elongation phase of preimplantation conceptuses is critical in this context because 1) 39% of blastocysts fail to elongate and 2) events occurring during this period are important for subsequent maintenance of pregnancy. The onset of elongation is marked by dynamic changes in the transcriptome of trophectoderm cells. Peroxisome proliferator-activated receptor gamma (PPARG), a nuclear receptor activated by binding of fatty acids, coordinates a sub-
stantial portion of these changes. In addition to having its own transcript expression increased during elongation, PPARG also promotes transcript expression of genes related to uptake, oxidation, modification, and de
ovo biosynthesis of fatty acids and prostaglandins. Lipid droplets of epithelial cells in the endometrium seem to be the major source of lipids for conceptus utilization. Therefore, endometrial lipid profile is likely to regulate PPARG activity in conceptuses and the success of elongation. Formation of lipid droplets is induced by progesterone and its composition can be altered by diet and health status of the cow. About 40% of dairy cows develop inflammatory diseases postpartum, which impairs conceptus elongation and increases the likelihood of pregnancy loss. Uterine environment is a major contributor for this cause of subfertility because these outcomes are also observed following embryo transfer. Inflammation alters lipid composition of tissues because such molecules are used as inflammatory mediators. Thus, changes in composition of endometrial lipids might be involved in subfertility of cows developing postpartum inflammatory diseases. Targeting of uterine lipid metabolism and PPARG activity during preimplantation conceptus development through health postpartum and nutraceutical diets are good strategies to improve survival of pregnancy in dairy cows.

Key Words: conceptus, lipids, inflammation

503 Reduction in oocyte developmental competence by stress is associated with alterations in mitochondrial function. Z. Roth*, Department of Animal Sciences, Robert H. Smith Faculty of Agricul-
ture, Food and Environment, the Hebrew University, Rehovot, Israel.

Stress can impact reproductive performance of lactating cows by tar-
getting the ovarian pool of follicles and their enclosed oocyte. Among the documented stressors are heat, environmental and food toxins, metabolic stress and pathogens. Oocytes collected during the hot season are of lower quality than those collected in the winter, as expressed by reduced cleavage rate and lower blastocyst formation. A similar pattern has been reported for oocytes exposed to endocrine-disrupting chemi-
cals or those collected from cows with mastitis or metritis. While the underlying mechanism might differ, accumulating evidence suggests that various stressors impair oocyte mitochondrial functioning. Within the oocyte, mitochondria are involved in ATP generation, calcium homeostasis, regulation of cytoplasmic redox, signal transduction and apoptosis. Summer heat stress is strongly associated with alterations in mitochondrial distribution, an increased proportion of highly polarized mitochondria, and impaired expression of mitochondrion-associated genes, in particular those encoding oxidative phosphorylation com-
plexes for ATP production. Thus, it is proposed that stress reduces ATP levels below the required threshold, compromising the progression of oocyte maturation. Oxidative phosphorylation in mitochondria is the major source of reactive oxygen species (ROS). Under physiological conditions, ROS are essential for nuclear maturation; however, disequi-
librium between ROS production and antioxidative capacity might lead to DNA damage and apoptosis, as documented for oocytes exposed to heat stress or environmental toxicants. The review provides new insights into the cellular and molecular responses of the oocyte to stress with an emphasis on the mitochondria. It discusses some strategies to mitigate the effects of stress on the mitochondria, such as incorporation of coenzyme Q10—a key component of the mitochondrial respiratory chain, administration of antioxidants and microinjection of healthy mitochondria. Exploring the oocyte’s cellular and molecular responses to a specific stress might enable the development of new strategies to mitigate its effects on fertility.

Key Words: stress, mitochondria, oocyte developmental competence
504 Effect of osmotic pressure on spermatozoa characteristics of cryopreserved buffalo bull (Bubalus bubalis) semen. A. Ijaz1, D. H. Mughal2, and U. Farooq3, 1Nur International University, Lahore, Punjab, Pakistan, 2University of Veterinary & Animal Sciences, Lahore, Punjab, Pakistan, 2The Islamia University of Bahawalpur, Bahawalpur, Punjab, Pakistan.

This study was designed to determine the effect of different osmotic pressures on spermatozoa characteristics for cryopreservation of buffalo bull semen using tris egg yolk extender (TEYE). Semen of Nili-Ravi buffalo bulls housed at Semen Production Unit, Qadirabad, Sahiwal, Pakistan, was collected at weekly interval for 10 weeks. Three solutions of tris-citric acid-fructose with osmotic pressures of 255, 275, and 295 mOsm/kg were used in extender preparation. Semen samples containing 20 × 10^6 spermatozoa were processed and stored at −196°C using liquid nitrogen. Post thaw analyses of spermatozoa included motility, viability, acrosomal integrity, plasma membrane integrity, DNA integrity, and lipid per-oxidation. One-way ANOVA was implied and in case of significant differences among groups, the Duncan’s Multiple Range Test was applied and results were considered significant at P < 0.05. Spermatozoa motility, acrosomal and DNA integrity were significantly (P < 0.05) affected by varying osmotic pressures of the solutions. Higher rate of spermatozoa motility, acrosomal and DNA integrity were recorded at osmotic pressure 275 and 295 mOsm/kg compared with 255 mOsm/kg. However, rate of spermatozoa viability, plasma membrane integrity and lipid per-oxidation was not affected at any of the osmotic pressure treatments. It is concluded that consideration of osmotic pressure of the solution used in extender preparation may be useful in improving the quality of cryopreserved buffalo bull semen.

Key Words: Nili Ravi buffalo, semen quality, osmotic pressure

505 Effects of sperm dosage on conception rates of sex-sorted sperm processed using SexedUltra procedures. M. D. Uitt1, B. Harstine1, L. Helser1, J. M. DeJarnette1, R. Lenz2, C. Gonzalez2, T. Gilligan3, J. Moreno2, and R. Vishwanath2, 1Select Sires Inc., Plain City, OH, 2Sexing Technologies, Navasota, TX.

Historically, the speed, expense, and efficiency of sperm sex-sorting procedures have dictated that a commercial product offering could only be economically viable if provided at relatively low sperm dosage (2 million sperm per straw). A lower conception rate was also accepted as part of this process and previous field trials have indicated only a marginal fertility increase when dosage was increased up to 10 million sperm per dose. However, recent technological improvements in sperm sorting efficiency and proprietary processing procedures that have enhanced sperm survival, collectively marketed as SexedUltra, may result in increased fertility in response to increased sperm dose rate. We hypothesized that increasing sperm dosage from 2 to 4 million sperm per straw with SexedUltra would increase conception rate in heifers. Using a split-ejaculate technique, ejaculates from 6 Holstein bulls were sex-sorted according to SexedUltra procedures and frozen at 2 and 4 million sperm per 0.25-mL straw or processed as unsorted sperm at 15 million sperm per 0.5-mL straw. Treatments were distributed among 16 herds for use at first or second service in Holstein heifers. Within herd, equal dosages of each treatment were allocated for each bull selected for use. Outcomes to artificial insemination (n = 5,476) were analyzed in a mixed model ANOVA with the fixed effect of treatment and random effects of herd, bull, and the 2-way interactions of herd and bull with treatment. Conception rates of 2 million (40.6%, n = 1,831) and 4 million (46.4%, n = 1,852) SexedUltra treatments were less (P < 0.05) than those obtained with unsorted sperm (57.8%, n = 1,793). The 4 million dosage of SexedUltra resulted in a greater (P < 0.05; Student’s t-test) conception rate compared with the 2 million dosage. Conception rates of SexedUltra are improved by a 4 million sperm dosage but remain significantly less than those obtained with unsorted semen.

