**W68** Nerve growth factor-β effects on steroidogenesis and angiogenic markers in the bovine pre-ovulatory follicle. J. L. Stewart1,2, L. Gao1, J. A. Flaws2, I. F. Canisso1,3, and F. S. Lima1,4,1. Department of Veterinary Medicine, University of Illinois, Urbana, IL, 2Department of Large Animal Clinical Science, Virginia Polytechnic Institute and State University, Blacksburg, VA, 3Department of Comparative Biosciences, University of Illinois, Urbana, IL.

Nerve growth factor-β (NGF) is a seminal plasma protein that induces ovulation and has a luteotrophic effect in camelduds, but little is known regarding the interactions of seminal plasma-derived NGF on the pre-ovulatory follicle in the bovine species. Our objectives were to assess the effects of purified bovine NGF on steroidogenesis and angiogenic markers in the bovine pre-ovulatory follicle. Two Holstein heifers were synchronized, and ovariectomy was performed via colpotomy when a pre-ovulatory follicle >12 mm was present. The pre-ovulatory follicle was excised from the ovary, fluid was aspirated, and the remaining tissue was dissected. The theca interna with adherent granulosa cells was peeled from the theca externa and surrounding stromal tissue and cut into small pieces (average weight: 5.3 ± 0.7 mg). The follicle tissue pieces (n = 24) were incubated in 0.5 mL of Eagle’s MEM culture medium supplemented with 1% i-glutamine, 1% nonessential amino acids, 1% penicillin-streptomycin, 1% insulin-transferrin-selenium, 10% fetal bovine serum, 40 ng/mL LH, 4 ng/mL FSH. Culture wells were either supplemented with 100 ng/mL NGF (n = 12) or left as an untreated control (n = 12). Medium was withdrawn and replaced with fresh medium at 3, 6, 12, 24, 48, and 72 h of culture and frozen at −80°C for hormone analysis and follicle tissue pieces were frozen to measure steroidogenic and angiogenic gene expression using qPCR. A general linear mixed model with repeated measures for hormone data and Kruskal-Wallis rank sum test for non-parametric data were performed. Treatment with NGF increased testosterone and (P < 0.01) and steroidogenic enzyme 17β-hydroxysteroid dehydrogenase (P = 0.04) in the follicle wall extracts. There was no effect of NGF on progesterone and estradiol (P ≥ 0.14) or other steroidogenic enzymes (P ≥ 0.31). Treatment with NGF reduced (P = 0.02) fibroblast growth factor 2 (Fgf-2) but did not alter other angiogenic factors (P ≥ 0.44). In conclusion, NGF affected steroidogenesis increasing testosterone production and reduced Fgf-2, a marker of cell remodeling.

**Key Words:** nerve growth factor-β, follicle, bovine

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**W69** Nerve growth factor-β increases small luteal cell and steroidogenic activity in the bovine corpus luteum. J. L. Stewart1,2, V. R. G. Mercadante3, N. W. Dias1, S. Stella1, L. Cunha*1, I. F. Canisso1, and F. S. Lima1,1. Department of Veterinary Medicine, University of Illinois, Urbana, IL, 2Department of Large Animal Clinical Science, Virginia Polytechnic Institute and State University, Blacksburg, VA, 3Department of Animal Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Nerve growth factor-β (NGF) is a seminal plasma protein that stimulates bovine theca cell proliferation and steroidogenesis in vitro and is luteotrophic in vivo, yet there is little is known on how it alters ovulation and corpus luteum formation in cattle. Our objectives were to assess the effects of systemic purified bovine NGF on vascularity and steroidogenesis of the pre-ovulatory follicle and subsequent corpus luteum (CL) in cattle. Holstein heifers (n = 12) were synchronized using a 5-d CIDR-Synch and randomly allocated to 1 of 2 treatment groups: (1) CONT, 12 mL of PBS, or (2) NGF, 250 μg purified NGF in 12 mL of PBS administered intramuscularly at time 0 (presence of pre-ovulatory follicle). A second replicate was performed using a crossover ~1 mo after the first replicate. Transectal ultrasonography with Doppler and blood sampling were performed every 4 h from 0 to 32 h to evaluate follicle size and vascularity, ovulation time, and estradiol concentrations. Ultrasonography was then performed daily to assess CL size and vascularity, and blood was obtained every 2 d to measure progesterone concentrations. On d 9 and 14, a CL biopsy was performed to assess mRNA expression of steroidogenic enzymes and LH receptor and the histological ratio of small to large luteal cells. Statistical analyses were performed using a general linear mixed model with repeated measures using R Version 3.4.3. Treatment with increased follicle diameter (P = 0.02) but did not alter estradiol (P = 0.95) or ovulation time (P = 0.42). Treatment with NGF tended to increase CL diameter (P = 0.10) and increased progesterone concentrations (P = 0.04). There was a higher percentage of small luteal cells (P < 0.01) and a tendency for increased LH receptor gene expression (P = 0.09) in NGF-treated heifers. Steroidogenic acute regulatory protein and 3β-hydroxysteroid dehydrogenase were increased (P ≤ 0.05) in NGF-treated heifers. Treatment with NGF did not alter the vascularity of the follicle or CL (P ≥ 0.16). Purified NGF interacted with the pre-ovulatory follicle and altered downstream CF formation and function.

