28 Mitochondrial genome diversity and association of mitochondrial protein gene expression with energy metabolism in dairy cattle. J. Dorji*[1,2], C. J. Vander Jagt[2], J. B. Garner[3], L. C. Marett[2], B. A. Mason[2], C. M. Reich[2], C. P. Prowse-Wilkins[2,4], R. Xiang[2,4], P. N. Ho[2], J. Pryce[1,2], B. G. Cocks[1,2], A. J. Chamberlain[2], I. M. MacLeod[2], and H. D. Daetwyler[1,2], [1]*School of Applied Systems Biology, La Trobe University*, *Bundoora, Victoria, Australia*, [2]*Agriculture Victoria, AgriBio, Centre for AgriBioscience*, *Bundoora, Victoria, Australia*, [3]*Agriculture Victoria, Ellinbank Dairy Centre*, *Ellinbank, Victoria, Australia*, [4]*Faculty of Veterinary & Agricultural Science, University of Melbourne*, *Parkville, Australia*.

Mitochondria are primarily organelles for cellular energy metabolism and have a maternally inherited genome encoding 37 genes. Proteins from these genes interact with mitochondrial proteins (MP) encoded by nuclear genes to enable mitochondrial functions. Given the key role of mitochondria in energy production, mutations affecting the expression of MP genes could have flow-on effects on important traits in cattle. Our study had 3 aims: first to assess the diversity of the mitochondrial genome of modern dairy cattle breeds, second, to characterize MP gene expression across tissues within animals, and third, to correlate MP gene expression in blood with feed efficiency in dairy cattle. Mitochondrial genome diversities (nucleotide and haplotype) were estimated based on selected variant positions across the genome. Overall, there was a low diversity in the dairy breeds studied. Broadly, the modern dairy cattle (e.g., Holsteins) were predominantly T3 (~95%, with > 10 evident subgroups) and to a lesser extent T2 and T1 haplogroups. Gene expression in tissue as was quantified by RNA sequencing. We used differential expression and co-expression analyses of genes in 29 tissues from 2 cows. We found consistent overexpression in high energy demand tissues (e.g., heart). This suggests that MP gene expression might also differ between animals that differ within tissue energy demands. We, therefore, measured gene expression in blood samples of 2 groups (14 dairy cows each) divergent for feed efficiency to analyze the differential gene expression and co-expression networks. There were 395 genes differentially expressed (DE) between high and low feed efficiency groups, of which 55 were MP genes. Furthermore, DE genes were significantly enriched for oxidative phosphorylation (OxPhos), an important pathway that generates cellular energy. However, none of the DE MP genes was from the mitochondrial genome. The association between feed efficiency and expression of MP genes involved in the OxPhos pathway was also evident in a weighted gene co-expression network analysis (r = 0.47, $P = 0.01$). Altogether, our study suggests that there is low mitochondrial genomic diversity among popular dairy breeds and MP gene expression may be associated with variation in traits linked to mitochondrial function.

Key Words: mitogenome, gene expression, cattle

29 Bioactivity of the endocannabinoid arachidonoylethanolamide in cultured bovine endothelial cells. C. C. F. Walker* and L. M. Sordillo, *Michigan State University*, *East Lansing, MI*.