Key Words: sex-sorted sperm, SexedUltra, sperm dosage

506 Comparative effect of a commercial and tris-citric-egg-yolk (TCEY) extenders on post-thaw semen quality of Nili-Ravi buffaloes. A. Sattar1, M. A. Khan1, S. Ali1, M. Ahmad1, A. Channa1, M. U. Mehmood1, A. Husnain1, and N. Ahmad1, 1Department of Theriogenology, University of Veterinary and Animal Sciences, Outfall Road, Lahore, Pakistan, 2Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Outfall Road, Lahore, Pakistan.

Semen cryopreservation is crucial to introduce high genetics in buffalo herds through artificial insemination. Success of artificial insemination in Nili-Ravi buffalo (Bubalus bubalis) is limited due to poor freezing ability. The objective of this study was to compare the effect of commercial (Triladyl) and Tris-citric-egg-yolk (TCEY) semen extenders on post-thaw semen quality of Nili-Ravi buffaloes. Semen was collected using an artificial vagina (42°C) twice a week from 3 mature regular donor bulls of Nili-Ravi buffalo (7 replicates). After evaluation, semen was pooled and divided into 2 equal aliquots. These semen aliquots were extended separately in Triladyl and TCEY extenders, to a final concentration of 40 × 10^6 sperm/mL. After filling in 0.5-mL French straws semen samples were frozen first in liquid nitrogen vapors using automatic freezer (IMV- France) and then stored in Liquid Nitrogen. Post-thaw motility (PTM), Plasma membrane integrity (PMI), Normal apical ridge (NAR), DNA integrity (DI), Live percentage (LP), Sperm abnormalities (SA) were studied using routine procedures. CASA (Pre-freeze and Post-Thaw) motility parameters and kinematics were also studied. Results were compared statistically using paired t-test. Post-thaw semen quality parameters were significantly better (P < 0.05) in Triladyl as compared with TCEY Extender. Pre freeze and post-thaw CASA motility parameters were also better in Triladyl semen extender. Pre-freeze CASA sperm motility kinematics VAP (μm/s), VSL (μm/s), LIN (%) and post-thaw CASA sperm motility kinematics VAP (μm/s), VSL (μm/s), and STR (%) were also found significantly (P < 0.05) better in Triladyl semen extender as compared with TCEY extender. It may be concluded that Triladyl semen extender may be a good choice for buffalo semen freezing as compared with TCEY extender.

Key Words: triladyl, TCEY extender, Buffalo semen

507 Triladyl improves post-thaw semen quality of Sahiwal bulls. A. Sattar1, S. Ali1, S. Fiyal2, M. A. Khan1, A. Rehman3, M. U. Mehmood1, A. Rehman1, and M. Z. Tahir1, 1Department of Theriogenology, University of Veterinary and Animal Sciences, Outfall Road, Lahore, Pakistan, 2Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Outfall Road, Lahore, Pakistan.

Semen cryopreservation is an important tool to improve the reproductive/ productive efficiency through artificial insemination (AI) in Sahiwal...
cattle (*Bos indicus*). The objective of present study was to compare the efficacy of commercially and traditionally used egg yolk based extenders (Triladyl vs. TCEY) on pre-freeze and post thaw semen quality of Sahiwal bull. For this purpose, semen from 5 Sahiwal bulls was collected using an artificial vagina (42°C) twice a week (replicates = 7). Ejaculates from each bull passing minimum criteria were selected for further processing. Then, semen samples of all bulls were pooled and 2 aliquots of pooled semen samples were made. Each aliquot was extended separately with specific extender, one with Triladyl and other with TCEY respectively. Final concentration of spermatozoa was kept 40 × 10^6 / mL. Before freezing, semen samples of both groups were evaluated for sperm motility parameters and kinematics by computer assisted semen analyzer (CASA). Diluted semen was cooled to 4°C, equilibrated for 4 h and filled in 0.5-mL French straws (20 × 10^6 sperm per straw). All semen straws were placed in automatic freezer (IMV-France) in liquid nitrogen vapors, then stored in liquid nitrogen. Post thaw motility (PTM), plasma membrane integrity (PMI), normal apical ridge (NAR), DNA integrity, live percentage, sperm abnormalities, mitochondrial activity and post thaw CASA motility parameters/kinematics were studied. Results were compared statistically using independent *t*-test. Pre-freeze CASA sperm motility parameters (Progressive %, Rapid %, Medium %, Slow %) and kinematics VAP (um/s), VSL (um/s), LIN (%) were significantly (*P* < 0.05) better in Triladyl than TCEY while post thaw CASA sperm motility parameters (motive %, progressive %, rapid %, medium %, slow %, static %) and kinematics VAP (um/s), VSL (um/s) and STR % were also significantly (*P* < 0.05) better in Triladyl than TCEY extender. Post thaw semen quality parameters containing PTM, PMI, DNA integrity, live percentage, sperm abnormalities and mitochondrial activity were also significantly better (*P* < 0.05) in Triladyl as compared with TCEY. It may be concluded that commercially available extender Triladyl improves post thaw semen quality than TCEY extender in Sahiwal bulls.

**Key Words:** Triladyl, TCEY, Sahiwal bulls

**508 Effect of royal jelly on post-thaw semen quality of Beetal bucks.** M. Kaleem1, A. Rehman1, M. Avais2, M. U. Mehmood1, and A. Sattar*1, 1Department of Theriogenology, University of Veterinary and Animal Sciences, Outfall Road, Lahore, Pakistan, 2Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Outfall Road, Lahore, Pakistan.

Artificial insemination (AI) is the best tool to improve the reproductive and productive performance of large/ small ruminants. Semen cryopreservation, an important step in AI, may result into damages to spermatozoa due to oxidation process. Different antioxidants have been used successfully in the extenders of buck semen. Royal jelly is also reported to be a good antioxidant for ram and buffalo bull semen. No prior study is available on the use of royal jelly in the extenders of buck semen. The objectives of this study were to evaluate and optimize the different concentration of royal jelly in semen extender and its effect on post thaw quality of Beetal buck sperm. A total of 20 one ejaculates were collected from 3 mature Beetal bucks during 7 successive weeks. At each collection, the ejaculates from 3 bucks passing the minimum criteria (with motility ≥60%, concentration >2 × 10^8 sperm/mL and abnormality rate < 10%) were pooled and divided into 5 aliquots. The aliquots were extended (60 × 10^6/0.5mL straw) at 37°C with Tris-Citric acid Egg Yolk (TCEY) extender with different concentrations of Royal Jelly (0, 0.5, 1.0, 1.5 or 2.0 mM). The extended semen samples were cooled to 4°C for 90 min, equilibrated at 4°C for 2 h, frozen in liquid nitrogen (LN2) vapor (4 cm above the LN2, for 7 min) and plunged into LN2 for storage. Post thaw semen evaluation assays, e.g., motility, plasma membrane integrity (PMI) using HOST, normal apical ridge (NAR) and sperm viability using FITC-PNA/PI and DNA integrity using acridine orange were evaluated using phase contrast and fluorescent microscopy. Data were analyzed through ANOVA under CRD and significant differences (*P* < 0.05%) were compared by using Duncan’s Multiple Range Test. Royal Jelly, at an inclusion level of 1.0 mM significantly (*P* < 0.05) improved post thaw motility, PMI, NAR, live percentage and DNA integrity as compared with control and other treatment groups. It is concluded that 1.0 mM concentration of royal jelly in TCEY semen extender may prove beneficial for buck semen.

