M13 Impact of palmitic acid and pH on ruminal NDF digestibility and fermentation in a continuous culture system. L. Padilla*, A. Sears, and F. Batistel, Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT.

Non-rumen bacteria incorporate exogenous long-chain saturated fatty acids to change membrane fluidity under low pH conditions. We hypothesized that rumen bacteria use a similar mechanism, thus, providing saturated fatty acids in the diet could support bacterial metabolism and growth and consequently enhance fiber digestibility. The objective of this study was to evaluate the effects of dietary palmitic acid and pH on ruminal NDF digestibility and fermentation. The study was conducted as a 2 × 2 factorial treatment arrangement in a replicated 4 × 4 Latin square using continuous culture fermenters (n = 8). Treatments were a control diet without supplemental fatty acids or the control diet plus 1.5% of palmitic acid factorized with normal pH (diurnally ranging from 6.4 to 7.0) or low pH (diurnally ranging from 6.0 to 6.4). The control diet (40 g DM/day) was a 50:50 orchardgrass hay:concentrate mixture that provided 5.8 g CP, 14 g NDF, 7.3 g starch, and 1 g fatty acids fed twice daily. The fatty acid treatment maintained the same nutrient input into the fermenters as the control except for fatty acids. Both buffer solutions (normal and low pH) were delivered continuously at rate of 10%/h. Data were analyzed using a mixed model including the fixed effect of pH, fatty acid, and its interaction, and the random effects of period and fermenter. Data are reported as least squares means with differences declared at P ≤ 0.05. No interaction between fatty acids and pH were observed for the variables measured. Compared with control, palmitic acid increased NDF digestibility (45.2 vs. 39.34%, P = 0.03). The lower pH decreased NDF digestibility in 8.2 percentage units compared with normal pH (46.4 vs. 38.16%, P < 0.01). Furthermore, lower pH decreased ammonia (7.30 vs. 5.64 mg/dL, P = 0.01) and total VFA concentration (168 vs. 138 mmol/dL, P = 0.02) compared with normal pH; palmitic acid did not affect ammonia nor total VFA concentration. Our preliminary data indicate that rumen pH and palmitic acid independently affect NDF digestibility and rumen fermentation. Palmitic acid supplementation increased ruminal fiber digestibility under low and normal pH conditions.

Key Words: fatty acids, rumen, pH

M14 Effects of fatty acid supplementation to prepartum dairy cows on immunity in dams and their offspring, A. Schroeder* and M. Eastridge, The Ohio State University, Columbus, OH.

The objectives of this study were to investigate the effects of fatty acid supplementation differing in ω-6:ω-3 to prepartum dairy cows on colostrum yield and quality and immunity of the dam and calf. The focus is on improving passive immunity and long-term health measures in calves. 53 nonlactating pregnant Holstein cows were allocated to blocked and randomized around 3 feeding groups considering parity and date of expected calving. All cows were fed ad libitum a TMR formulated at 14% CP and a DCAD of −7.3 mEq/100 g. The TMR consisted of corn silage (42.6%), grass haylage (28.4%), concentrate mix (27.3%), and top-dressed with 1 of 3 treatments consisting of 50% corn and 50% of the fat supplements (1.7%: −0.227 kg/d; R4 (n = 18) with 23 g/d Prequel and 91 g/d Strata (Virtus Nutrition LLC, Coralcor, CA; ω-6:ω-3 (R) = 4); R6 (n = 18) with 64 g/d Prequel and 50 g/d Strata; and R8 (n = 17) with 91 g/d Prequel and 23 g/d Strata. Treatments were initiated at 21 d before expected parturition, at which time the cows were moved from group housing to individual maternity stalls. Feed offered and refused from individual cows were used to determine daily DMI; BW and body condition score (BCS) were determined at the beginning of the trial and weekly until calving. Blood was collected from the jugular vein when calving was imminent. Fresh animals were milked within 2 h of calving; 4L of fresh colostrum from the dam were bottle fed to each calf, and samples of colostrum and blood from the calf were collected at hour 0 and blood again at 48 h. The mixed model procedure of SAS was used for data analysis with block as the random variable. Data with P < 0.05 was regarded as significant and P < 0.15 a trend. DMI tended (P = 0.11) to be higher for R4 than R6 and R8 at 12.1, 11.6, and 11.6 kg/d, respectively. BW (718 kg) and BCS (3.38) of the dams neither differed by treatment nor was there a treatment by week interaction. Yield (6.1 kg) and Brix reading (26.1) of the colostrum was similar among treatments. Calf birth weight (42.3 kg) also was similar among treatments. Immune function measures will include concentrations of IgG in both the dam and calf and lymphocyte blastogenesis in the calf. Other than for DMI on dams, physical measures were similar among treatments, but further investigation on immune function will provide additional insight to whether ω-6:ω-3 can impact fetal and neonate development.