**Key Words:** bovine, luteotrophic, nerve growth factor-β

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**W70** Pegbovigrastim treatment alters gene expression profiles of leukocytes in Simmental and Holstein cows after calving. V. Lopreai1, A. Minuti2,3, D. Britti1, F. Trimboli1, F. Piccioli Cappelli2, J. L. Voor1, and E. Trevisi*2,3. Interdepartmental Services Centre of Veterinary for Human and Animal Health, Department of Health Science, Magna Graciea University, Catanzaro, Italy, 1Department of Animal Sciences, Food and Nutrition (DIANA), Università Cattolica del Sacro Cuore, Piacenza, Italy, 3Proteomics and Nutrigenomics Research Center (PRONUTRIGEN), Università Cattolica del Sacro Cuore, Piacenza, Italy, 4Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL.

Pegbovigrastim is a commercial long-acting analog of bovine granulocyte colony-stimulating factor. We hypothesized that pegbovigrastim (Imrestor; Elanco) administered at approximately 7 d before expected calving and within 24 h after calving affects expression profiles of genes involved in leukocyte function. Cows were randomly allocated into 1 of 2 groups: control [CTR; 13 Holstein (7 primiparous and 6 multiparous) and 13 Simmental (6 primiparous and 7 multiparous)] and pegbovigrastim [PEG; 13 Holstein (6 primiparous and 7 multiparous) and 13 Simmental (6 primiparous and 7 multiparous)]. Blood was collected on d 3 after calving in PAXgene tubes (Preanalytix) to measure mRNA expression of 36 genes. The final data were normalized using the geometric mean of 3 internal control genes: ACTB, SDHA, and YWHAZ. Data were subjected to ANOVA and analyzed with PROC MIXED of SAS. Treatment, breed, parity, and their interaction were the fixed effects. Compared with CTR, PEG cows had greater expression (P < 0.01) of genes involved in recognition and immune modulation (CD14, CD16, MYD88, TLR2, and TLR4), cell adhesion (ITGB2, ITGAL, TLN1, SELT, SELPLG, and CD44), antimicrobial activity (MMP9, LTF, LYZ, and LCN2), and inflammation (CASP1, TNFRSF1A, IL1B, IL1R, IL18, IRAK1, NLRP3, and S100A8). Pegbovigrastim also led to lower expression of RPL13A, ALOX15, IL8, TLN2, and TNF (P < 0.05). Expression of IDO1, RPL13A,
W71 A lateral flow-based portable platform for quantification of circulating concentrations of progesterone. M. Masello*, E. M. Schillkowsky1, Z. Lu2, D. Erickson2, J. Gavalchin1, and J. O. Giovannone1, 1Department of Animal Science, Cornell University, Ithaca NY, 2Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca NY.

Our objective was to develop and validate a disposable fluorescence-based test strip coupled with a portable imaging device for quantification of plasma progesterone (P4). First, we developed and optimized a competitive lateral flow immunoassay (LFA) test strip to measure P4 in bovine plasma. The LFIA design included a sample pad, a conjugate pad that dry-stores Rphycoerythrin-anti-P4 conjugates, a glass-fiber spacer pad, a nitrocellulose membrane with printed test and control lines, and a cellulose-fiber absorbent pad. To perform a test, 20 µL of plasma and 50 µL of running buffer (RB) were added on the sample pad, and the test strip was left in a light-free environment. After 3 min, 45 µL of RB were added to initiate sample flow. After allowing 15 min to stabilize the colorimetric signal, strips were inserted into an LFIA reader to determine P4 concentration based on test-to-control-line signal (T/C ratio). The reader was linked to a laptop to interpret and display results. In a series of experiments (n = 6), the ability of the LFIA to predict the presence of a functional (P4 ≥ 1 ng/mL) corpus luteum (CL) in bovine plasma samples was evaluated. For each experiment, a calibration curve was constructed using plasma with known concentrations of P4 (0.1–3.7 ng/mL; n = 5). There was a linear relationship between average T/C ratio and P4 levels (average R2 = 0.82; range 0.44–0.99). The T/C ratio decreased as P4 concentrations increased. Next, plasma samples from lactating dairy cattle (n = 58) were tested in triplicate to predict the presence or absence of a functional CL using a radioimmunoassay for P4 as reference test. Overall, the LFIA assay correctly classified 90% (P < 0.01; 95%CI 79–96) of the samples, with 93% sensitivity (P < 0.01; 95%CI 77–99), 86% specificity (P < 0.01; 95%CI 68–96), 87% positive predictive value (P < 0.01; 95%CI 70–96) and 93% negative predictive value (P < 0.01; 95%CI 76 to 99). The agreement between the LFIA and the reference test was substantial (kappa = 0.79; 95%CI 0.64–0.95; P < 0.01). The intra-assay CV averaged 16.0% (range 0.5–42.8%). These data suggest that the current LFIA system can accurately predict the presence of a CL based on circulating concentrations of P4. Supported by USDA-NIFA Project 2016–08814.