**Key Words:** royal jelly, Beetal buck, cryopreservation

**509 Dietary supplementation of conjugated linoleic acids on sperm quality and freezability in bovines.** M. S. Liman1, C. L. Cardoso1, D. C. Holm1, S. de Bruyn2, B. Gasparrovi2, V. Franco2, V. Longobardi2, and G. Esposito*1, 1Faculty of Veterinary Sciences, University of Pretoria, Pretoria, South Africa, 2University of Naples, Federico II, Naples, Italy.

Feeding rumen-protected isomers of conjugated linoleic acid (CLA) to fresh dairy cows improves fertility by reducing the postpartum interval to first ovulation and enhancing blood IGF-I levels. To our knowledge, there are no studies on the effect of CLA dietary supplementation on semen quality and freezability in bovines. Fourteen bulls blocked by breed, age, BW and BCS were randomly assigned to 2 groups: control (CTL) and CLA (50g/day). CLA was supplemented for 10 weeks and samples of blood, seminal plasma and ejaculate were collected twice a week on −2, −1wks before supplementation, 4 and 5wks (during supplementation) and 11,12wks (after supplementation). Blood and seminal plasma were analyzed for IGF-I; ejaculate from each bull was frozen in 6 subgroups to which CLA isoamers were added to the semen extender as follow: CTL (no addition), CLA9,11 50μM, CLA9,11 100μM, CLA10,12 50μM, CLA10,12 100μM, CLA mix. The sperm was analyzed for %motility, %progressive, average pathway velocity, straight-line velocity, curvilinear velocity, beat cross frequency, straightness, linearity, using the CASA system; morphology trough eosin-nigrosin staining; viability, mitochondrial activity and oxidative stress using the flow cytometer with live/dead viability kit, mitotrope JC-1, 2',7'-dichlorodihydrofluorescein diacetate and TO-PRO-3 dyes. Data were analyzed by ANOVA for repeated measures. Preliminary results show that dietary CLA supplementation decreased the total volume of the ejaculated (*P* < 0.05), increased sperm concentration (*P* < 0.05) and increased plasma and seminal plasma IGF-I levels (*P* < 0.001) compared with the CTL. Sperm from CLA bulls had increased beat cross frequency (*P* < 0.05) and decreased oxidative stress (*P* < 0.1). The results support the authors’ hypothesis of dietary CLA supplementation improving semen quality and bull performance. Investigation on the effect of CLA addition into the semen extender and its interaction with CLA dietary supplementation is ongoing.

**Key Words:** CLA, semen evaluation, IGF-I

**510 Expression of TGF-β superfamily genes in bovine embryos developed in vivo from oocytes exposed to endogenous (bovine) or exogenous (porcine) luteinizing hormone.** A. Behrouzi*1, A. Ruiz-Sanchez2, M. G. Colazo2, and D. J. Ambrose1,2, 1Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, 2Livestock Research Section, Alberta Agriculture and Forestry, Edmonton, AB, Canada.
In previous work, using porcine luteinizing hormone (pLH) in lieu of gonadotropin-releasing hormone (GnRH), for synchronizing ovulation, improved pregnancy rates (42 vs. 28%) in dairy cows, without increasing post-ovulation progesterone concentrations. Later, we found that a prolonged and elevated LH profile of pLH cows increased the expression of BMP-15, GDF-9, TGF-β1 proteins and genes associated with TGF-β superfamily pathway; these factors promote cumulus expansion and oocyte competence during preovulatory stage of the follicle. In the present study, we hypothesized that pLH exposure would alter the activation of the TGF-β pathway genes, which are involved in the development of preimplantation bovine embryos. Cyclic nonlactating Holstein cows (n = 16) were subjected to ovarian stimulation with 400 mg FSH and received either 100 μg GnRH or 25 mg pLH 24 h after the last FSH injection. Cows were artificially inseminated 12 and 24 h after GnRH or pLH injection. Serial blood samples were collected from before giving GnRH/pLH until 20 h after, for plasma LH analysis using an anti-bovine LH monoclonal antibody that cross-reacts equally with both bovine and porcine LH. Ova/embryos were recovered nonsurgically 6.5 d post-insemination. Total RNA was extracted from 3 to 4 morulae of each cow and qRT-PCR was performed with 3 biological replicates per treatment. Plasma LH concentrations and qRT-PCR data were analyzed using MIXED and GENMOD procedures of SAS, respectively. When LH concentrations (ng/mL) were compared in GnRH- and pLH-treated cows, mean LH was greater in GnRH- than pLH-cows (5.2 ± 0.2 vs. 1.3 ± 0.2) from 30 min until 4 h post-treatment. However, mean plasma LH from 5 to 20 h post-treatment was higher (\( P < 0.001 \)) in pLH- than in GnRH-treated cows (2.1 ± 0.1 vs. 0.6 ± 0.1). The relative abundance of PRO6KB-2, SMAD-2, and ACVR-1 in morulae, was about 2-, 3- and 3-fold higher, respectively, in cows subjected to pLH than GnRH treatment, but expression of THBS-4, BMP-2, BMPR1A and INHBA genes did not differ. Results indicate that a prolonged, higher-than-basal LH profile in pLH-treated cows increased the TGF-β signaling cascade and its components in bovine embryo, which may explain improved pregnancy rate following pLH-induced ovulation reported previously.

**Key Words:** porcine, LH, embryo, TGF-β
Production, Management and the Environment Symposium: Greenhouse Gas Emissions from Dairy Operations

512 Greenhouse gas emissions from confined dairy production systems. A. B. Leytem*1 and E. Kebreab2, 1USDA-ARS, Kimberly, ID, 2University of California, Davis, CA.

The main sources of GHG emitted on dairies consist of enteric CH4 production, N2O production from housing, and both CH4 and N2O production from manure management systems. The US greenhouse gas (GHG) inventory estimates that dairy production comprises 37% of total enteric methane (CH4) emitted as well as 52% and 34% of the total CH4 and nitrous oxide (N2O) emitted from manure management, respectively, making it an important sector of agricultural GHG emissions. Confined dairy production systems typically consist of cattle housed either in barns or open-lots with the manure intensively managed in a variety of systems. While enteric CH4 production is one of the main sources of GHG generated on farms, depending on the manure management strategies employed, manure derived GHG can exceed that of enteric CH4 production. On farm data collected from different housing types and manure management strategies in the western US reported daily CH4 production rates ranging from 0.75 to 1.72 kg/animal unit (AU), with the majority of emissions from the cattle housing during the cold months and from the manure management system in the warmer months. Emissions of N2O from the production facilities were lower at approximately 200 g/AU per day, with the majority from the housing area. Current inventory methods tend to either over or underestimate on farm emissions depending on the climatic region and time of year. This underscores the importance of obtaining annual on farm data when comparing on farm emissions to inventory estimates. Improvements in the inventory calculations via validation with on-farm measurements and modeling need to be done to improve these methodologies to accurately quantify emissions from this sector.

