The objective of this study was to evaluate the effects of heat stress on the fecal microbiome of lactating dairy cows. We hypothesized there would be an increase in the richness and diversity of the bacterial communities in the feces of heat stressed cows. Six Holstein cows were housed in tie stalls in an environmental chamber. Cows averaged 175 ± 7 d in milk, 1.5 ± 0.5 parities and 36.3 ± 3.7 kg/d of milk and were fed and milked twice-daily. Cows were allowed 5-d acclimation to the chambers (d −5 to 0; temperature humidity index (THI) ~65) and were then subjected to constant heat stress for 16d (d0 to 16; THI ~76), followed by a 9d recovery period (d16 to 24; THI ~66). Feed and water were available ad libitum. Fecal samples were collected per rectum on d −1, 0, 6, 13, 16, 20 and 24. Samples were immediately frozen at −20°C. Fecal DNA was extracted using PowerFecal kits (Qiagen), the V4 hypervariable region of the 16S rRNA gene was sequenced using the Illumina MiSeq platform, and operational taxonomic units (OTUs) were assigned to the SILVA database using BLAST based on a 97% nucleotide identity to evaluate richness and composition of fecal bacterial populations. Number of OTUs, and diversity assessed by the Shannon Index (Bonferroni r-test), increased approximately 20% and 5%, respectively, during heat stress compared with d −1 (P < 0.05, ANOVA). Diversity returned to d −1 levels by d 20 whereas the total number of OTUs was not fully restored until d 24. Principal component analysis (Bray-Curtis distances) revealed that community composition was similar during acclimation but diverged during heat stress and into the recovery period (P < 0.001, PERMANOVA). Results revealed individual OTUs displaying markedly different patterns of abundance across the experiment (P < 0.05). For example, compared with the acclimation period, Bacteroides species abundance increased 112% by d 16 of heat stress, then increased an additional 15% by d 24. We conclude that heat stress altered the fecal microbiome of lactating dairy cows affecting both diversity and abundance of individual OTUs. Altered fecal microbiome may impact gut health or environmental pathogen loads.

Key Words: bacteria, hyperthermia, metagenomics

M11 Evaluating the impact of stage of life on anti-Müllerian hormone in dairy cattle. K. Alward1, W. Graves1, R. Palomares2, A. Nelson1, and J. Bohlen1, 1Animal and Dairy Science, University of Georgia, Athens, GA, 2College of Veterinary Medicine, University of Georgia, Athens, GA.

Anti-Müllerian hormone (AMH) is produced by granulosa cells, found in early, antral follicles on the ovary. Higher circulating AMH concentrations are indicative of a larger number of viable follicles present and published data shows that heifers with high AMH have longer productive lives than low AMH counterparts. The objective of this study was to examine whether stage of life at sampling affects AMH level and to describe changes in AMH level from virgin heifers through calving and early lactation. Virgin Holstein heifers (n = 111) meeting minimum weight and height requirements were enrolled pre-breeding at 13–15 mo of age. Upon enrollment, blood was collected and analyzed for AMH (Ansh Labs, Webster, TX) and transrectal ultrasonography was performed to record antral follicle count (AFC). In addition, presence of corpora lutea (CL) were noted for cyclicity status and any reproductive tract anomalies were recorded. Heifers were inseminated upon standing estrus with all service data recorded. After calving, blood was collected at 5–20 d fresh and at 45–60 d in milk (DIM) to analyze for AMH concentration. Transrectal ultrasonography was also performed at 45–60 DIM and the same parameters were recorded as previously described. Following sampling, heifers were split based on AMH concentration to form a HIGH AMH group (>275 pg/mL) and LOW AMH group (<275 pg/mL) for analysis. AMH was consistent across stages of life with animals maintaining their categorization as HIGH or LOW throughout all 3 sampling times (P < 0.0001). As heifers, the high AMH categorization correlated with fewer services per conception (P < 0.0001). AMH and AFC were positively correlated when evaluated as heifers and at 45–60 DIM in the lactating herd (P < 0.0001). AMH was in the highest concentration within the heifer group (P < 0.0001) while the 45–60 DIM sample was higher than fresh (P < 0.0001). This data
indicates that calving may temporarily depress AMH concentrations; however, these animals recover and maintain their AMH categorization.

Key Words: anti-Müllerian hormone, stress, fertility

M12 Effects of timing of artificial insemination and use of semen extenders on fertility of dairy heifers subjected to timed artificial insemination. C. C. Figueiredo*1, D. Z. Bisinotto1, R. C. Chebel1, R. Le Boucher2, S. Camugli2, E. Schmitt2, C. Arnoult2, W. W. Thatcher1, and R. S. Bisinotto1, 1University of Florida, Gainesville, FL, 2INV-Technologies, L’Aigle, France, 3University of Grenoble Alpes, Grenoble, France.