Key Words: Met and Arg availability, mechanistic target of rapamycin (mTOR) signaling pathway, circadian clock

W73 Novel phospho-proteomic analysis of abdominal and subcutaneous adipose tissues from dairy cows supplemented with conjugated linoleic acid during the transition period. M. Zachutt1, G. Kra1, Y. Levin2, A. Trösch2, L. Vogel1, M. Gnoth3, and H. Hamm4, 1Agriculture Research Organization, Volcani Center, Rishon LeZion, Israel, 2The Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Rehovot, Israel, 3BASF SE, Lampertheim, Germany, 4FBN, Dummerstorf, Germany.

Phospho-proteomics is a new frontier in omics-research that reveals the full repertoire of phosphorylation sites in proteins. Conjugated linoleic acid (CLA) supplementation reduces energy output in milk, which could increase fat storage in transition cows, but it is also known to promote lipolysis in adipose tissue (AT). Our objective was to elucidate the effect of CLA on protein activation in AT of transition cows by phospho-proteomics. Ten rumen cannulated Holstein cows were fed a corn silage-based TMR with low fat content and daily abomasally supplemented from wk 9 prepartum until slaughtering at 63 d postpartum with coconut oil (CTL, n = 5; 45.5% C12:0; 16.9% C14:0; 76g/d) or Lutalin (CLA, n = 5; C9, t11 and t10, c12, 10g/d). Subcutaneous (S) and abdominal (A) AT were collected and frozen (−80°C). Proteins were
extracted for global quantification of the phospho-proteome by enrichment of phospho-peptides (PP) by Fe immobilized metal ion affinity chromatography (Fe-IMAC) followed by discovery analysis. Data were analyzed for the effect of fat depot (S vs. A), treatment (CLA vs. CTL), and their interaction by 2-way ANOVA, and bioinformatics analysis was conducted by Ingenuity (Qiagen). Overall, CTA cows had lower fat and energy output in milk compared with CTL based on weekly milk components, and had more total and abdominal AT than CTL (P < 0.005). A total of 5,919 PP were identified in AT. The abundance of 854 PP (14.4%) was statistically different between CLA and CTL (P < 0.05, FC ± 1.5). Increased protein phosphorylation, i.e., higher abundance of several PP, was found in lipid-metabolism proteins in CTA vs. CTL: 7 PP were more abundant in acetyl-CoA carboxylase 1 (ACACA); 9 PP in fatty acid synthase (FASN); 8 PP in hormone sensitive lipase (HSL); and 3 PP in perilipin (PLIN). Increased total abundance of FASN and HSL was found by immunoblots in CTA vs. CTL AT (P < 0.02). Top canonical pathways enriched in CLAAT were protein kinase A signaling and insulin receptor signaling. These novel findings provide insight to the molecular mechanism by which CLA might stimulate both lipogenesis and lipolysis in AT.

Key Words: proteomics, adipose, CLA

W74 Effect of 2-hydroxy-4-(methylthio)butanoate supplementation on rumen bacterial populations in dairy cows when exposed to diets with risk for milk fat depression. D. Pitta*,1, N. Indugu1, B. Vecchiarelli1, M. Baldwin1, and K. Harvatine2, 1University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA, 2The Penn State University, University Park, PA, 3Provimi, North America, Brookville, OH.