Vascular endothelial cells are crucial inflammatory-mediating cells susceptible to compromised barrier integrity during coliform mastitis. Endocannabinoid arachidonoylethanolamide (AEA) is a lipid mediator that can affect barrier integrity through modulation of network formation, proliferation, and viability in human and rodent endothelial cells during endotoxin challenge. We hypothesized that the endocannabinoid AEA will increase barrier integrity of primary cultured bovine aortic endothelial cells (BAEC) challenged with lipopolysaccharide (LPS). Cells were treated with varying AEA concentrations (10 n*M* to 5 μ*M*) for up to 12 h following pre-treatment with 25 ng/mL LPS. Endothelial barrier integrity was continuously assessed by recording electrical resistance using an electric cell-substrate impedance sensing system and reported resistance was normalized to a media control. Cell proliferation and viability were assessed using commercially available plate-based assays. All AEA treatments were compared with LPS control group and analyzed using the ANOVA procedure in SAS9.4. Physiological-relevant concentrations (<1 μ*M*) increased proliferation and viability of BAEC by up to 21.5 ± 2.283% and 19.37 ± 2.564%, respectively, at 1 and 2 h post-treatment ($P < 0.05$). Normalized electrical resistance was increased for 2 h after AEA treatment at 100 n*M* (0.105 ± 0.0137 Ω) and 500 n*M* (0.123 ± 0.015 Ω) ($P < 0.05$). No differences in resistance were noted for any other treatments <1 μ*M* ($P > 0.05$). Doses >1 μ*M* decreased electrical resistance, proliferation, and viability for up to 2 h post-treatment ($P < 0.05$). All treatment effects were lost at 6 or 12 h ($P > 0.05$). Physiological AEA concentrations of <1 μ*M* improve barrier integrity of BAEC when challenged with LPS, in contrast to the potential toxicity associated with super-physiological concentrations of >1 μ*M*. Ongoing projects are focused on the mechanistic effects of AEA on endothelial cell barrier integrity with the goal of reducing the severity of coliform mastitis.

Key Words: coliform mastitis, endocannabinoids, endothelial cells

30 Effect of feeding *Camelina sativa* cake on rumen microbiota and gene expression in follicular cells in dairy Italian Holstein Friesian heifers. F. Omodei Zorini*[1], M. Dell'Anno[1], G. Pennarossa[1], G. Morini[2], G. Ranzenigo[3], V. Caprarulo[1], L. Rossi[1], P. Cremonesi[4], B. Castiglioni[4], F. Biscarini[4], and G. Invernizzi[1], [1]*Department of Health, Animal Science and Food Safety 'Carlo Cantoni', University of Milan*, *Milan, Italy*, [2]*Department of Veterinary Medical Sciences, University of Parma*, *Parma, Italy*, [3]*DVM*, *Varese, Italy*, [4]*Institute for Biology and Biotechnology in Agriculture (IBBA), NRC*, *Lodi, Italy*.

Omega-3 PUFAs have unique role in several biological systems in mammals. However, its impact on rumen microbial environment could represent a critical point for both rumen welfare and its availability for the animal. The aim of the study was to evaluate the effects of the inclusion in the diet of a terrestrial vegetable and sustainable source of omega-3 PUFAs (*Camelina sativa* cake) in dairy heifers on rumen microbiota and gene expression of fertility markers in follicular cells. The trial was conducted at the tie stall dairy barn of the Experimental Farm of Animal Production Research and Teaching Centre of Lodi, University of Milan, Italy and lasted 56 d. Sixteen Italian Holstein Friesian heifers 12 mo old were divided in 2 homogeneous groups randomly allocated to 2 treatments: CAME (n = 8) receiving the basal diet supplemented with 800 g/head/day of camelina cake and CTR (n = 8) receiving the basal diet supplemented with an isonitrogenous and isoenergetic soybean-based premix. Basal diet consisted of a dry TMR composed by alfalfa hay, durum wheat middlings, sugar cane molasses, corn meal, soybean hulls, rice bran, sunflower meal and minerals (15.15 PG, 42.63 NDF, 19.43 starch on DM basis). Performances were recorded weekly, feed intake daily. Rumen and follicular content samples were collected at d 0, 28 and 56 of the trial. Follicular developmental competence was assessed by RT-PCR analysis. Performance data were analyzed by PROC MIXED of SAS for repeated measures. No differences were detected for live BW, FCR and BCS, but an interaction between diet and time was observed ($P \leq 0.05$) on DMI with higher values for CAME at d 34, 51, 52 and 55 compared with CTR (14.44 ± 0.93 kg vs. 10.47 ± 0.93 kg; 13.36 ± 0.93 kg vs. 10.81 ± 0.93 kg; 13.21 ± 0.93 kg vs. 10.86 ± 0.93 kg; 13.99 ± 0.93 kg vs. 11.15 ± 0.93 kg). Higher expression levels for *HAS2*, *GREM1*, *LHCGR* and *FSHR* genes were detected in CAME group compared with CTR, suggesting a positive effect of treatment diet. Rumen microbiota was influenced by dietary treatment both at 28 and 56 d showing significant α diversity values.

