Animal Health II

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 (explanatory 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 (< 0.01; greatest difference on D0: 163 units/d), MY (P < 0.01; 275) had reduced RT (< 0.01; greatest difference on D0: 163 units/d), MY (< 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. 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: amisutins, antimicrobial resistance, milk microbiome

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. Oikonomou4, E. C. R. Bonsaglia1,2, M. S. Gomes1, I. F. Canisso1, Z. Zhou1, S. F. Lima3, V. L. M. Rall2, E. F. Garrett1, G. Oikonomou4, C. R. Bicalho5, and F. S. Lima6,1, 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, Actinobacter, 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

T13 The effect of 2,4-thiazolidinedione on lipid-soluble vitamins in lactating goats induced with subclinical mastitis. C. Y. Tsai*1, F. Rosa2, M. Bionaz3, and P. Rezamand1, 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 receptor 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...
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. Plaizier1, 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(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) ceftriaxone 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 ceftriaxone 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 ceftriaxone 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 ceftriaxone.

**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. Moridi1, J. S. Osorio2, J. Lohakare2, C. Estill1, and M. Bionaz3, 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. TNFa 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. Probé1, O. Bogado Pascottini*2, S. LeBlanc2, G. Opsomer3, and M. Hostens3, 1Central Laboratory, Veterinary Teaching Hospital, University of Milan, Lodi, Italy, 2Population Medicine, Ontario Veterinary College, University of Guelph, Guelph,
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 for each MD and MD+ are depicted in Table 1. Setting the health cows as reference, the 120 DIM hazard ratios for culling were MD 2.1; MD+ 2.9; TWIN 2.8; TWIN+ 3.0; MF 3.3; MF+ 4.6; RFM + 2.7; MT + 1.8; KT + 3.3; DA 5.5; DA+ 4.5; MAST 3.1; and MAST+ 2.3 (all P ≤ 0.01). In conclusion, the presence of MD or MD+ during the transition period was associated with increased culling rate in the first 120 DIM. The culling hazard was greater when a MD was complicated with another metabolic disease (MD+). In this case, the study performed in a well-managed large farm, uncomplicated cases of RFM and MT did not have an influence on the 120 DIM culling risk.

Key Words: dairy cow, metabolic disease, survival analysis

T18 Risk factors for subclinical mastitis in grazing dairy cows. R. R. Daros*, M. J. Hötzel, S. J. LeBlanc, J. A. Bran, A. J. Thompson, and M. A. von Keyserlingk, 1Animal Welfare Program, Faculty of Land and Food systems, University of British Columbia, Vancouver, BC, Canada, 2Laboratório de Etiologia Aplicada e Bem-Estar Animal, Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil, 3Population Medicine, Ontario Veterinary College, University of Guelph, Canada, Guelph, ON, Canada.

Mastitis is one of the greatest health challenges facing the dairy industry. Knowledge of potential risk factors is key to designing preventive protocols to manage this disease. The aims of this study were to determine risk factors for subclinical mastitis (SCM) in grazing cows. A total of 42 dairy farms in southern Brazil were visited twice, 4 mo apart. All farms used a rotational grazing system with 2 to 3 fresh paddocks per day. Breeds were Holstein, Jerseys and their crosses. At each visit, milk samples from every quarter from every lactating cow were subjected to a California Mastitis Test (CMT). Cows having at least one quarter with a CMT score ≥1 were considered positive for SCM. Individual cows were also locomotion scored (LS; scale 1 to 5, LS ≥ 3 as lame)

while exiting the parlor. Continuous data were categorized to improve model fit as follows – DIM: early (<100 DIM), mid (101 to 200 DIM) or late (>200 DIM) lactation; BCS: thin (BCS < 2.75), adequate (2.75 ≤ BCS ≤ 3.5) or over-conditioned (BCS > 3.5); and parity: primiparous and multiparous. A multivariable logistic regression model (cow nested within farm as random effects) was used to identify risk factors for SCM.

A total of 2,606 cows were assessed for SCM, however only 1,987 cows with complete data were used in the model. Prevalence of SCM was 47%. Risk factors for SCM included DIM, parity and lameness status. Mid-lactation and late-lactation cows were 1.4 (95% CI: 1.1 – 1.9; P < 0.01) and 2.2 (95% CI: 1.7 – 2.8; P < 0.01) times more likely to have SCM than early-lactation cows. Primiparous cows were less likely (OR = 0.4; 95% CI: 0.3 – 0.5; P < 0.01) to have SCM than multiparous cows. Lameness also increased the likelihood of a cow having SCM by 1.7 times (95% CI: 1.3 – 2.0; P < 0.01). Over-conditioned cows tended to have increased likelihood (OR = 1.5; 95% CI: 0.9 – 2.1; P = 0.09) 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