Diet-induced milk fat depression (MFD) is a condition marked by a reduction in milk fat yield achieved by altering dietary conditions including increasing unsaturated fatty acids and fermentability of carbohydrates. 2-Hydroxy-4-(methylthio) butanoate (HMTBa) is a methionine analog that has been observed to reduce diet-induced MFD in dairy cows. Our hypothesis was that the reduction in diet-induced MFD by HMTBa was due to changes in the rumen microbiota. To test this, 22 high-producing cannulated Holstein dairy cows were arranged in a randomized block design and assigned to either control or HMTBa supplementation (0.1% of diet DM). Cows were then exposed to 3 diets with either a low risk (32% NDF, no added oil; fed d 1 to 14), a moderate risk (29% NDF and 0.75% soybean oil; fed d 15 to 21), or a high-risk (29% NDF and 1.5% soybean oil; fed d 21 to 28) for diet-induced MFD. Rumen samples were collected on d 0, 14, 21, and 28, DNA extracted, V1-V2 region of the 16S rRNA gene PCR amplified, sequenced on Illumina MiSeq platform, and subjected to bacterial diversity analysis using the QIIME pipeline. The α diversity estimates (species richness) and Shannon diversity were decreased in control compared with HMTBa (P < 0.05). Bacterial community composition also differed between control and HMTBa based on both unweighted unifrac (presence/absence) and weighted unifrac (relative abundance of commonly detected bacteria) distances (P < 0.01). Within the HMTBa group, there were no differences between d 0 and d 14, 21 and 28; however, in the control group, d 0 samples were different (P < 0.05) from d 14, 21 and 28. Bacterial genera including Dialister, Megaphasphaera, Lachnospira, and Sharpea were increased in control compared with HMTBa (P < 0.05). Interestingly, these genera were positively correlated with milk fat trans-10, cis-12 CLA and trans-10 C18:1, isomers associated with MFD. It can be concluded that diet-induced MFD is accompanied by significant alterations in the bacterial community and HMTBa supplementation reduced rumen microbial perturbations when increasing dietary risk factors.

Key Words: biohydrogenation, milk fat depression, rumen bacteria

W75 Effect of postpartum meloxicam administration to ewes on inflammatory status, plasma fatty acid concentrations, and oxylipid biosynthesis. K. E. Olagaray*,1, L. M. Sordillo2, J. C. Gandy2, T. H. Swartz1, C. Youngs3, and B. J. Bradford1, 1Kansas State University, Manhattan, KS, 2Michigan State University, East Lansing, MI, 3Iowa State University, Ames, IA.

The effect of postpartum meloxicam administration on ewe inflammatory status and plasma fatty acid (FA) and oxylipid concentrations were evaluated in 34 Hampshire and Hampshire × Suffolk ewes rearing singles or twins. After lambing, ewes (94 ± 17 kg BW) were sequentially assigned within type of birth to control (n = 16) or meloxicam orally administered on d 1 and 4 of lactation (MEL; 90 mg, n = 18). Blood was sampled on d 1 (before treatment) and d 4, and plasma was analyzed for haptoglobin (Hp) by colorimetric assay, FA profile by LC-MS, and oxylipids by LC-MS/MS. Results were analyzed in a mixed model with d 1 values as covariates. Plasma Hp concentrations tended to be less for MEL ewes (P = 0.06), and a covariate x treatment effect (P = 0.04) showed that CON ewes with greater d 1 Hp concentrations were also elevated on d 4, which was not the case for MEL. Among FA, MEL increased arachidonic acid (AA) concentration by more than 4-fold in ewes rearing singles (P < 0.01 main effect and interaction). MEL decreased concentrations of 9,10-DiHOME, PGG2, 8-iso-PGE2, and 8,9-DHET (P < 0.05). Nine oxylipids had interactions of treatment with d 1 Hp (P < 0.05), all of which revealed positive associations between d 1 Hp and d 4 oxylipid concentrations for CON, but neutral or negative relationships for MEL. MEL decreased 13-HODE/13-oxoODE (P = 0.04), tended to increase 9-HODE/9-oxoODE ratio (P = 0.06; both dependent on d 1 values), and tended to decrease 13-HODE/9-HODE ratio (depending on d 1 Hp, P = 0.08), indicating progressive metabolism of linoleic acid-derived oxylipids occurred by enzymatic oxidation after MEL treatment. The diversity of affected oxylipids suggested that MEL not only inhibited cyclooxygenase activity, but may also have reduced cytochrome P450 and nonenzymatic oxidative reactions, potentially due to an improved redox state. In conclusion, postpartum MEL treatment of ewes decreased plasma concentrations of Hp and several oxylipids, with the greatest effect in ewes with biomarkers reflecting a greater inflammatory state before treatment.

Key Words: nonsteroidal anti-inflammatory drug, inflammation, lactation

W76 Adipose tissue and plasma fatty acid profile during the peripartum period differ by parity but not by late-lactation dietary fatty acid profile. K. A. Weld*,1, C. Bradley2, J. Davidson2, and H. M. White1, 1University of Wisconsin-Madison, Madison, WI, 2Purina Animal Nutrition, Gray Summit, MO.

Some dietary fatty acids (FA) may influence adipose tissue FA composition. The objective of this study was to compare the long-term effects of late lactation fat feeding on subcutaneous adipose and plasma FA profiles during the peripartum period in multiparous (MP) cows. To determine if there is an influence of mobilization cycle, a secondary objective was to compare MP and primiparous (PP) adipose tissue FA profile. Multiparous cows were assigned to either a C16 (pure palmitic acid + concentrate pellet) or C18 (concentrate pellet with 50% FA [78% C18, 22% C16]) supplemented diet (2.3% DM added FA) for the last
W77 Calcium propionate supplementation leads to broad downregulation of hypothalamic pathways in lambs. M. Vallatic-Riboni*, H. A. Lee-Ragel2, G. D. Mendoza1, and J. J. Loor3,1

Department of Animal Sciences, University of Illinois, Urbana, IL, 2Universidad Autónoma de San Luis Potosí, Facultad de Agronomía, San Luis Potosí, México, 3Universidad Autónoma Metropolitana, Xochimilco, México City, México.