Approximately 25% of dairy heifers subjected to timed AI are observed in estrus 24 h before prescheduled AI, creating an asynchrony between ovulation and insemination. Objectives were to evaluate the effects of timing of AI and use of 2 semen extenders (SE) on pregnancy per AI (P/AI) and pregnancy loss. Holstein heifers (≥13 mo of age, body weight ≥350 kg, wither height ≥122 cm) received GnRH and an intravaginal insert containing progesterone on study d−8. Inserts were removed 5 d later and heifers received injections of PGF2α on d−3 and −2, followed by GnRH on d 0. Heifers were assigned randomly to receive timed AI with untreated semen on d 0 (72 h after first PGF2α positive control; 72-CON; n = 103), timed AI with untreated semen on d−1 (48 h after first PGF2α; negative control; 48-CON; n = 100), timed AI on d−1 with SE1-treated semen (48-SE1; n = 98), timed AI on d−1 with SE2-treated semen (48-SE2; n = 102). A total of 4 bulls were used. Sample size was sufficient to detect a 20%-percentage point difference among treatments (55 vs. 35%; α = 0.05; β = 0.20). Heifers were fitted with automated estrus detection monitors. Pregnancy was diagnosed by transrectal ultrasonography 29 and 54 d after AI. Data were analyzed by logistic regression. Statistical models included fixed effects of treatment and enrollment week. Orthogonal contrasts were used to assess the effects of day of AI (72-CON vs. 48-CON+48-SE1+48-SE2), use of semen extenders (48-CON vs. 48-SE1+48-SE2), and extender type (48-SE1 vs. 48-SE2). Alteration of estrus between 24 and 48 h after first PGF2α was detected in 66.6% of heifers. Pregnancy per AI on d 29 (72-CON = 60.1, 48-CON = 55.4, 48-SE1 = 47.6, 48-SE2 = 52.3%) and 54 (72-CON = 77.8, 48-CON = 73.1, 48-SE1 = 63.0, 48-SE2 = 70.7%) was greater (P < 0.001) for heifers inseminated on d 0 compared with d−1; however, no effect semen extender or extender type was observed. Treatment did not affect pregnancy loss (72-CON = 31.4, 48-CON = 7.9, 48-SE1 = 7.3, 48-SE2 = 5.6%). Hastening AI by 24 h decreased likelihood of pregnancy, which was not improved by the use of semen extenders.

Key Words: semen extender, fertility, sperm lifespan

M13 Using 30-h in vitro NDF digestibility of feedstuffs in ration formulation: evaluation of predictions for milk and methane production in lactating dairy cows. K. C. Krogstad*1, D. L. Morris1, P. J. Kononoff1, and K. J. Herrick2, 1Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE, 2POET Nutrition, Sioux Falls, SD.

In vitro NDF digestibility (NDFd) is often used to determine forage quality, and its increased availability has spurred interest for use in ration formulation procedures. The objectives of this study were to compare the predictions of metabolizable energy (ME) available milk and methane production when default NDFd values or lab-determined 30-h NDFd were used in the Cornell Net Carbohydrate and Protein System (CNCPS; v 6.5). We hypothesized that the use of 30-h NDFd would improve ME-available milk and methane predictions from CNCPS. The 30-h NDFd was measured on forages and byproducts included in diets fed to lactating dairy cows. Predictions of carbon dioxide production and DMI were also evaluated. Treatment means (n = 32) originating from 8 energy balance studies were assembled to evaluate the prediction of ME allowable milk and methane production when either a default value or observed 30 h NDFd was entered into the feed library. Of the 32 means, 26 were made on Jersey cows and 6 were made on Holstein cows. Our data set averaged 18.8 ± 2.26 kg of DMI, 25.8 ± 6.00 kg of milk, and 15.1 ± 2.65 L of methane/kg of milk. Headbox-style indirect calorimeters were used to measure methane and carbon dioxide production in all studies. Comparison statistics were generated using R (v 3.5.2) and predictions were evaluated based upon the root mean square prediction error (RMSPE). A concordance correlation coefficient (CCC) was used to evaluate the agreement between the predicted and observed values. Carbon dioxide and the mean of the upper and lower bounds of predicted DMI resulted in CCC of 0.48 and 0.75 and RMSPE of 8.8% and 20.5% of the mean, respectively. The use of lab-determined NDFd resulted in a decreased CCC (0.87 to 0.82) and an increased RMSPE (10.7% to 12.5% of the mean) for ME allowable milk but an increased CCC (0.55 to 0.62) and reduced RMSPE (18.4% to 15.7% of the mean) for methane production. These results suggest that determination of NDFd may not be advantageous in predicting milk yield but may improve prediction of methane production.