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 (EffertCep (EF) and EffertCepSG (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 EffertCep tablets were added to 1893 mL (0.5 gal) of water. One EffertCepSG 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. Both solutions 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). 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
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

**T20  Associations of dry-off management and somatic cell count in robotic milking systems.** F. H. Padua, M. T. M. King* , 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:** antioxidant, mineral, vitamin

**T22  Effect of somatic cell count around service on the fertility of grazing dairy cows.** N. Lorenti1, R. Rearte2,5, M. Giuliodori3, and R. de la Sota4,5. 1Práctica Privada, Brandens, 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+) 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,

**Key Words:** robotic milking, dry off management, somatic cell count
Table 1 (abstract T22). Logistic regression model assessing the odds of conception at first artificial insemination (FAI) in grazing dairy cows (n=6642)  

<table>
<thead>
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<th>Model</th>
<th>N</th>
<th>CR (%)</th>
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<th>95% CI</th>
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<td>41.3</td>
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<td>0.79–1.10</td>
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<tr>
<td>Chronic case</td>
<td>1,403</td>
<td>38.0</td>
<td>0.85</td>
<td>0.74–0.99*</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.

**Key Words:** robotic milking, dairy cow, hyperketonemia

T24 Can the use of ceftiofle 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.

Ceftiofle is a 4th generation cephalosporin that is approved in Brazil to treat Streptococcus agalactiae subclinical mastitis (SM). Nonetheless, ceftiofle 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 ceftiofle (CEFIMM), or intramuscular ceftiofle (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, ceftiofle, 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. simulans, 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 x 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 pasA 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 R² = 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 peripartal dairy cows fed ethyl-cellulose rumen-protected methionine is associated with better performance and immunometabolic status. F. Batistet*, B. Saremì, C. Parys, E. Trevisi, and J. J. Loor, 1University of Illinois at Urbana-Champaign, Urbana, IL, 2Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany, 3Università Cattolica del Sacro Cuore, Piacenza, Italy.

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 = (Albumin - 17.71)/1.08 + (Cholesterol - 2.57)/0.43-(Bilirubin - 4.01)/1.21] 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 DMI (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, postpartum DMI (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 paroxonase (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*, A. Delia Libera, and H. Sullivan, 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 method was fitted utilizing 3 combinations of potential variables: milk components and DairyComp 305 data), or all variables, resulting in 9 models. All models were evaluated 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). The ANN method performed the greatest, particularly the ANN-all with an r², RMSE, and AUC of 0.23 ± 0.02, 0.45 ± 0.07, and 0.81 ± 0.01, respectively. PLS models performed intermediately 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. Paudyal*, G. Pena2, P. Melendez3, A. Villarreal1, N. Roman-Muniz1, and P. Pinedo1, 1Colorado State University, Fort Collins, CO, 2Advanced Animal Diagnostics, Morrisville, NC, 3University of Missouri, Columbia, MO, 4Afimilk USA, Fitchburg, WI.

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. Quarters 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 LR, 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 leukocytes 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. Firth*, C. Schleichert, A. Käsbohrer, and W. Obritzhauser, 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 \( (P = 0.264, \text{Chi}^2 \text{ test}) \). The correlation between the use of antibiotics at dry off and whether the CMT was regularly used was not significant \( (P = 0.807, \text{2-sided Fisher’s exact test}) \). 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
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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 2×, 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 cows 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. 23.6% \( (P < 0.05) \). During fall, cows with BCS ≥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 \( (P > 0.05) \). 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* 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, CD172a was 95.2% ± 0.4% and monocytes became undifferentiated macrophages 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
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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 \( (\text{IMR} n = 90; \text{CON} n = 97) \) and multiparous cows \( (\text{IMR} n = 230; \text{CON} n = 224) \ 7 \text{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 

\( P < 0.0001 \) \). 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*, 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. Khidoyatov3, 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 (NFκB1; $P = 0.02$) 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 - Invitik. 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 spp. 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

**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 academic 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 1 January 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):

\[
#TD_{100} = \frac{\sum_{i=0}^{n} \text{amount of active substance (mg)}}{\text{DDD × prod days (d)} \times \text{std weight (kg)} \times 100}
\]

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

**T39 Explaining farmers’ adaptation of preventive measures against mastitis—An application of Theory of Planned Behavior.** N. Lind*1, H. Hansson1, U. Emanuelson2, 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

**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. Fourcas2, and R. Rupp1, 1INRA GenPhySE, Castanet-Tolosan, France, 2Université de Toulouse 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 to 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 mammary 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 Vries2, L. W. Tauer3, 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 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, $736 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

T42 **Cow-level risk factors for clinical and subclinical mastitis in New York dairy cattle.** A. M. Miles*, J. A. A. McArt, P. D. Virkler, and H. J. Huson, Cornell University, Ithaca, NY.

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 d 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 PUFA 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 ω-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.