Our objective was to investigate the effect of propionate on the hypothalamic regulation of food intake in the context of the hepatic oxidation theory in ruminants. Nine Rambouillet lambs (27.93 ± 4.6 kg) were randomly assigned to one of three experimental diets (n = 3/diet): T1, a control diet containing 93% alfalfa hay and 7% molasses, without supplementation of Calcium-Propionate (CaPr); T2, T1 + 35 g/kg DM of CaPr; and T3, T1 + 30 g/kg DM of CaPr. Diets were offered ad libitum as a total mixed ration. The lambs were housed in individual cages equipped with feed and water bowls. Lambs were first adapted to their diets (without CaPr) for 10 d, and the experimental period lasted 42 d (lambs will receive treatments during this period). On d 42, lambs were euthanized, the hypothalamus collected, and RNA was extracted and sequenced on the Illumina HiSeq 4000 system, generating an average of 50 million reads/sample, of which 75% where uniquely mapped, with 56% of gene-assigned read, with a total of 15608 uniquely identified genes. A linear model with CaPr as fixed and animal as random effect was fitted. Differentially expressed genes (DEG) were declared at fold change ≥ 2 and P-value ≤ 0.05. The Dynamic Impact Approach was used for pathway analyses using the KEGG database. Two contrast yielded the highest number of DEG: T2 + T3 vs T1 (overall effect of CaPr infusion vs control), and T3 vs T1 (3.5% CaPr vs control), with 1200 and 919 DEG, respectively. Independently of the contrast, pathway analysis revealed a broad downregulation of all pathways in animals that received CaPr. The analysis highlighted an extended downregulation of all hypothalamic activities in ewes that received CaPr infusions, independently of the dose. Further work is needed to assess the hypothalamic mechanisms involved in feed intake regulation.

Key Words: propionate, hypothalamus, RNA-seq

W78 Comparison of telomere lengths in blood leukocytes and in nasal and vaginal epithelial cells from water buffaloes (Bubalus bubalis) of different ages. K. Seibt1, S. Häussler2, D. Vecchio3, E. DeCarlo2, F. Ceciliani3, and H. Sauerwein*1,1

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Telomeres are short and repetitive sequences at the ends of linear chromosomes which shorten with every cell division in vitro. Telomere length (TL) is reported to decrease with age and stress. The domesticated water buffalo (Bubalus bubalis) is the second important milk-producing animal worldwide. The productive lifespan of water buffalo cows is reported to be longer than that of dairy cows (Bos taurus). With this background, we aimed to compare in TL in blood leukocytes obtained from water buffaloes across different ages. In addition, we tested the suitability of assessing TL in DNA derived from nasal and vaginal swabs as potential non-invasive alternatives for blood telomeres. The respective samples were collected from 20 calves (3 mo of age), 20 heifers (2 years old), 20 cows (first lactation, 3 years old age), and 13 cows (third lactation, about 5 years old) TL was assessed by qPCR with β-globin as reference. One-way ANOVA with Bonferroni post-hoc tests was used to test for differences between the age groups. Pearson correlations were calculated to assess associations of TL obtained in different sample matrices (blood versus swabs). The TL in blood leukocytes from water buffalo calves, heifers, and from cows in their first lactation was not different, but shorter telomeres were observed in cows in their third lactation. The results thus support an age-dependent decrease of TL in water buffaloes. The TL recorded in leukocytes were weakly correlated with TL measured in DNA from nasal swabs (r = 0.327; P = 0.025), but not with TL from vaginal swabs. The weak and absent correlation of TL in nasal and vaginal epithelial cells, respectively, and the unease of collecting nasal swabs from buffaloes, incapacitated these swabs as suitable alternatives for blood cells when assessing TL.