Key Words: methane, nitrous oxide, emissions

513 Greenhouse gas emissions from pasture-based dairy production systems. G. J. Lanigan*1, W. Burchill1, J. Humphreys2, P. Forrestal3, and K. G. Richards1, 1Teagasc, Johnstown Castle, Wexford, Ireland, 2Teagasc, Moorepark, Fermoy, Ireland.

The global population is projected to increase to 9.1 billion by 2050 and in combination with improved living standards in developing countries will result in increased meat and dairy demand. Simultaneously there is an urgent requirement to reduce anthropogenic greenhouse gas (GHG) emissions to curb global warming. Livestock production produces 14.5% (7.1 Gigatonnes CO2-e yr−1) of total global GHG emissions, with dairy production comprises 20% of sectors emissions. The principal GHG sources from pasture-based dairy production are methane (CH4) from enteric fermentation and manure management, nitrous oxide (N2O) associated with animal nitrogen (N) excretion and mineral fertiliser application to soils and CO2 associated with land-use change (principally deforestation). Mitigation of methane emissions is mainly focused on improving animal and herd efficiency although there is less scope for dietary manipulation and use of better feeds compared with confinement systems. Improvements in genetic merit and adoption of economic breeding indices are key tools in reducing CH4 emissions by maintaining production levels with fewer animals and/or the reductions in replacement rate. Greater absolute reductions in emissions can be achieved in terms of reductions in methane/ammonia/N2O from manure treatment and altered application techniques and timing, altered fertiliser type and timing (for N2O), increased nitrogen use efficiency and the use of urease and nitrification inhibitors. In addition, emissions can also be substantially offset by optimal grassland management that can enhance carbon sinks.

Key Words: greenhouse gas, mitigation, sequestration

514 Manure greenhouse gas emissions: Prediction and mitigation. S. O. Petersen*, Aarhus University, Tjele, Denmark.

While enteric methane (CH4) is the main source of greenhouse gas (GHG) emissions from dairy farms, the potential for CH4 and N2O emissions from manure is also significant. Dairy manure is typically managed in liquid form, but management practices range from year-round daily spreading to mandatory storage to recycle manure nutrients for crop production. In the former case, CH4 emissions will be small while direct and indirect N2O emissions are high. In the latter case CH4 emissions during storage dominate the GHG balance of manure management, and manure nitrogen (N) may substitute fertilizer N. The GHG mitigation options available will depend on the baseline situation, and therefore prediction and mitigation at farm level requires a dynamic description of site conditions, management and associated emissions. Currently GHG inventories are based on guidelines from the Intergovernmental Panel on Climate Change (IPCC). With this methodology GHG emissions from manure management are calculated using statistical data and annual emission factors for a limited number of housing systems and climates. Each category must represent a range of site-specific conditions and practices, and the uncertainty of emission factors is therefore high. Country-specific emission factors may be adopted, but verification is costly and inflexible, and effects of management changes are not easily documented. Mechanistic models have been proposed to predict CH4 and N2O emissions during manure storage, but data requirements prevent routine use. An empirical model of CH4 emissions during storage is currently explored which is based on daily time steps and few input parameters. The model allows for experimental verification and uncertainty estimation, and is suitable for investigating effects of management on GHG emissions. Water balance is key to control of N2O emissions during storage, where populations of nitrifiers and denitrifiers can develop in natural crusts on stored manure. After field application soil water content is also critical by determining the redistribution of slurry constituents and subsequent turnover. Models to predict N2O emissions must account for the distribution of reactive C and N in manure, and the balance between oxygen supply and demand.

Key Words: manure management, methane, nitrous oxide

Ruminant production systems are important contributors to anthropogenic methane emissions. Globally, there is a large body of enteric methane emission data. The GLOBAL NETWORK (GN) project was established to collate and analyze methane emission and mitigation data for ruminants. Two separate databases have been developed: mitigation database and prediction database. The objective of the mitigation database is to summarize and recommend science-based enteric methane mitigation options to stakeholders. This database consists of 1,800 experimental treatment means from 410 publications. The goal of the prediction database, which consists of individual animal data, is to develop robust enteric methane emission prediction models for various ruminant species (dairy and beef cattle, sheep) and nutritional, animal, and farm management scenarios. The dairy cattle prediction database currently contains 5,899 individual animal observations from 159 studies from North and South America, Europe, and Oceania. Development of enteric methane prediction models was conducted using a sequential approach, by incrementally adding available information to develop models with increasing complexity. In total, 11 models were developed. Methane emission (g/d, per DMI, or per milk/energy-corrected milk yields) was predicted by fitting linear mixed models including random effect of study nested within the random effect of continent. As expected, a global methane emission (g/d) model with a greater number of independent variables fitted the data best [Root mean square prediction error as a percentage of mean observed value (RMSPE) = 13.4%]. Inputs were DMI, dietary concentrations of ether extract (EE) and NDF, milk fat and protein content, and cow BW. The predictive ability of fitted models was evaluated through cross-validation. Less complex models requiring only DMI, or DMI plus NDF or EE concentrations had predictive ability similar to more complex models (RMSPE = 14.0 to 14.3%). These prediction models, along with recommendations from the mitigation database analysis, provide robust enteric methane inventory and mitigation options for ruminant farming systems.

**Key Words:** livestock, methane, prediction

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**Modeling greenhouse gas emissions from dairy farms.** C. A. Rotz*, USDA-ARS, University Park, PA.

Evaluation and mitigation of greenhouse gas emissions from dairy farms requires a comprehensive approach that integrates the impacts and interactions of all important sources and sinks. This approach requires some form of modeling. Types of models commonly used include empirical emission factors, process-based emission factors and process-level simulation. If properly applied, each type can be useful in evaluating dairy farm emissions. Important emission sources include the animal, manure in the housing facility, manure storage, pasture and cropland soil, and machinery operations. Important gases emitted are methane (CH₄), nitrous oxide (N₂O) and carbon dioxide (CO₂). Methane and N₂O are normally converted to CO₂ equivalents using a global warming index and summed with anthropogenic CO₂ to get a total net emission. From a global warming perspective, enteric CH₄ from the animals is normally the major dairy farm source followed by manure storage emissions and soil emissions during feed crop production. Emissions from a free stall barn are small, but those from open lots or bedded pack facilities become more important. Manure storage is often an important source where storage type, manure dry matter content and temperature have a major effect on the amount and form of emissions. Nitrous oxide emissions from pasture and cropland can also be important due to the high global warming potential of this gas. These emissions are related to temperature and the nitrogen and moisture contents of the soil. Anthropogenic CO₂ emissions from fuel combustion and lime decomposition are relatively small compared with other sources, but they are still important. Ammonia is also an important emission. It is not a greenhouse gas, but some of the ammonia and nitrates lost to the environment are transformed to N₂O providing an indirect emission source. For a full accounting, emissions occurring during the production of resources used on the farm, such as fuel, electricity, fertilizer and feed, must also be considered. Models representing the integration of these sources have become important tools for assessing best management practices to mitigate dairy farm emissions.

**Key Words:** whole farm, model, greenhouse gas
Production, Management, and the Environment V

517 Relationship between bulk tank fat and true protein test and milk fatty acid composition. D. M. Barbano*1, M. E. Carabeau2, H. M. Dann2, and R. J. Grant2, 1Cornell University, Ithaca, NY, 2Miner Institute, Chazy, NY.