Key Words: immunity, fatty acids, prepartum

M15 Empirical modelling of vitamin B₁₂ duodenal flow in lactating dairy cows. V. Brisson*, C. L. Girard#, J. A. Metcalf³, D. S. Castagnino¹, J. Dijkstra², and J. L. Ellis¹, ¹University of Guelph, Guelph, ON, Canada, ²Agriculture and Ag Food Canada, Sherbrooke, QC, Canada, ³Trouw Nutrition Canada, Guelph, ON, Canada, ⁴Wageningen University and Research, Wageningen, the Netherlands.

Unlike other B vitamins, vitamin B₁₂ is not found in plants and is manufactured only by bacteria. Therefore, supply to the dairy cow, unless provided via supplementation, will mainly be the result of B₁₂ manufactured by ruminal microbes. The duodenal flow of B vitamins therefore represents the amount of vitamin available for absorption by the ruminant, which can be used for essential metabolic functions and milk production. However, diet composition may affect ruminal synthesis and the resulting duodenal flow (DF) of vitamin B₁₂ due to alterations to fermentation and ruminal conditions. Therefore, the objective of this study was to conduct a meta-analysis describing how diet composition affects DF of vitamin B₁₂. Data were collected from 340 individual lactating cows involved in 16 published studies. Saved diet and duodenal samples from these studies were subsequently reanalyzed for B vitamin content to create the database used in the present study. Potential driving variables considered included (DM basis) dietary organic matter (%), NDF (%), starch (%), crude protein (%), and DMI (kg/d). The meta-analysis was conducted in 3 steps, followed by statistical evaluation of the resulting empirical models. A Spearman correlation matrix was constructed between all potential driving variables to assess for collinearity between X variables, and guide model creation. Then, using Cook’s distance statistic (Proc MIXED), outliers were determined and removed. Finally, a suite of potential models (with study treated as a random effect) were developed in GLIMMIX. Where models were statistically significant, evaluation was completed using root mean square prediction error (RMSPE) and concordance correlation coefficient (CCC) to determine the sources of error. The best performing model was: B₁₂DF (mg/d) = −7.87 (±2.46) + 0.29 (±0.056) × DietNDF(%) + 0.44 (±0.042) × DMI (kg/d); RMSPE: 41.1%, CCC: 0.268. In conclusion, DF of B₁₂ was positively impacted by both the overall DMI and the dietary NDF content of the diet. This information may be used to better understand supply of vitamin B₁₂ to the modern dairy cow, in relation to requirements, to improve milk production efficiency.

Key Words: vitamin B₁₂, meta-analysis, duodenal flow

M16 Genome-wide association study and functional analyses of clinical and subclinical ketosis in Holstein cattle. R. A. N. Soares*, G. Vargas, F. S. Schenklen, and E. J. Squires, University of Guelph, Guelph, ON, Canada.

Ketosis affects high yielding cows and it is one of the most frequent metabolic diseases in dairy cows causing economic losses. Therefore,
finding genetic markers for gene variants associated with resistance to ketosis is of interest to genetically select for less susceptible cows. The aim of this study was to identify and investigate genomic regions associated with clinical and subclinical ketosis in Holstein cattle. To achieve this, weighted single step genome-wide association study (wssGWAS) was performed considering 4 traits: clinical ketosis in first (CK1) and later lactations (from 2 to 5; CK2), and subclinical ketosis in first (SCK1) and later lactations (from 2 to 5; SCK2). The estimated breeding values (EBV) from 77,277 cows and 7,704 bulls were de-regressed and used as pseudo-phenotypes in the GWAS. The wssGWAS model was: y* = µ + (EBV) from 77,277 cows and 7,704 bulls were de-regressed and used as pseudo-phenotypes in the GWAS. The wssGWAS model was: y* = µ + Z α + e, where y* is the vector of pseudo-phenotypes; µ is the overall mean; Z is a matrix that relates animals to pseudo-phenotypes; α is the vector of additive genetic effects and e is the vector of random residuals. The top-20 genomic regions explaining the largest proportion of the genetic variance were investigated for putative genes associated with the traits through functional analyses. Regions of interest were identified in chromosomes 2, 5 and 6 for CK1, 3, 6 and 7 for CK2, 1, 2 and 12 for SCK1 and 20, 11 and 25 for SCK2. The highest proportion of genetic variance explained by a region was located on BTA2 for SCK1. The chromosomes 2, 5 and 6 for CK1, 3, 6 and 7 for CK2, 1, 2 and 12 for SCK1 and 20, 11 and 25 for SCK2. The highest proportion of genetic variance explained by a region was located on BTA2 for SCK1. The highlighted genes potentially related to clinical and subclinical ketosis included ACAT2 and IFG1. Enrichment analyses of the candidate genes for the traits showed molecular functions and biological processes that are associated with fatty acid metabolism, synthesis and degradation of ketone bodies and inflammatory response. Several genomic regions and SNPs related to susceptibility to ketosis in dairy cattle, which were previously described in other studies were confirmed here. In addition, some novel potential regions were found that would warrant further investigation on their potential association with clinical and subclinical ketosis.