Key Words: camelina cake, fertility, rumen microbiota

31 One plus one is … three? Evidence for a compounding effect of long-chain fatty acids on peroxisome proliferator-activated receptor

activity. S. Busato* and M. Bionaz, *Oregon State University*, *Corvallis, OR*.

Peroxisome proliferator-activated receptors (PPAR) are transcription factors with known nutrigenomic response to fatty acids. In ruminants, PPAR control the expression of genes involved in lipid and glucose metabolism, anti-inflammatory response, and milk fat synthesis. While information on the in vitro potency to activate PPAR of some individual fatty acids exists, we were interested in exploring the activation of PPAR by a wider range of fatty acids and their combination. We hypothesized that the activation of PPAR with the combination of fatty acids is larger than the sum of the effect of individual fatty acids. We assessed in BFH-12 cells (immortalized bovine hepatocytes) the dose-response of 10 fatty acids common in bovine nutrition. PPAR activation was assessed by a gene reporter containing luciferase (3xPPAR response element) and normalized by renilla. Doses from 0 to 500 μM were applied using a HP D300e digital dispenser. Results were analyzed using GLM of SAS with dose as main effect and replicates (n = 4) as random. Response to each fatty acid was variable both in extent and in dose at maximum activation, with palmitic, stearic and dodecanoic acid showing the greatest impact on PPAR activation (3.5-fold, 3.7-fold and 4.3-fold vs. untreated control, respectively). Conversely, octanoic, myristic and linoleic acid displayed little to no effect. Cells were then treated with 2 fatty acids in combination, using only the 6 with the highest impact on PPAR activation, at the dose that maximizes PPAR activation. As hypothesized, the impact of 2 fatty acids in combination was greater than the sum of the activation of each individual fatty acid. The combinations of 3 fatty acids, each at a dose equal to one third of the optimal individual dose, resulted in a similar magnitude of effect on PPAR activation as compared with the combination of 2 fatty acids, while minimizing the total amount of fatty acids present in the medium. Our data indicates a greater nutrigenomic effect via PPAR of a mixture vs. single fatty acids, paving the way for the effective activation of PPAR isotypes in dairy cows through dietary means.

Key Words: long-chain fatty acids, peroxisome proliferator-activated receptors (PPAR), nutrigenomics

32 Effects of dietary organic acid and plant botanical supplementation on growth performance in Holstein calves challenged by heat stress. A. B. P. Fontoura*[1], V. Sáinz de la Maza-Escolà[1,2], B. N. Tate[1], J. T. Siegel Nieves[1], A. T. Richards[1], F. Wang[1,3], L. F. Wang[1,4], M. E. Van Amburgh[1], E. Grilli[2,5], and J. W. McFadden[1], [1]*Cornell University*, *Ithaca, NY*, [2]*University of Bologna*, *Bologna, Italy*, [3]*China Agricultural University*, *Beijing, China*, [4]*Henan Agricultural University*, *Zhengzhou, China*, [5]*VetAgro S.p.A*, *Reggio Emilia, Italy*.