Our objective was to determine the relationship between bulk tank fat and protein concentration and the concentration of denovo (C4 to C14), mixed origin (C16 and C16:1), and preformed fatty acids (>C18) expressed as g per 100 g milk and milk unsaturation expressed as double bonds per fatty acid. Bulk tank milks from 46 Holstein farms were collected and analyzed for fat, true protein, and milk fatty acid composition determined using mid-IR milk analysis over a period of 16 mo with a Delta FTA mid-IR milk analyzer. Milk sample collection was done by the licensed milk truck driver as part of the official sampling for payment testing at the St Albans Cooperative (St Albans, Vermont) and the samples in the study were the same samples used to determine fat and protein for payment. The number of milk samples analyzed per farm per month ranged from a minimum of 3 to as many as 20 within a month. Farms producing more milk per day were sampled more frequently. Mean monthly average bulk tank fat concentration (Y) increased (P < 0.05) with increased de novo fatty acid concentration (X) (Y = 2.297X + 1.84; r² = 0.88), while the bulk tank milk fat concentration was not related to concentration of preformed fatty acids (Y = 0.8005X + 2.4179; r² = 0.07). Milk fat (Y = −8.583X + 6.4213; r² = 0.69) and true protein (Y = −2.5588X + 3.8812; r² = 0.34) concentration both decreased (P < 0.05) with increasing milk fatty unsaturation expressed as double bonds per fatty. Bulk tank milk true protein concentration increased with increasing de novo fatty acid concentration (Y = 0.8005X + 2.4179; r² = 0.53). For a Holstein farm to achieve a >3.80% bulk tank fat test, the denovo fatty acid concentration needs to be > 0.305 double bonds per fatty acid. Higher denovo and mixed origin fatty acid concentration was related to higher milk fat and true protein concentration while preformed fatty acids was not.

Key Words: milk fat, milk true protein, de novo fatty acid


The annual or seasonal rhythm of milk yield and composition is important for dairy producers and it may represent an underlying adaptation of the cow to yearly changes. It is well appreciated that milk fat and protein concentration peak during the winter and reach a nadir in the summer. Summarized monthly production data from individual Federal Milk Marketing orders has suggested that the region of the US may impact the difference between mean and peak (amplitude) fat and protein concentration and the timing of peak production (acrophase). Less data is available on yields of milk, fat and protein. Our objective was to determine the seasonal rhythm of milk production and the effect of US region at the herd level. Monthly DHIA records of all herds in Pennsylvania, Minnesota, Texas and Florida from the years 2003 to 2016 were obtained from Dairy Records Managements Systems. Milk yield, fat and protein yield, and fat and protein concentration were fit to the linear form of the cosine function with a 12-mo period using a linear mixed effects model in ASreml. Model parameters included the fixed effects of state, cosine parameters, the interaction of state and cosine parameters, and breed and the random effects of herd and year. A zero-amplitude test was performed to determine the fit of the linear form of the cosine function. Milk yield and fat and protein yield and concentration fit a cosine function in all 4 states, indicating an annual rhythm (P < 0.001). The amplitude of the rhythm of milk yield varied by state, and was lower in PA (2.8 kg) and MN (2.4 kg) compared with TX (6.9 kg) and FL (8.1 kg; P < 0.05). Fat and protein yield similarly showed a greater amplitude in the southern versus northern states (P < 0.05). The concentrations of fat and protein was opposite, with greater amplitudes occurring in MN and PA than in TX and FL (P < 0.05). The acrophase of milk yield, fat and protein yield, and concentration also varied by state, but all peaked between October and March (P < 0.05). Results suggest that region of the US impacts annual production rhythms, with a greater yearly variation in milk, fat and protein yield occurring in the south.

Key Words: annual rhythms, milk synthesis, yearly pattern

519 Relationship of mid-lactation feed efficiency with early and late lactation body condition score in Holstein dairy cows. L. Hardie*, K. Maxwell1, M. VandeHaar2, and D. Spurlock1, 1Iowa State University, Ames, IA, 2Michigan State University, East Lansing, MI.

The objective of this study was to investigate the relationship between feed efficiency in mid-lactation primiparous cows with change in body condition score (BCS) measured in late first parity and early second parity. Individual daily feed intakes, daily milk production, weekly body weight (BW), weekly BCS, and weekly samples for milk component analysis were collected over 8 weeks on 173 primiparous Holstein cows between 50 and 215 d in milk (DIM). For each cow, 3 measures of feed efficiency were calculated: the ratio of milk to feed (MtoF), calculated as her average milk energy (MilkE) output divided by her average dry matter intake (DMI); gross efficiency (GE), calculated as the ratio of the sum of MilkE and energy in body weight change (BWCE) divided by gross energy consumed; and residual feed intake (RFI), calculated as the regression of DMI on MilkE, metabolic body weight, and BWCE. Measures were adjusted for replicate and DIM. Weekly BCS were observed during late first parity and the first 45 DIM in second parity and used to estimate BCS at the start and end of each time period along with the change in BCS. For each feed efficiency measure, BCS traits were compared between the 18 most feed efficient and inefficient cows. Between feed efficiency group, mean RFI differed by 3.62 kg, GE by 0.10, and MtoF by 0.30 Mcal/kg per day. At dry off, low RFI (feed efficient) cows carried significantly more body condition than high RFI cows (P < 0.05). RFI and GE will likely identify cows that maintain body condition throughout any time period for GE. In conclusion, defining feed efficiency as MtoF may favor cows prone to greater body condition loss during early lactation.

Key Words: feed efficiency, body condition score
520  Comparison of growth and meat quality of Holstein and crossbred dairy steers grazing two cover cropping systems.  H. Phillips*1, B. Heins1, K. Delate2, and B. Turnbull2, 1University of Minnesota, Morris, MN, 2Iowa State University, Ames, IA.

Body weights and carcass measurements for Holstein and crossbred organic dairy steers were compared for growth while grazing 2 different cover cropping systems. Bulls calves were born at the University of Minnesota West Central Research and Outreach Center organic dairy from March to May 2015 and assigned to 1 of 3 replicated breed groups at birth. Breed groups were: crossbreds comprised of Montbéliarde, Holstein, and Viking Red (MVH; n = 10), crossbreds comprised of Jersey, Normande, and Viking Red (NJV; n = 9), and purebred Holstein (HOL; n = 10). Steers grazed either winter wheat (WW) or winter rye (WR) cover crops planted the previous fall. The WW and WR cover crops were planted in September 2015 on 2 adjacent 10 acre plots. In April 2016, each breed group was randomly assigned to either cover crop and grazed rotationally until June 2016 for a total of 7 weeks. Steers were weighed individually on the first and last day of grazing and twice during the grazing season for a total of 4 weights. Steers were harvested in 2 groups at an average age of 16 mo. Statistical analysis was with PROC MIXED of SAS with forage, breed, and the interaction of forage and breed as fixed effects and the individual steer within the forage and breed interaction as a random effect. For body weights, the HOL and MVH steers were heavier (P < 0.02) than the NJV steers throughout the grazing season. For cover crops, HOL and MVH steers did not differ (P > 0.30) in weight between cover crops throughout the grazing season. However, NJV steers grazing WW tended to be heavier (P < 0.09) than NJV steers grazing WR throughout the grazing season. For average daily gain (ADG), breed groups did not differ (P > 0.12) throughout the grazing season. At harvest, MVH and HOL steers weighed more (P < 0.05) than NJV steers, and steers grazed on WW (483 kg) weighed more (P < 0.05) than steers grazed on WR (458 kg). Dressing percent, marbling score, back fat, ribeye area, and yield grade were not different (P > 0.10) between breeds or cover crops. In summary, steer breeds gained weight comparably to each other on cover crops and had similar carcass characteristics.