Key Words: neutral detergent fiber, digestibility, Cornell Net Carbohydrate and Protein System (CNCPS)

M14 Ruminal degradation and intestinal digestibility of hydrolyzed feather meal with and without blood. K. Buse*, D. Morris, and P. Kononoff, University of Nebraska-Lincoln, Lincoln, NE.

Hydrolyzed feather meal (HFM) is a readily available, high protein feedstuff that can be used as a cost-effective dairy feedstuff. Because the production process may vary, the chemical composition of HFM may also vary. Additionally, some processes may incorporate blood into the final product. The objective of this study was to evaluate the ruminal and intestinal digestibility of HFM originating from processes that differ in their inclusion of blood. Ten samples of HFM, 5 without blood (FM) and 5 with blood (FMB), were collected from 10 different production plants across the United States. Two multiparous lactating Holstein cows fitted with rumen and proximal duodenal cannulas were used to quantitate rumen undegradable protein (RUP), and RUP digestibility (dRUP) by employing the mobile bag technique. Approximately 1.5 g of each was weighed into 10 N-free nylon bags with a mean pore size of 50 μm and a dimension of 5 × 10 cm and incubated in the rumen for 16 h. A subset of rumen bags were then used to determine RUP. The remaining bags were placed in a pepsin-HCl bath for 3 h and then inserted in the duodenal cannula of each cow. Bags were recovered in the feces and used to quantify RUP. Data were analyzed as a complete randomized design to test the effect of blood inclusion on RUP and dRUP of HFM. The CP content was similar (P = 0.57) between FMB and FM averaging 49.5 ± 0.90%. The RUP content of FM tended (P = 0.13) to be greater than FM (81.6 vs. 74.1 ± 3.19%). The dRUP was not different (P = 0.77) averaging 61.1 ± 2.36% across treatments. There was also no difference detected (P = 0.40) between FMB and FM in total-tract DM (P = 0.40) and CP (P = 0.52) digestibility averaging 74.2 ± 3.34 and 69.4 ± 4.07%. Results of this study suggest that although there are modest differences in chemical composition in hydrolyzed feather meal associated with the
inclusion of blood, very little differences are observed in either ruminal or intestinal digestion of protein.

**Key Words:** intestinal digestibility, rumen degradation, rumen undegraded protein

**M15  Effect of feeding switchgrass hay to dairy cows during the dry period.** J. F. Rivera1, S. W. Gee1, J. C. DeBruyn2, A. Heeg3, M. Thimmanagari3, and A. J. Carpenter1, 1Department of Animal Biosciences, University of Guelph, Ridgetown, ON, Canada, 2Ontario Ministry of Food, Agriculture, and Rural Affairs, Guelph, ON, Canada.

Switchgrass (*Panicum virgatum*) is a native tall grass species. Hay from this forage is high in fiber and low in potassium, making it a promising feed for dry cows. Our objective was to evaluate the effect of feeding switchgrass hay or straw in a single dry period ration. Holstein cows (*n* = 36) were randomly assigned to one of two “controlled energy” diets at dry-off. Forage in the TMR consisted of an approximately 50:50 ratio of corn silage and either switchgrass hay (SGR) or wheat straw (CON). Cows were fed twice daily during the dry period, and refusals were weighed once daily. After calving, cows entered the lactating herd, and daily milk yield was collected up to 21 DIM. Ketones were measured with a cow-side test at 7, 10, 14, and 21 DIM, and BCS was recorded once weekly from the beginning of the dry period through the first month of lactation. Data were analyzed using PROC MIXED in SAS, with the random effect of cow and the fixed effects of calving month, diet, parity, days relative to calving (DRC), and the interactions of diet with parity and DRC. There was a significant effect of parity and DRC on DMI, BCS (prepartum and postpartum), and milk yield (*P* ≤ 0.05). Ketones were affected by parity (*P* = 0.01) but only tended to differ due to DRC (*P* = 0.07). Prepartum DMI was not affected by diet (CON = 14.8 ± 0.86 kg/d, SGR = 15.4 ± 0.70 kg/d; *P* = 0.20), nor was daily milk yield (CON = 31.2 ± 1.62 kg/d, SGR = 32.9 ± 1.31 kg/d; *P* = 0.31). Postpartum ketone levels also were not affected by diet (CON = 1.2 ± 0.14 mmol/L, SGR = 0.8 ± 0.12 mmol/L; *P* = 0.20). There was no overall effect of diet on BCS prepartum (CON = 3.45 ± 0.070, SGR = 3.39 ± 0.056; *P* = 0.59), although postpartum BCS differed due to dry period diet (CON = 3.24 ± 0.070, SGR = 3.01 ± 0.057; *P* = 0.02). Interactions between diet and DRC or parity were not significant (*P* ≥ 0.11), except for DMI, where DRC × diet was significant (*P* < 0.01). Although cows who received SGR in the dry period mobilized more body fat in the month following parturition than CON, cows on both treatments maintained a healthy BCS, and overall there were no apparent negative effects of feeding switchgrass in the dry period.