Key Words: telomere length, water buffaloes, age

W79 Post-ruminal choline supply during a feed restriction-induced negative nutrient balance alters components of hepatic mechanism target of rapamycin (mTOR) signaling and plasma amino acids in Holstein cows. D. N. Coleman*1, A. Abdelmaksoud2, R. Bucktrout1, Y. Liang1, M. Miura1, and J. J. Loor3,1

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The objective was to investigate the effects of post-ruminal choline (CHL) supply during a feed restriction-induced negative nutrient balance (NBB) on hepatic abundance and phosphorylation of mTOR (mechanistic target of rapamycin)-related signaling proteins and plasma AA concentrations. Ten primiparous rumin-cannulated Holstein cows (158 ± 24 DIM) were used in a replicated 5 × 5 Latin square design with 4 d of treatment and 10 d of recovery (14 d/period). Treatments were unrestricted intake with abomasal infusion of water, restricted...
intake (R; 60% of net energy for lactation requirements to induce NNB) with abomasal infusion of water (R0) or R plus abomasal infusion of 6.25, 12.5, or 25 g/d CHL ion. Liver tissue was collected on d 5 after infusions ended and blood on d 1–5. Statistical contrasts were A0 vs. R0 (CONT1), R vs. the average of CHL dose (CONT2) and tests of linear and quadratic effects of CHL dose. Although R tended to decrease the ratio of p-mTOR:total (t) mTOR (CONT1; P = 0.08), ratios of p-RPS6KB1:tRPS6KB1, p-EFF2:EEF2, and p-EIF2:EIF2 were greater (P < 0.05). Among those, supply of CHL led to decreases in p-EFF2:EEF2 (CONT2; P = 0.04), p-EIF2:EIF2 (P < 0.001) and tended to decrease p-EIF4BP1:EIF4BP1 (P = 0.07). However, the effect was quadratic only for p-EFF2:EEF2 (P = 0.02) and p-EIF2:EIF2 (P < 0.001), reaching a nadir at 6.25 to 12.5 g/d CHL ion. The ratio of p-RPS6KB1:tRPS6KB1 was not affected by supply of CHL and was close to 2-fold higher at 25 g/d CHL vs. A0. Plasma Met concentration decreased with R (CONT1; P = 0.001) but increased linearly with CHL (P = 0.03). Restriction also increased plasma 3-methyl-histidine (CONT1; P < 0.001). Data suggest that dephosphorylation of EEF2 due to enhanced CHL supply along with greater p-RPS6KB1 potentially helped maintain or increase protein elongation during NNB. This idea is partly supported by the increased circulating Met. However, changes in initiation factors and initiation binding proteins indicated that CHL did not enhance initiation of protein synthesis.

Key Words: amino acid, protein synthesis

W80 Lipogenic effects of trans-10,cis-12 and cis-9,trans-11 conjugated linoleic acids on 3D cultured omental and subcutaneous adipocytes derived from lactating dairy cows. J. Geldersma*1, J. Laguna1,2, A. Lock2, and G. Contreras1, 1Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, 2Animal Science, East Lansing, MI.

The anti-lipogenic effects of trans-10,cis-12 conjugated linoleic acid (T10C12) are well characterized in monogastric species. Similarly, the impact of abomasal infusion of T10C12 on milk fat synthesis in lactating dairy cows is well established. However, the effect of T10C12 on the lipogenic capacity of bovine adipocytes from omental (OM) and subcutaneous adipose tissues (AT) of lactating dairy cows is unknown. Our objective was to evaluate the effects of T10C12 on the lipogenic activity of 3D cultured bovine adipocytes. OM and tailhead (TH) AT samples were collected from 5 lactating non-gestating mature Holstein dairy cows. AT samples were digested with collagenase type II to harvest stromal vascular cells. Preadipocytes were selected by outgrowth of plastic adherent cells and then seeded 3 dimensionally using collagen type I gels. Cells were induced to differentiate using standard adipogenic medium for 14 d. During induction, adipocytes were supplemented with 50 μM T10C12 or 50 μM cis-9,trans-11 (C9T11) using fatty acid free bovine serum albumin (BSA) as carrier. Triglyceride (TAG) accumulation was evaluated quantitatively (unit = μM/μg DNA) using a fluorometric adipogenic kit. Statistical analysis was performed using linear mixed models. Independent of treatment, adipocytes from TH accumulated more TAG than those from OM (28.3 ± 2.92 vs. 20.2 ± 2.92; P < 0.01). Across sites, adipocytes treated with T10C12 had less TAG (18.1 ± 3.21) than those exposed to BSA (25.8 ± 3.21) or C9T11 (28.8 ± 3.21; P < 0.05). TH adipocytes treated with C9T11 accumulated more (P < 0.05) TAG (35.17 ± 4.12) than those exposed to BSA (27.9 ± 4.12). In turn, T10C12 treated TH adipocytes had lower TAG content (21.9 ± 4.12) than cells that were BSA treated. OM adipocytes exposed to T10C12 (14.3 ± 3.71) had lower TAG compared with those treated with C9T11 (22.3 ± 3.71) and BSA (23.9 ± 3.71). Results suggests that T10C12 has a marked anti-lipogenic effect in bovine adipocytes from OM and TH, similar to that observed in monogastric species, and that C9T11 may enhance lipogenesis in the subcutaneous TH AT depot of lactating dairy cows.