Key Words: steer growth, cover crops, organic beef

522  Progesterone profile of lactating dairy cows with reference to production and cyclicity during P4 supplementation.  R. S. Balouch*1, S. Abbas2, and A. H. Shahzad2, 1L&D, Punjab, Lahore, Pakistan, 2UVAS, Lahore, Lahore, Pakistan.

Aim of current research was to investigate the progesterone (P4) profile in milk and plasma of lactating dairy cows during intravaginal P4 device insertion. Cows (n = 56) were randomly blocked based on milk yield (low: < 36 L [25–35 L; n = 26] vs. high: > 36 L [37–49 L; n = 30]) and cyclicity (presence or absence of visible corpus luteum [cyclic, n = 28 vs acyclic, n = 28]) to generate 4 groups: high producing-cyclic (n = 16), low producing-cyclic (n = 12), high producing-acyclic (n = 14) and low producing-acyclic (n = 14). Immediately after collection of milk and blood samples, CIDR was inserted intravaginally to all enrolled cows. Milk and blood samples were collected on d 1, 2, 4, 5 and 7 post CIDR insertion. Data were analyzed by using the repeated measures analysis of the mixed procedure of SAS. Serum and milk P4 concentrations were maintained above physiological threshold levels (>1 ng for serum and ≥ 15 ng in milk) during CIDR insertion in acyclic cows. There were cyclicity (P < 0.01) and cyclicity X day (P < 0.01) effects for serum and milk P4 concentrations. In this regard, serum and milk P4 concentrations were lower (P < 0.01) in acyclic cows (3.24 ± 0.48 ng/mL in serum; 13.67 ± 1.19 ng/mL in milk) than those in cyclic cows (6.49 ± 0.49 ng/mL in serum; 22.66 ± 1.23 ng/mL in milk). There was a cyclicity, milk yield and day interaction (P < 0.01) for milk P4 profile. Milk P4 profile did not differ between low and high producing acyclic cows; however, it was higher (P < 0.01) in high producing-cyclic cows than those in low producing-cyclic cows. In conclusion, serum and milk P4 profile was lower in acyclic cows in comparison with cyclic cows; however, serum and milk P4 concentrations were elevated to physiological threshold levels following P4 administration in acyclic cows regardless of milk yield.

Key Words: progesterone profile, milk production, cyclicity
Dairy Foods Symposium: Chr. Hansen Symposium: Microbial Ecology of Cheese

523 Dairy species from non-dairy sources: Their genomic and metabolic diversity and potential applications in cheese. O. McAuliffe*, Teagasc Food Research Centre, Fermoy, Cork, Ireland.

The widespread dissemination of species of the lactic acid bacteria (LAB) group in different environments testifies to their extraordinary niche adaptability. Members of the LAB are present on grass and other plant material, in dairy products, on human skin, and in the gastrointestinal and reproductive tracts. The selective pressure imparted by these specific environments is a key driver in the genomic diversity observed between strains of the same species originating from different habitats. Strains which are exploited in the dairy industry for the production of fermented dairy products are often referred to as ‘domesticated’ strains. These strains, which initially may have inhabited a non-dairy niche, have become specialized for growth in the milk environment. In fact, comparative genome analysis of multiple LAB species and strains has revealed a central trend in LAB evolution: the loss of ancestral genes and metabolic simplification toward adaptation to nutritionally-rich environments. By contrast, ‘environmental’ strains, defined as those from plants, animals and raw milk, exhibit diverse metabolic capabilities and lifestyle characteristics when compared with their ‘domesticated’ counterparts. Owing to the limited number of established dairy strains used in the production of fermented foods today, there is an increasing demand for novel strains, with concerted efforts to mine the microbiota of natural environments for strains of technological interest. Numerous studies have focused on uncovering the genomic and metabolic potential of these organisms, facilitating comparative genome analysis of strains from different environments and providing insight into the natural diversity of the LAB, a group of organisms that is at the core of the dairy industry. The natural biodiversity which exists in these environments may be exploited in dairy fermentations to expand flavor profiles, to produce natural ‘clean label’ ingredients or to develop safer products.

Key Words: niche adaptability, domesticated strains, environmental strains


Cheese ripening and flavor development is a dynamic process and for mature cheeses the evolution of flavor and texture can often be slow. The ripening of cheese is largely controlled by intricate biochemical reactions mediated by several enzymes coming from milk, residual coagulant, starter and secondary bacteria as well as the non-starter bacteria. The flavor and texture characteristic of each cheese variety is a result of a series of microbiological and biochemical reactions the extent of which is dependent on the environmental conditions in the cheese – moisture, pH and salt content. However, variations in milk quality, plant hygiene, non-starter flora, moisture and salt levels can result in inconsistencies and loss of control over the ripening of cheese at industrial scale. To overcome some of these inconsistencies and achieve some control over the development of flavor and texture of many cheese types, Chr-Hansen has developed robotics assisted high throughput screening methods to characterize strains of bacteria, better understand their needs and their interactions to increase consistency and robustness of cultures. The focus of this talk will be on the omics and automation methods used to characterize individual strains for a range of phenotypes (acidification, flavor and texture potential), compounding design to identify optimal culture combinations, enhanced knowledge of their mode of action to manage which bacterial components, and to what proportions, are required for specific functionalities.

Key Words: cheese, ripening, methods

525 Interaction of starter cultures and nonstarter lactic acid bacteria (NSLAB) in the cheese environment. G. LaPointe*, University of Guelph, Guelph, ON, Canada.

The microbiota of ripening cheese is dominated by lactic acid bacteria, which are either added as starters and adjunct cultures, or originate from the production and processing environments (non starter or NSLAB). After curd formation and pressing, starters have reached high numbers, but their viability then decreases due to lactose depletion, salt addition, low pH and temperature. Starter autoysis releases cellular contents, including nutrients and enzymes, into the cheese matrix. During ripening, NSLAB may attain cell densities up to 8 logs of colony-forming units after 3–9 mo. Depending on the species and strains, their metabolic activity may contribute to defects or inconsistency in cheese quality as well as to the development of typical cheese flavor. Studies using qPCR and RT-qPCR have shown that the starters survive and dominate the cheese microbiota over 6 mo. The lowering costs of high throughput sequencing have contributed to understanding the changing composition of the cheese microbial community. The availability of gene and genome sequences has enabled targeted detection of specific cheese microbes and their gene expression over the ripening period. The application of RT-qPCR has revealed how the expression of genes encoding peptide transporters and peptidases of Lactobacillus paracasei is stimulated in mixed culture compared with pure culture in cheese slurry. Integrated systems biology is needed to combine the multiple perspectives of post-genomics technologies to elucidate the metabolic interactions among microorganisms. Future research should delve into the variation in cell physiology within the microbial populations, as spatial distribution within the cheese matrix will lead to microenvironments that could impact localized interactions of starters and NSLAB. Microbial community modeling can contribute to improving the efficiency and reduce the cost of food processes such as cheese ripening.