Our objectives were to evaluate the effects of heat stress (HS) and dietary organic acid and plant botanical (OA/PB) supplementation on growth in calves. In a completely randomized design, 62 bull and heifer calves were assigned to 1 of 5 groups (n = 12–13/group): thermoneutral conditions (TN-Con), HS conditions (HS-Con), thermoneutral conditions pair-fed to HS-Con (TN-PF), HS with low-dose OA/PB (75 mg/kg of BW; 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride; AviPlus R; Vetagro, Italy; HS-Low), or HS with high-dose OA/PB (150 mg/kg of BW; AviPlus R; HS-High). Supplements were delivered as a twice daily bolus via the esophagus wk 1 through 13 of life; all calves received boluses equivalent for triglyceride. Post weaning, calves (62 ± 2 d; 91 ± 10.9 kg) remained in thermoneutral conditions (temperature-humidity index [THI]: 60 to 69) for a 7-d covariate period. Thereafter, calves remained in TN conditions or were moved to HS conditions (THI: 75 to 83) for 19 d. Clinical assessments and BW were recorded, and blood was sampled. Organs from HS-Con and TN-Con were harvested at trial completion. The mixed model included fixed effects of BW at birth, treatment, time, and their interaction. Rectal and skin temperatures, and respiration rates were greater in HS-Con, relative to TN-Con ($P < 0.01$). Dry matter intake (DMI) and average daily gain (ADG) were lower in HS-Con, relative to TN-Con ($P < 0.01$). Comparing HS-Con and PF-Con, ADG and gain:feed were similar. Plasma fatty acids were elevated in TN-PF versus all other groups ($P = 0.04$; not observed for HS-Con). Liver and small intestine weights were lower in HS-Con, relative to TN-Con ($P = 0.03$ and 0.15, respectively). DMI was greater with HS-Low, relative to HS-Con ($P < 0.01$). ADG for HS-Low and HS-High were not different from HS-Con or TN-Con (i.e., effect was intermediate). Compared with HS-Con, calves fed OA/PB tended have greater gain:feed ($P = 0.08$). We conclude that reductions in DMI account for losses in growth during HS and dietary OA/PB supplementation enhances HS resilience in calves.

Key Words: calf, heat stress, organic acid

33 Feeding rumen-protected lysine prepartum increased energy-corrected milk in Holstein cows during early lactation. L. K. Fehlberg*[1], A. R. Guadagnin[1], B. L. Thomas[1], Y. Sugimoto[2], I. Shinzato[2], and F. C. Cardoso[1], [1]*University of Illinois*, *Urbana, IL*, [2]*Ajinomoto Co. Inc*, *Tokyo, Japan*.

Balancing for AA in the diet can optimize milk yield and composition; however, there is little information on the requirement of AA, specifically Lys, during the transition period. This experiment was conducted to determine the effects of feeding rumen-protected Lys (RPL; AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL) prepartum (0.54%DM of TMR), postpartum (0.395%DM of TMR), or both on performance. Seventy-five multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, expected calving date, and body condition score during the far-off dry period were assigned to 1 of 4 dietary treatments in a randomized, complete block design with a crossover of diet with RPL (L) or without (C). Treatments consisted of TMR with RPL prepartum and postpartum (LL), with RPL prepartum and without postpartum (LC), without RPL prepartum and with postpartum (CL), and without RPL prepartum and postpartum (CC). Cows were milked 2 × per d and milk samples were taken on 7 ± 1.3, 14 ± 1.4, and 28 ± 1.1 d relative to calving. Milk yield and dry matter intake (DMI) were obtained daily. Statistical analyses were performed using MIXED procedure of SAS. Cows in L had greater ($P = 0.03$) BW (823 ± 3kg for wk −2 and 785 ± 3kg for wk −1) during the −2 wk before calving compared with those in C (814 ± 3kg for wk −2 and 775 ± 3kg for wk −1). Postpartum BW (717 ± 6kg) was greater ($P = 0.05$) and DMI (18.12 ± 0.74kg) tended ($P = 0.08$) to be greater for cows in LL and LC compared with those that were in CL and CC (706.5 ± 6 and 16.84 ± 0.74 kg, respectively). Energy-corrected milk (48.7 ± 1.9 kg/d), 3.5% fat-corrected milk (50.1 ± 2.1 kg/d), milk fat (1.93 ± 0.09 kg/d), milk true protein (1.41 ± 0.05 kg/d), milk casein (0.64 ± 0.04 kg/d), and milk lactose yields (2.07 ± 0.08 kg/d) were greater ($P \leq 0.04$) for cows in LL and LC compared with those that were in CL and CC (44.2 ± 1.9, 45.2 ± 2.1, 1.71 ± 0.09, 1.30 ± 0.05, 0.54 ± 0.04, 1.88 ± 0.08 kg/d, respectively). In conclusion, cows that consumed RPL prepartum tended to increase DMI postpartum and increased energy-corrected milk and milk component yields.