**Key Words:** dry period, body condition score, switchgrass hay.

**M16  Effect of weaning and supplemental butyrate on nutrient transporter expression in Holstein calves.** R. Hiltz1, D. McCurdy1, K. Klander2, S. Moreland2, and A. H. Laarman1, 1Department of Animal and Veterinary Science, University of Idaho, Moscow, ID, 2Nutriad Inc., Hampshire, IL.

This study examined the effect of the weaning transition and supplemental sodium butyrate—a primary stimulator of rumen development—on rumen fermentation and volatile fatty acid transporter (VFA) abundance. Holstein bull calves (*n* = 36; age = 10.7 ± 4.1d) were assigned to 1 of 4 treatment groups: 2 pre-weaning groups, animals fed either milk only (PRE-M) or milk, calf starter, and hay (PRE-S); and 2 post-weaning groups: animals fed milk, calf starter, and hay either without supplementation (POST-S) or with 1% wt/wt supplemental butyrate during the weaning transition (POST-B). Milk was provided at 1200 g/d; starter, water, and hay were provided ad libitum. Weaning transition occurred in POST-S and POST-B by reducing milk replacer to 800 g/d in wk 7 and 400 g/d in wk 8, 0 g/d at wk 9 and harvest at wk 10. Rumen pH was measured continuously for 7 d before harvest. At harvest, rumen fluid was analyzed for VFA and rumen tissue was analyzed for VFA transporters. Data were analyzed in SAS with fixed effect of treatment and, where appropriate, repeated effect of week. Between PRE-M and PRE-S, total VFA concentrations increased (11.8 ± 5.8 vs. 35.6 ± 5.6 mM, *P* < 0.01), mean rumen pH was unaffected (6.16 ± 0.83 vs. 7.44 ± 0.79, *P* = 0.28), and MCT1 expression was unaffected (6.70 ± 135 vs. 8.39 ± 143 × 105 A.U., respectively, *P* = 0.30). Between PRE-S and POST-S, total VFA concentrations increased (35.6 ± 5.6 vs. 154.3 ± 15.0 mM, *P* < 0.01), but mean rumen pH was unaffected (7.44 ± 0.79 vs. 6.39 ± 0.19, respectively; *P* = 0.48), as was MCT1 expression (8.39 ± 1.43 vs. 7.28 ± 1.35 A.U., respectively; *P* = 0.58). Between POST-S and POST-B, total VFA concentrations were unaffected (154 ± 15 vs. 131 ± 16 mM, *P* = 0.23), and mean rumen pH decreased (6.39 ± 0.19 vs. 5.83 ± 0.18, *P* = 0.05), while MCT1 expression was unaffected (7.28 ± 1.35 vs. 8.17 ± 1.59 ×105 A.U., respectively; *P* = 0.61). Expression of MCT1 was unaffected by changes in calf starter intake or rumen pH and is not correlated with average daily gain during the weaning transition. These data suggest improvements in nutrient transport may be driven by other transporters or mechanisms.

**Key Words:** weaning, transporter expression, switchgrass, Holstein calves.

**M17  Supplementation of serotonin or fluoxetine impacts bioenergetics in dairy calves.** S. L. Field*, M. G. Marrero, A. L. Skibiel, B. Dado-Senn, and J. Laporta, Department of Animal Sciences, University of Florida, Gainesville, FL.