Key Words: lactococci, lactobacilli, cheese

526 Interactions of production environment microbiota with food and beverage fermentations: Lessons for cheese production. D. A. Mills*, Department of Food Science & Technology, University of California, Davis, CA.

Cheese production is a useful model to study food ecosystem dynamics as these fermented products illustrate opposing roles of adventitious microbes involved—as spoilage agents and as beneficial members of the microbial consortium—both of which influence final product quality. Recently, application of rRNA marker gene surveys to define the modes of microbial transmission across space and time in cheese production has provided unique insight into these important commercial fermentations. Cheese fermentations are well known to be initiated by starter cultures, however recent studies suggest that adventitious microbiota is influenced by environmental factors thus potentially contributing to the “regional
character” often attributed to specific products. Moreover, advances in sensor technology now allows simultaneous monitoring of food production facilities for various environmental parameters including: temperature, relative humidity, volatile organic carbon, CO₂, dust accumulation and human traffic. Integration of sensor data with microbiota surveys provides unique insight into mechanisms of microbial dispersal and persistence throughout seasonal or process-related environmental changes. Elucidating microbial ecosystems and spatial characteristics present in cheese production environments identifies the fundamental drivers of microbial biogeography with practical implications for all food production systems.

**Key Words:** cheese, microbiota, environment

527 **Diversity and dynamics of surface-ripened cheese microbiomes: Implications for cheese quality and safety.** B. E. Wolfe*, Department of Biology, Tufts University, Medford, MA.

Despite the long history of producing and consuming surface-ripened cheeses, we are just beginning to understand the diversity of microbes that negatively and positively affect the quality and safety of these cheeses. I will explain the genomic and experimental approaches that my research team is using to dissect microbiome diversity and dynamics in the rinds of surface-ripened cheeses. Metagenomic and genomic approaches demonstrate species and strain-level variation that contributes to the diversity of cheese aesthetics and flavors and highlight the widespread abundance of non-starter culture bacteria and fungi in surface-ripened cheeses. Experimental approaches demonstrate the dynamic interactions occurring within cheese rind microbial communities and highlight how these interactions can be managed to create specific cheese communities. I will also describe our efforts to diagnose the microbial origins of common cheese rind defects and how we are collaborating with chemists to identify the sensory impacts of specific cheese microbes. Ongoing work is uncovering the potential risks of antibiotic resistance genes and opportunistic pathogens that can occur in the rinds of some cheese varieties. Collectively, our work is uncovering a previously unknown diversity of microbes in cheese rinds and providing key data on how to manage and manipulate these microbes to improve the quality and safety of traditional cheeses.
In 2015, under the leadership of ADSA Past President Scott Rankin, the ADSA Board of Directors approved a strategic initiative that posits an overarching goal “to attract and foster the best minds affecting the global dairy discipline through increasing the strength of our community.” International members represent a key constituency within the ADSA community. The Teagasc-Moorepark/University College Cork Cheese Symposium arose out of this strategic initiative as an action item aimed at strengthening a sense of community for our international members. The basic concept is to use organizationally defined symposia to encourage partnerships between ADSA and international organizations that are known for their outstanding research in dairy food science. The ultimate goal of such partnerships is to afford our international colleagues new and welcoming opportunities to work and learn together with our North American ADSA members and industry partners in areas of mutual benefit. Dwindling resources to support basic research in dairy food science are affecting scientists globally, yet the need for basic dairy food research to meet global challenges, and the need to leverage global intellectual and infrastructural resources for the broader good, have never been greater. The Teagasc-Moorepark/University College Cork Cheese Symposium is a pilot effort that aims to (1) showcase some of the finest cutting-edge cheese science globally; (2) serve as a venue to encourage scientist-to-scientist connections and explore potential opportunities for international partnership at the organizational level; and (3) offer the cheese industry a platform to shape/influence future cheese research. An example of a positive outcome of this pilot effort would be a partnership that is mutually beneficial on both sides of the Atlantic, that strengthens the ADSA international community, and that demonstrates the potential for replication with other international organizations that are known for their outstanding research in dairy food science.

**Key Words:** cheese, science, international

This article presents a brief history of cheese, a description of the Irish cheese industry, a history of research on cheese, the research of the author on cheese and suggested aspects of cheese that warrant further research.

**Key Words:** cheese, Ireland

The biochemical pathways through which flavor compounds develop in cheese during ripening are conventionally grouped into 3 major pathways: (i) proteolysis and amino acid catabolism, (ii) lipolysis and fatty acid metabolism and (iii) the metabolism of lactose and of lactate and citrate. Considerable work has been done in University College Cork over recent decades, together with our colleagues in Teagasc Moorepark and elsewhere, into pathways of proteolysis including study of the role of indigenous enzymes, effect of novel coagulants and identification of the many peptides that are produced from the caseins during ripening. More recently, the effect of oxidation-reduction potential on cheese ripening has also been studied and shown to influence the production of certain volatile flavor compounds. The ripening of hard cheeses such as Cheddar is a slow and expensive process and so its acceleration has attracted considerable work in recent years. Many approaches to accelerated ripening have been investigated, but elevated temperatures have been shown to be the simplest and most effective. Seminal work done in Cork in the 1950s and 1960s into the milk salts system has been extrapolated to cheese in more recent years when it was discovered that the softening of Cheddar cheese early in ripening was correlated closely to the equilibrium between soluble and casein-bound calcium, which corresponds to the equilibrium that exists between soluble and colloidal calcium in milk. Further research has indicated that it is possible to modify cheese texture by controlling this equilibrium. Recent work on the functionality of low-fat cheese has concentrated on the use of hydrocolloids to improve its texture. Translucency is functional property of low-fat cheese that has also been studied in some depth. Factors that affect this parameter include temperature, levels of total and insoluble calcium, TiO₂, homogenization and addition of annatto. This presentation will provide an overview of the results of our work on the ripening of Cheddar cheese.

**Key Words:** cheese ripening, proteolysis, cheese texture

Recent advances in next-generation DNA sequencing have revolutionised our understanding of numerous microbial environments. These approaches have been employed with increasing frequency to study food-associated microbiota, including cheese. Initially many such studies were curiosity driven, but are now beginning to be used to investigate the microbial basis for microbial related food quality and safety issues. Here we describe our research in this area, with a particular focus on our investigation of the cheese-pinking phenomenon. The nucleic-acid based approaches used for this study revealed a microbial basis for this phenomenon and, armed with this knowledge, can provide a means of preventing/controlling the problem.

**Key Words:** cheese, microbiome, DNA

Cheese, a product of microbial fermentation may be defined as a protein matrix entrapping fat, moisture, minerals and solutes as well as dispersed bacterial colonies. The cheese matrix is an immensely complex and dynamic system, particularly during ripening. Knowledge gaps persist relating to the influence of manufacture parameters on structural and physicochemical characteristics of the matrix, on levels of inhomogeneity of these parameters within individual cheese blocks and in turn on their influence on the metabolic activity of entrapped bacteria. The advent of recent and more sophisticated analytical techniques, particularly in the fields of microstructure, microscopy and flow cytometry, now offers the opportunity to gain a deeper understanding of these factors during cheese ripening. This review considers levels of
in homogeneity of physico-chemical parameters such as pH observed at local level within cheese matrices and the influence of manufacture processes, including salting, on the in situ metabolic activity of starter bacteria within the cheese matrix. In addition it explores the influence of supplementation of curd with milk fat globule membrane material on subsequent cheese microstructure, ripening and sensory quality. Overall, a greater understanding of the influence of cheese manufacture parameters on microstructure and starter metabolic activity will facilitate the manufacture of cheeses with enhanced quality and consistency.