Key Words: lysine, milk protein, transition period

34 Effects of rumen undegradable protein and amino acid sources and replacing forage or non-forage fiber in postpartum cows on production. A. W. Tebbe* and W. P. Weiss, *Ohio Agricultural Research and Development Center, The Ohio State University*, *Wooster, OH*.

Greater metabolizable protein (MP) supply from rumen undegradable protein (RUP) improves production in fresh cows. However, using one RUP source instead of blends may worsen AA imbalances as RUP (% of MP) increases. Replacing forage instead of non-forage fiber with RUP may also increase MP supply by increasing intake. Our objective was to determine whether high MP diets using one or a blend of RUP and AA sources and whether substituting forage NDF (fNDF) rather than non-forage NDF with RUP improves production in fresh cows. In a randomized block design, 40 primigravid and 40 multigravid Holsteins were blocked

by calving date and fed a common diet (11.5% CP). After calving to 25 DIM, cows were fed 1 of 4 diets: 1) deficient MP (DMP, 17% CP, 24% fNDF), 2) adequate MP met with high inclusion of treated soybean meal (AMP, 20% CP, 24% fNDF), 3) adequate MP met with a blend of RUP and rumen protected AA (Blend, 20% CP, 24% fNDF), or 4) Blend but replacing fNDF rather than non-forage NDF with RUP (Blend-fNDF, 20% CP, 19% fNDF). Cows were fed a common diet (17% CP) from 26 to 92 DIM. Data were averaged by week and analyzed with models with fixed effects of diet, week (repeated), parity, their interactions and random effects of block nested within parity. During treatment, Blend vs. AMP tended to increase DMI (17.4 vs. 16.4 kg/d; $P = 0.098$) but not for Blend-fNDF (17.2 kg/d; $P = 0.79$). Blend and AMP had similar DMI from 26 to 50 DIM (19.2 vs. 20.1 kg/d; $P = 0.16$). For milk, fat and protein, AMP and Blend increased yields 2.1, 0.05 and 0.14 kg/d, respectively, vs. DMP ($P \leq 0.06$) but AA profile and fNDF interacted with parity. Blend vs. AMP had similar yields during treatment, but energy-corrected milk and fat yields increased ($P < 0.01$) 5.6 and 0.33 kg/d from 26 – 92 DIM in multiparous cows only. Blend-fNDF vs. Blend decreased ($P \leq 0.01$) milk and milk fat yields 2.8 and 0.32 kg/d in multiparous cows only. Lower milk fat yield ($P = 0.01$) carried over until 92 DIM (1.85 vs 1.65 kg/d). Blends of RUP and AA are better for fresh cows fed high MP, especially multiparous cows. Multiparous cows may also require more fNDF than primiparous cows.

35 Reproductive outcomes associated with delayed clinical cure of metritis in dairy cows. C. Figueiredo*[1], V. Merenda[1], E. de Oliveira[2], F. Lima[2], R. Chebel[1], K. Galvao[1], J. Santos[1], and R. Bisinotto[1], [1]*University of Florida*, *Gainesville, FL*, [2]*University of California*, *Davis, CA*.