Key Words: conjugated linoleic acid, adipocyte, lipogenesis

W81 Increasing supply of methionine and arginine at constant Thr:Phe, Lys:Thr, Lys:His, and Lys:Val ratios alters inflammatory and oxidative stress responses during a lipopolysaccharide challenge in bovine mammary epithelial cells. H. Dai1,2, D. N. Coleman1, L. Hu3, I. Martinez-Cortés1,4, X. Shen2, and J. J. Loor1, 1University of Illinois, Urbana, IL, 2Nanjing Agricultural University, Nanjing, Jiangsu, China, 3Yangzhou University, Yangzhou, Jiangsu, China, 4National Autonomous University of Mexico, Mexico City, Mexico.

We evaluated whether increased supply of Met and Arg alone or in combination altered inflammation and oxidative stress responses in primary bovine mammary cells (BMEC) challenged with lipopolysaccharide (LPS). The BMEC (n = 4 replicates/treatment) were incubated for 12 h with control medium (IPAA, Lys:Met 2.9:1, Lys:Arg 2:1) or medium supplemented with Met (LM2.5; Lys:Met 2.5:1, Lys:Arg 2:1), Arg (LA; Lys:Met 2.9:1, Lys:Arg 1:1), or LM2.5+LA (Lys:Met 2.5:1, Lys:Arg 1:1). Ratios of Thr:Phe (1.05:1), Lys:Thr (1.8:1), Lys:His (2.38:1), and Lys:Val (1.23:1) were constant across treatments. Cells were then incubated with or without LPS (1,000 ng/mL) for another 6 h. Data were analyzed as a 2 × 2 factorial arrangement of treatments with the MIXED procedure of SAS 9.4. Target genes were measured by RT-PCR and proteins by Western blotting. LPS downregulated mRNA abundance of the amino acid transporters SLC7A5, SLC7A1 and SLC36A1. However, Arg supply alone attenuated the downregulation of SLC36A1 mRNA and SLC7A1 mRNA. LPS upregulated mRNA abundance of KEAP1 and protein abundance of CUL3, and both of them inhibit the antioxidant transcription factor NFE2L2 which was associated with lower protein abundance of NFE2L2 and the antioxidant proteins NQO1 and GPX1. Protein abundance of phosphorylated p65 (RELA; NFKB1 activator) was greater after LPS stimulation, but the response was attenuated by supply of Met alone. Similar responses to LPS and increased supply of Met plus Arg were observed for the ratio of p-RELA to total RELA. mRNA abundance of proinflammatory cytokines and chemokines (IL1B and CXCL2) was greater after LPS stimulation, but the response was attenuated with greater supply of Met and Arg alone. Although greater supply of Met and Arg could not rescue the inhibition of antioxidant mechanisms controlled by NFE2L2, overall, the data suggest that these amino acids dampened the proinflammatory responses triggered by LPS. One of the underlying mechanisms was through the control of NFKB1 activity and abundance of proinflammatory cytokines and chemokines.

Key Words: amino acid, lactation, mastitis

W82 Identification of novel real-time quantitative PCR reference genes for bovine corpus luteum via whole-transcriptome RNA sequencing. M. A. Mezera*1, L. Wenli2, D. J. Koch2, A. Edwards2, C. A. Gammara1, R. S. Gennari1, V. E. Gomez-Leon1, R. Reis Domingues1, A. D. Beard1, and M. C. Wiltbank1, 1University of Wisconsin- Madison, Madison, WI, 2USDA Dairy Forage Research Center, Madison, WI.

Real-time quantitative PCR (RTqPCR) is a valuable tool to study gene expression in tissues. However, tissues from dynamic physiological states pose challenges to normalization, and thus analysis, as traditional reference genes may be unstable across physiologic states. The
corpus luteum (CL) is dynamic throughout luteolysis and maintenance in pregnancy. Thus, there is a need to identify stable genes throughout physiologic conditions for use as references genes in RT-qPCR analysis. Stable genes were discovered with whole transcriptome RNA sequencing (RNA-seq) and validated with RT-qPCR. CL biopsies collected from 5 states were subjected to RNA-seq analysis: CL from pregnant animals during (n = 5, d 20 ± 0) and after (n = 4, d 55.3 ± 3.4) secretion of interferon-tau, and CL before (n = 10), during (n = 8), and after (n = 5) functional luteolysis (luteolytic progression based on circulating progesterone and prostaglandin F2α metabolite). Potential reference genes were identified by ANOVA analysis of normalized read counts calculated by Cufflinks. Seventy-seven genes had a P-value greater than 0.1 and standard deviation less than 20% of mean read count. Genes were further analyzed with the R/Bioconductor package DESeq2 by randomly assigning samples to 2 groups. The 6 genes with the highest P-values were further analyzed (RPL4, UQCRFS1, COX411, RPS4X, SSRI3, and CST3) with RT-qPCR analysis of an independent set of CL samples from first (n = 4) and second month (n = 4) pregnant cows, and CL before (n = 5), during (n = 5), and after (n = 5) functional luteolysis. Gene stability from PCR was calculated with the algorithms geNorm and NormFinder. Four genes were consistently more stable than ACTB and GAPDH, the most common reference genes in bovine CL literature, regardless of physiologic state based on RT-qPCR analysis: RPL4, COX411, SSRI3, and RPS4X. In CL tissue from pregnancy, CST3 had highest stability. The identification of these novel reference genes will aid accurate normalization of RT-qPCR results. Furthermore, analyses shed light into the effects of luteolysis and pregnancy on stability of gene expression in the CL.