**Key Words:** cheese, microstructure, bacterial metabolic activity

### 533 Effect of dairy cow diet on the milk composition and processing characteristics of milk.

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The effect of diet on the composition, rennet gelation and heat stability of bovine milk from a spring-calved dairy herd was evaluated during 2015 and 2016. Fifty 4 cows (mean calving date, mid-February) from the Institute’s herd were allocated to one of 3 dairy treatments. Each treatment group comprised 18 cows and the groups were balanced with respect to age, lactation number, genetic merit and breed. The 3 dietary treatments were imposed from mid-February (1 d in lactation, DIL 1) to November (DIL 300): grazing grass-only pasture (G), grazing grass-clover pasture (GC) or indoors-offered total mixed ration (TMR). In 2015, milk samples were collected from each of the 3 treatments at 3 week intervals during the period June–November (133–294 DIL) and analyzed for gross composition, protein profile (reversed phase HPLC), casein micelle size (Malvern Zetasizer Nano ZS), rennet gelation (low-strain oscillation rheometry) characteristics at pH 6.55 and heat stability (140°C) over the pH range 6.2 – 7.2. In 2016, samples were again collected from each of the diet treatments and evaluated for Mozzarella cheesemaking characteristics in mid- (May–June, 94–115 DIL) and late- (Oct-Nov, 234–262 DIL) lactation. Results from 2015 showed that diet significantly affected milk composition (contents of true protein, total calcium, ionic calcium, casein micelle size) and rennet-gelation. Cheesemaking studies in showed that diet significantly affected Mozzarella yield, while having little, or no, effect on composition, texture of unheated cheeses, and cooking characteristics of heated cheese.

**Key Words:** cow, diet, milk

### 534 Profiling the flavour of dairy products from grass-based versus non-grass based milk production systems.

K. N. Kilcawley*, Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.

Dairy products from the milk of cows grazing natural swards rather than those fed preserved forages have perceived ‘added value’ among food producers and consumers based on healthiness, sensory experience and environmental acceptability. To date data to substantiate or reject such perceptions is lacking, especially in relation to sensory perception. The main focus of this presentation is to outline the impact of different forages on the sensory and volatile characteristics of milk and dairy products from on-going research in Ireland. Milk and dairy products were produced from 54 Friesian lactating cows divided into 3 distinct groups; 18 outdoors on perennial ryegrass pasture (grass), 18 outdoors on perennial ryegrass/white clover (grass/clover) and 18 indoors on total mixed ration (TMR) over a season. A chemometric approach was used to correlate volatiles with specific sensory characteristics and to monitor changes in volatiles during dairy processing and/or storage. Overall differences in forage can directly and directly impact on the volatile profiles of dairy products, some of which also affect the sensory characteristics. However, differences in volatile profiles due to forage can also be eliminated or masked during the processing and/or storage of some products. This presentation also focuses on different volatile extraction techniques, advances in gas chromatography mass spectrometry and in data processing in relation to targeted and untargeted volatile analysis of dairy products.

### 535 Cheese: Nutrition and health.

T. Beresford* and S. Sertalic, Teagasc, Cork, Ireland.

Cheese, of which there are over 1,000 varieties is a nutritious food which when consumed as part of an overall balanced diet can contribute a significant portion of the daily requirements for protein and fat as well as several important minerals and vitamins. Depending on variety a 50g serving can provide between 2 and 19g of protein and 2 and 23g of fat with an associated energy intake of between 56 and 226 kcal. Cheese is a particularly good source of calcium in a bioavailable form and one serving depending on variety can provide up to 400mg of calcium equivalent to 38% of daily needs. Similarly, a serving can provide up to 500IU and 0.19mg of vitamin A and B2 respectively or 10% of daily needs of each vitamin. However, as cheese contains added sodium and it is a relatively high fat energy dense food, there is some concern that its consumption should be limited. NaCl is added to cheese during manufacture and is a necessary part of the process. However, it is generally recommended that sodium intake should not exceed 2,000mg per day and depending on variety a serving of cheese will contribute from 15 to 700mg. Furthermore, most public health organisations currently recommend reduction in total fat and in particular saturated fat in the western diet. In cheese such as Cheddar 66% of the fatty acids are saturated, 30% are monounsaturated and 4% are polyunsaturated. However, many human studies revealed that cheese intake resulted in lower total and LDL cholesterol concentration, including reduction of triglycerides. Moreover, cheese intake had no impact on cardiovascular health and an inverse correlation between cheese intake and myocardial infarction, as well as an inverse association with the risk of stroke was reported. Most cheeses undergo extensive proteolysis during ripening resulting in the release of a diversity of peptides and amino acids. It has been demonstrated that cheese extracts rich in peptides and amino acids can encode a range of beneficial bioactivities including recent research from our group which reveals antioxidant, satiating and induction of insulin secretion activities. In conclusion, there is increasing evidence that eaten as part of a balanced diet cheese can make an overall positive contribution to nutrition and health.

**Key Words:** cheese, nutrition, health

### 536 Interfacing next-generation cheese research with industry needs: A strategic challenge.

J. Lucey*, Wisconsin Center for Dairy Research, University of Wisconsin-Madison, Madison, WI.

Over the past hundred years, we have seen remarkable developments in cheese science including aspects like the characterization of milk proteins, rennet coagulation explained, defined starter cultures, advent of genomic techniques, detailed knowledge of the biochemistry of ripening, and control of functionality. These developments have helped to fuel the worldwide growth of the cheese industry, as well as the tremendous increase in the size of manufacturing plants. The needs of industry
depend on the country, as well as the type of company, and its cheese types. Some ongoing industry needs are greater efficiency and consistency of production, better control of flavor, development of targeted flavors, cheesemaking processes that provide highest quality whey, and cheese with improved health/wellness characteristics. Unfortunately, industry is often unaware of the latest research developments and many feel that most current research efforts cannot be directly applied to meet their individual company needs. Researchers often appear uninterested in addressing industry needs (or they do not have the time to visit plants or have open discussions with them). To bridge this gap we need more opportunities, or structures, that allow industry to engage with researchers, and we need incentives (like funding) for researchers to tackle real industry needs. At our center, we include staff with industry experience in all our research teams, to help bring an applied perspective to projects. Industry problems like quality defects are a useful example where discussions can lead to very challenging research projects that can allow researchers to apply modern techniques to solve an issue, while still generating new scientific understanding. Benefits to industry of greater engagement in the research area include more focused/relevant projects as well as better access to highly trained technical research staff. We live in a time where there is an amazing array of analytical capabilities that are available to answer important scientific questions related to cheese science. How best to exploit this opportunity is a strategic challenge to both researchers and the dairy industry.

Key Words: cheese science, industry needs
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Numbers following names refer to abstract numbers. A number alone indicates an oral presentation; an M preceding the number indicates a Monday poster and a T indicates a Tuesday poster. Orals are listed first, followed by Monday and Tuesday posters in numeric order.

The author index is created directly and automatically from the submitted abstracts. If an author's name is entered differently on multiple abstracts, the entries in this index will reflect those discrepancies. Efforts have been made to make this index consistent; however, error from author entry contributes to inaccuracies.

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