Approximately 20% of cows treated for metritis fail to resolve clinical signs shortly after antimicrobial therapy. Objectives were to evaluate reproductive outcomes, uterine health, and estrous cyclicity associated with delayed clinical cure of metritis. This prospective cohort study included data from 4 experiments performed between 2012 and 2018 in 6 Florida dairies. Metritis was characterized by presence of watery, fetid, reddish-brownish vaginal discharge (VD; VD = 5) within 21 DIM (d 0). Cows with metritis were treated with ampicillin or ceftiofur and paired with counterparts without metritis (NoMet; n = 2,906). On d 11, cows with metritis with VD <5 were classified as cured (MetC; n = 1,136) and those with VD = 5 were classified not cured (MetN; n = 279). Incidence of purulent vaginal discharge (PVD) was evaluated at 32 ± 7 DIM using the Metricheck device and estrous cyclicity was evaluated via 2 ultrasonographic exams 10 to 14 d apart, with the last exam between 50 and 67 ± 3 DIM. Binary variables were analyzed by logistic regression. Hazard of pregnancy and time to pregnancy were evaluated by Cox's proportional hazard regression models. Services per conception (SPC) was assessed by ANOVA. Incidence of PVD was greater ($P < 0.001$) for MetN compared with MetC and NoMet (93.2, 79.2, and 43.2%). Proportion of cyclic cows was smaller ($P < 0.02$) for MetN compared with MetC and NoMet (67.2, 79.9, and 91.8%). Pregnancy per AI (MetN = 28.2, MetC = 29.2, NoMet = 31.8%) and pregnancy loss (MetN = 13.1, MetC = 11.5, NoMet = 13.5%) after first AI did not differ ($P > 0.35$) among groups. SPC was greater ($P = 0.04$) for MetN compared with NoMet and intermediate for MetC (3.43, 2.98, 3.15). Hazard of pregnancy was smaller ($P < 0.001$) for MetN compared with MetC (AHR = 0.72; 95% CI = 0.49 to 0.94) or NoMet (AHR = 0.59; 95% CI = 0.49 to 0.70), and for MetC compared with NoMet (AHR = 0.82; 95% CI = 0.74 to 0.90). Mean days to pregnancy for MetN, MetC, and NoMet were 211, 183, and 170. Delayed clinical cure of metritis was associated with impaired subsequent uterine health, delayed resumption of ovulation postpartum, and decreased hazard of pregnancy.

Key Words: uterine disease, health, pregnancy

36 Variation in bovine colostrum fat content is related to specific lipid species. R. N. Klopp*[1], A. Suarez-Trujillo[1], C. R. Ferreira[2], T. M. Casey[1], and J. P. Boerman[1], [1]*Department of Animal Sciences, Purdue University*, *West Lafayette, IN*, [2]*Metabolite Profiling Facility, Bindley Bioscience Center, Purdue University*, *West Lafayette, IN*.

Colostrum is an essential source of nutrients, energy, and antibodies for neonates. Studies across multiple species associate long-term programming effects of colostrum on health, fertility and production capacity. However, the quality of colostrum varies extensively across cows and herds, including fat content, which is essential for calf temperature regulation and thermogenesis. Understanding what is potentially driving the variation of fat content in colostrum may enable the development of approaches to standardize quality to ensure nutrient needs of calves are met. The objective of this study was to determine if there is a relationship between the percent fat of colostrum and the amount of specific lipids in colostrum. Colostrum was collected from 16 multiparous cows within 2 h of calving and immediately frozen at −20°C until analysis. Colostrum fat percent was measured using the creamatocrit approach, 5.46 ± 2.20% (mean ± SD) and range from 0.92 to 8.36%. The Bligh and Dyer protocol was used to extract lipids from the colostrum and multiple-reaction monitoring (MRM) profiling was used to measure colostrum lipids on an Agilent 6410 QQQ mass spectrometer (Agilent Technologies). MRM-profiling was divided into 2 phases, a discovery and screening phase. For discovery phase a pooled colostrum sample was profiled, and lipids with intensities ≥1.3-fold in relation to the blank were selected for the screening phase. Individual colostrum samples were profiled for selected lipids. Data were uploaded into MetaboAnalyst 4.0 for statistical analysis following autoscaling for normalization. Correlation analysis, evaluating non-triglycerides, indicated a significant relationship between percent fat and multiple lipids in colostrum, including phosphatidylcholine (PC) 32:1 ($P = 0.004$, $r = 0.67$), PC 32:0 ($P = 0.02$, $r = 0.57$), PCo 40:0 ($P = 0.01$, $r = −0.62$), and PCo 42:4 ($P = 0.05$, $r = −0.50$), sphingomyelin (SM) d18:0/24:0 ($P = 0.02$, $r = 0.59$), and SM d18:1/24:0 ($P = 0.03$, $r = 0.55$). These findings suggest that specific colostrum lipids are related to percent fat, however further studies are required to determine regulatory mechanisms for colostrum fat synthesis.

Key Words: colostrum, fat, lipidome

37 Effect of hyperketonemia on circadian patterns of blood metabolites and milk predicted constituents in dairy cows. C. Seely*[1], K. Bach[1], D. Barbano[2], and J. McArt[1], [1]*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University*, *Ithaca, NY*, [2]*Department of Food Science, College of Agriculture and Life Sciences, Cornell University*, *Ithaca, NY*.