**Key Words:** association study, ketones, negative energy balance

**M17** Mammary blood vessel development in response to estradiol administration in heifer calves, N. R. Hardy*1, K. M. Enger1, M. L. Eastridge2, L. E. Moraes3, and B. D. Enger1, 1The Ohio State University, Department of Animal Sciences, OARDC, Wooster, OH, 2The Ohio State University, Department of Animal Sciences, Columbus, OH.

Mammary blood flow is central to mammary growth, development, and productivity, but development of the vasculature network is poorly understood. The objective of this study was to determine how the vascular system adapts to mammary growth by inducing different levels of mammary growth and examining 2 regions of the mammary tissue. Holstein heifer calves received 12 daily injections on the days immediately preceding euthanasia at 82 d of age. Treatments were control (n = 4, CON), short-term estradiol (n = 4, SHORT), and long-term estradiol (n = 4, LONG). CON calves received corn oil injections while SHORT calves received 9 injections of corn oil followed by 3 injections of estradiol; LONG calves received 12 estradiol injections. Mammary tissues were collected from the center and edge parenchymal regions of all right rear mammary glands to quantify the tissue area of various tissue structures, the percentage of proliferating epithelial cells, and the number and form of blood vessels. Data were analyzed using PROC MIXED with the fixed effects of calf treatment and parenchymal region, and calf nested within treatment as a random effect. Results showed LONG calves had a greater tissue area occupied by epithelium (34.0% ± 1.5, P < 0.05) than CON and SHORT calves (21.4% and 23.0% ± 1.5, respectively), while CON and SHORT calves were similar. Edge parenchyma had a greater percentage of proliferating epithelial cells than center parenchyma across all treatment groups. Within the edge region, LONG calves had the greatest percent of proliferating epithelial cells (P < 0.05). Blood vessel number per unit of tissue area was greater in center than edge parenchyma (395 vs 295 ± 19 vessels/mm2, P < 0.001, respectively); the corresponding vessel surface area/unit of tissue area followed this same pattern (23,140 vs 18,166 ± 1088 μm/mm2, P < 0.001). These vessel measures were not affected by estradiol treatment (P > 0.1). These results show there is a large difference in blood vessel number in the center versus the edge parenchyma and estradiol treatment elicits mammary growth but not necessarily increases in blood vessel formation.

**Key Words:** angiogenesis, growth, endothelium

**M18** Effects of physically effective undigested neutral detergent fiber and rumen fermentable starch on lactation performance and total tract digestibility of lactating cows, K. M. Smith*1, A. Obata2, K. Hirano2, H. Uchihori2, S. Y. Morrison1, and R. J. Grant1, 1Miner Institute, Chazy, NY, 2ZEN-NOH, Tokyo, Japan.

Multiparous cows (n = 16) were used in a 4 × 4 replicated Latin square design to evaluate the effect of feeding different dietary concentrations of 240-h physically effective undigested neutral detergent fiber (peuNDF240) and rumen fermentable starch (RFS) on intake, milk yield and composition, and total-tract digestibility (TTD). Diets differed in peuNDF240 and RFS by inclusion of different corn silage hybrids and cornmeal amount. Treatments were 1) low peuNDF240 (6.4%DM), low RFS (16.7%DM; LULR) 2) low peuNDF240 (6.1%DM), high RFS (19.2%DM; LURH) 3) high peuNDF240 (8.6%DM), low RFS (16.9%DM; HULR) 4) high peuNDF240 (8.0%DM), high RFS (19.0%DM; HUHR). On d 19–28 of each 28-d period, samples for dry matter intake (DMI), milk yield, milk composition, behavior, rumen pH, rumen fluid, and TTD were taken. Data were summarized by period and analyzed with model effects of diet, period, and replicate using MIXED procedure of SAS (v.9.4). Cow within replicate was a random effect. No significant treatment differences (P > 0.05) were observed for (mean ± SEM) DMI, total chewing time (809 ± 14 min/d), daily mean rumen pH (6.1 ± 0.1), total ammonia (7 ± 10 mg/dL), and total VFA (125 ± 3 mM). Cows fed LULR had higher 3.5% fat-corrected milk (FCM) compared with cows fed LUEHR with HI diets being intermediate (Table 1). The LULR diet had greater TTD of neutral detergent fiber (NDF) compared with HI diets, and LUEH diets had lower TTD of starch compared with HI diets. Overall, this data shows that there is a balance when adding fermentable starch to diets with highly digestible fiber in the physically effective fraction, and lesser effect in diets with less digestible fiber in the physically effective fraction.