Serotonin (5-HT) is a monoamine that regulates energy balance through the modulation of insulin and lipid metabolism. Here, we hypothesize that manipulating 5-HT pathway by administering Fluoxetine (FLX, a 5-HT reuptake inhibitor) or 5-hydroxytryptophan (5-HTP, a 5-HT precursor), would improve energy metabolism in pre-weaned dairy calves. Bull Holstein calves (21 ± 2 d) were fed milk replacer (8 L/d) with saline (CON, 8 mL/d n = 8), FLX (40 mg/d, n = 8) or 5-HTP (90 mg/d, n = 8) for 10 consecutive d in a complete randomized block design. Blood samples were collected before (d-1), daily during treatment administration (d1 to 10) and during a 14-d withdrawal period (at d2, 3, 4, 7, 14) to measure insulin and NEFA concentrations. Calves were euthanized after the 10-d treatment or after the 14-d withdrawal period to harvest pancreas and adipose tissue. Gene expression of 5-HT receptors (5-HTR), 5-HT transporter (SERT) and tryptophan hydroxylase (*TPH1*) was measured by real-time PCR. Data were analyzed by period using 1- and 2-way ANOVAs in R. Insulin had a treatment by day interaction (*P* < 0.01), where 5-HTP group had higher circulating concentrations compared with the CON, particularly on d 5, 6, 7 and 9 of treatment; but had only a day effect during withdrawal period (*P* < 0.001). Circulating NEFA concentrations were not different during treatment or withdrawal period (*P* > 0.10). After treatment period, adipose gene expression of 5-HT1A tended to be downregulated in the 5-HTP group (*P* < 0.10), but after withdrawal period 5-HT1F was upregulated by 5-HTP and FLX (*P* < 0.01) when compared with CON. After treatment period, there was a tendency for downregulation of pancreatic gene expression of SERT (*P* < 0.08) and 5-HT1F (*P* < 0.10), in FLX and 5-HTP group, respectively, when compared with CON. After withdrawal, serotonin pancreatic receptors were not differentially expressed (*P* > 0.11). Manipulating serotonin bioavailability of dairy calves increases circulating insulin and modifies the expression of serotonin receptors in the pancreas and adipose tissue.

**Key Words:** adipose, pancreas, serotonin
Comparison of IgG absorption in calves fed a commercial colostrum replacer or supplement maternal colostrum. A. J. Lopez¹, C. M. Jones², A. J. Geiger³, and A. J. Heinrichs¹, ¹Department of Animal Science, The Pennsylvania State University, University Park, PA, ²Department of Dairy Science, The University of Wisconsin, Madison, WI, ³Zinpro Corporation, Eden Prairie, MN.

Successful passive transfer of antibodies in neonatal calves can be achieved by feeding maternal colostrum (MC) or colostrum replacer (CR). An alternative could be a supplemented low-quality maternal colostrum (CS). The objective of this study was to determine if a commercial colostrum product (Premolac PLUS Bovine IgG, Zinpro Corporation, Eden Prairie, MN) fed to replace or supplement MC could lead to adequate IgG levels and apparent efficiency of absorption (AEA) 24 h after birth in neonatal dairy calves. Holstein calves (n = 20/treatment; TRT) were separated from their dam after birth and randomly assigned to 1 of 2 levels of CR (110 g or 150 g of IgG/L), low-quality colostrum (41 g IgG/L) supplemented with CR (154 g IgG fed) or MC (106 g IgG/L; 401 g IgG fed; positive control) within 1.5 h of birth. Colostrum was obtained from first (MC TRT) or second and third milking (CS TRT) of cows from The Pennsylvania State University dairy. Then, it was pooled in 2 batches and analyzed for total IgG concentration using radial immunodiffusion. Blood samples were taken before feeding colostrum and 24 h after birth and analyzed for serum total protein, total IgG, hematocrit, and Brix. In the statistical model, data were analyzed as a completely randomized design. Hematocrit was tested as a covariate but not used in the final model. Reported means are followed by their standard errors. Feeding 150 g of IgG in CR led to higher 24 h serum IgG values than feeding 110 g of IgG (16.90 ± 1.09 and 12.79 ± 1.08 mg/mL, respectively; P < 0.01). MC had higher 24 h IgG values than CS (27.04 ± 1.07 and 22.33 ± 1.08 mg/mL, respectively; P < 0.01). Serum IgG levels were statistically different between CR and MC (P < 0.01), but both had average values above 10 mg/mL IgG. Calves fed CS had greater AEA than calves fed MC (54.58 ± 2.39 and 24.38 ± 2.36%, respectively; P < 0.01). Among calves fed CR with 110 g or 150 g of IgG, AEA was not different (P = 0.68), but was greater than previous reports (40.47 and 40.09%, respectively). Total protein and Brix had a strong correlation (r = 0.98, P < 0.01). These results indicate that CR can be an alternative to MC or a supplement to colostrum with low IgG.

Key Words: calf, colostrum, immunoglobulin G