Estimates of milk and blood constituents by Fourier-transform mid-infrared (FTIR) analysis of milk offer a promising tool to monitor energy deficit in dairy cows. We sought to explore the (1) diurnal changes in plasma nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) and FTIR estimates of milk BHB and milk predicted blood NEFA (pbNEFA), (2) correlation between plasma BHB and NEFA, and (3) effect of hyperketonemia (HYK) on circadian patterns of plasma and milk metabolites. Multiparous Holstein cows (n = 28), between 3 and 9 DIM, were fit with jugular catheters and blood samples were collected every 2 h for 5 d. Cows were milked thrice daily (0600, 1400, 2200 h) and milk samples were collected at every milking for the same 5 d. Cows were fed daily at 0900 h and offered ad lib access to a TMR. Plasma NEFA and BHB was quantified by enzymatic analysis and milk BHB and pbNEFA was estimated by FTIR. Cows were retrospectively grouped as HYK positive (n = 13) if plasma BHB was ≥1.2 mmol/L for ≥3 study days or HYK negative (non-HYK; n = 15) if plasma BHB was ≥1.2 mmol/L for ≤2 study days. Explanatory models were used to analyze plasma and milk metabolites over time and differences in metabolites between HYK groups. Models analyzing metabolites over time included the random effect of cow and fixed effect of time; those analyzing differences between groups included the random effect of cow and fixed effects of HYK group, time, and HYK group × time.

The correlation between plasma NEFA and BHB was analyzed by calculating the area under the curve for total plasma NEFA and BHB. Plasma BHB and NEFA, milk BHB, and pbNEFA all changed throughout the day ($P < 0.001$). The amplitude of change in plasma BHB was greater within a day for the HYK cows than the non-HYK cows ($P = 0.009$). Plasma NEFA and BHB were positively correlated (r = 0.81), suggesting that accounting for diurnal variation increased the correlation of plasma metabolites. Our results support the use of FTIR estimates of milk constituents as a tool to monitor energy deficit and suggest that time relative to feeding should be considered when analyzing both plasma and milk metabolites.

Key Words: hyperketonemia, Fourier-transform mid-infrared (FTIR)

38 Isoprostanes reduce production of reactive oxygen species and apoptosis in a bovine model of oxidative stress. A. K. Putman*, J. C. Gandy, and L. M. Sordillo, *Michigan State University College of Veterinary Medicine*, *East Lansing, MI.*

Oxidative stress is associated with several economically important diseases in dairy cattle and results in damage to tissue macromolecules. Isoprostanes (IsoP) are molecules generated from interactions between free radicals and membrane phospholipids, thus serving as excellent indicators of free radical-mediated lipid damage during times of oxidative stress. In dairy cattle, IsoP have been detected throughout the lactation cycle, during both health and disease. While IsoP are recognized as excellent biomarkers of oxidative stress, their physiological role remains largely unknown. The vascular endothelium is a primary target of lipid peroxidation during oxidative stress. Thus, this experiment aimed to determine the effect of the most extensively studied IsoP, 15-F2t-IsoP, on bovine endothelial cells during oxidative stress conditions. Bovine aortic endothelial cells (BAEC) were incubated in the presence of 10 nM 15-F2t-IsoP alone and in combination with known oxidizers 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and lipopolysaccharide (LPS). 15-F2t-IsoP decreased ROS production in BAEC incubated with AAPH for 12 h compared with cells incubated with AAPH alone. Additionally, 15-F2t-IsoP decreased apoptosis in BAEC incubated with LPS for 12 h when compared with cells incubated with LPS alone. The results of this study indicate that 15-F2t-IsoP may have a cytoprotective role during times of oxidative stress. Future studies should be directed toward investigating if IsoP alter other factors associated with vascular damage during oxidative stress, such as endothelial cell barrier integrity. This research benefits the industry by providing insight into how a well-known biomarker of oxidative stress in dairy cattle may contribute to the pathophysiology of economically important diseases.

Key Words: isoprostane, oxidative stress