**Table 1 (Abstr. M18).**

<table>
<thead>
<tr>
<th>Item</th>
<th>LULR</th>
<th>LURI</th>
<th>HULR</th>
<th>HUHR</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>29.7</td>
<td>29.4</td>
<td>29.4</td>
<td>29.2</td>
<td>0.7</td>
<td>0.56</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>53.1*</td>
<td>52.0b</td>
<td>51.2b</td>
<td>51.5b</td>
<td>1.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.59</td>
<td>3.48</td>
<td>3.74</td>
<td>3.60</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>FCM, kg/d</td>
<td>53.8*</td>
<td>51.5b</td>
<td>52.9b</td>
<td>52.2b</td>
<td>1.3</td>
<td>0.05</td>
</tr>
<tr>
<td>TTD NDF, %</td>
<td>62.0*</td>
<td>60.1b</td>
<td>58.6b</td>
<td>57.6b</td>
<td>0.59</td>
<td>0.006</td>
</tr>
<tr>
<td>TTD starch, %DM</td>
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<td>98.5b</td>
<td>98.9b</td>
<td>98.7b</td>
<td>0.14</td>
<td>0.002</td>
</tr>
</tbody>
</table>

aLeast squares means within row without common superscript differ (P ≤ 0.05).

**Key Words:** undigested fiber, rumen fermentable starch, physically effective fiber

**M19** Effects of probiotics, enzymes, and yeast combinations on ruminal fermentation in a dual-flow continuous culture system. S. Bennett*1, J. A. Arece-Cordero1, V. L. N. Brandao2, J. R. Vinyard3, B. Agustinho4, H. F. Monteiro1, L. Tomaz2, R. Lobo1, and A. P. Facioli1, 1University of Florida, Gainesville, FL, 2State University of Maringá, Maringá, Paraná, Brazil, 3Sao Paulo State University, Sao Paulo, Sao Paulo, Brazil.

The objective of this study was to evaluate the changes in ruminal fermentation when the diet is supplemented with different combinations of probiotics, enzymes, and live yeast. Our hypotheses were (1) inclusion of additives would increase nutrient digestibility and volatile fatty acid concentration, and (2) increasing additive doses would lead to further im-
provements in digestibility. Diets were randomly assigned to 8 fermentors in a replicated 4 × 4 Latin square with four 10-d experimental periods, consisting of 7 d for diet adaptation and 3 d for sample collection. Diets contained 50:50 forage:concentrate and fermentors were fed 106 g of dry matter per day divided equally between 2 feeding times. Treatments were control; bacterial culture/enzyme blend (1.7 mg per day); bacterial culture/enzyme blend with live yeast (49.76 mg per day); and double dose of the bacterial culture/enzyme blend with live yeast treatment (99.53 mg per day). The bacterial culture/enzyme blend contained 5 strains of live bacteria with a concentration of 10^{10} cfu (Lactobacillus animalis, Propionibacterium freudenreichii, Bacillus licheniformis, B. subtilis, and Enterococcus faecium) and 3 enzymes (amylase, hemicellulase, and xylanase). The yeast component was Saccharomyces cerevisiae. On d 8 and 9, samples were collected for pH, redox, volatile fatty acid (VFA), lactate, NH_{3}-N and digestibility measurements. Statistical analysis was performed using the GLIMMIX procedure of SAS with fermentor, square and period as random effects. Significance was declared at \( P \leq 0.05 \). No effects were observed for pH, redox, NH_{3}-N, acetate, isobutyrate, valerate, total VFA, acetate:propionate, nutrient digestibility or N utilization. Furthermore, no effects were observed for the hourly propionate molar proportion. Within the pooled effluent samples, butyrate increased with the inclusion of additives when compared with the control while propionate had a tendency to decrease. In conclusion, the addition of probiotics, enzymes, and yeast to the diet increased butyrate concentration.

Key Words: butyrate, Lactobacillus, Saccharomyces cerevisiae