Key Words: corpus luteum, PCR

W83 Whole-transcriptome RNA-seq analysis of the corpus luteum throughout physiologic luteolysis in dairy cows. M. A. Mezera1, L. Wen2, C. A. Gamarra, R. S. Gennari, A. Edwards, A. B. Prata, and M. C. Wilbank1, 1University of Wisconsin-Madison, Madison, WI, 2USDA Dairy Forage Research Center, Madison, WI.

Corpus luteum (CL) regression is required for ovulation, with incomplete luteolysis associated with decreased fertility. Changes in gene expression have been correlated with CL from known days of the cycle and circulating progesterone (P4), and from exogenous prostaglandin F2α (PGF) induced regression. However, characterization of gene expression in physiologic regression with knowledge of PGF exposure is lacking. To address this, daily CL biopsies were collected with bihourly blood samples for 74 h from d 18–21, and circulating P4 and PGFM (PGF metabolite) analyzed. Hormone profiles were used to classify biopsies in 3 groups: control: CL unexposed to PGF in the previous 24 h (C; n = 6); EL: CL with exposure to ≥1 PGFM pulse (n = 8); and LL: when P4 < 1 ng/mL (n = 5). Whole transcriptome RNA-seq raw data were aligned to bovine reference genome (NCBI, UMD3.1) with TopHat. Followed by differential gene expression analysis using Cufflinks. Compared with control, 173 genes were differentially expressed (DE: Q < 0.05) in EL (103 upregulated, 70 downregulated), while 4615 were DE in LL (2455 upregulated, 2160 downregulated), with 161 genes DE in both EL and LL. Of these, 145 (84%) DE genes in EL displayed an increased fold change from control in LL compared with EL, showing most DE genes in early regression are further up or downregulated as regression progresses. For upregulated genes, enriched pathways in LL included hemostasis (P = 4.0E-8), extracellular matrix organization (P = 4.9E-8), and innate immune system (P = 2.1E-6), while pathways in downregulated genes were enriched in cholesterol biosynthesis (P = 7.1E-12), metabolism of lipids and lipoproteins (P = 1.0E-17), and steroid biosynthesis (P = 3.2E-6). While these results are largely confirmatory of pathways associated with luteolysis, this is the first study to characterize physiologic regression with serial biopsies and known PGF exposure. Furthermore, the small number of genes altered after small PGF pulses but before the drop in P4 associated with luteolysis suggests further inquiry into the role of small PGF pulses in physiologic luteolysis is warranted.

Key Words: corpus luteum, luteolysis

W84 Feeding NutriTek improves udder health and systemic response during a Streptococcus uberis mastitis challenge in mid-lactating dairy cows. M. Vailati-Riboni1*, D. Coleman, V. Lopreato1, A. Alharthi1, R. Bucktrout1, E. Trevisi4, I. Yoon4, and J. J. Loor1, 1Department of Animal Sciences, University of Illinois, Urbana, IL, 2Department of Animal Sciences, Food and Nutrition (DIANA), Università Cattolica del Sacro Cuore, Piacenza, Italy, 3Department of Animal Sciences, Interdepartmental Services Centre for Veterinary and Animal Health, Department of Health Science, Magna Gracie University, Catanzano, Italy, 4Department of Animal Sciences, University of Illinois, Catanzaro, Italy.

Eighteen mid-lactation multiparous Holstein cows (n = 9/group) were used to determine the effects of a Saccharomyces cerevisiae fermentation product (NTK, NutriTek, Diamond V, Cedar Rapids, IA) on the response to a mastitis challenge. Cows were fed the control diet (CON) or CON supplemented with 19 g/d NTK for 45 d (phase 1, P1), and then infected in the right rear quarter with 2500 cfu of S. uberis (phase 2, P2). Antibiotic treatment was started 36 h post-challenge until the end of P2 (9 d post challenge). Milk yield (MY) and DMI were recorded daily. Milk samples for somatic cell count were collected 3 times daily, and rectal and udder temperature, heart and respiration rate were recorded every 6 h during the challenge period. Blood samples for metabolites and immune function analysis were collected at 0, 15, and 36 h post-challenge. Data were analyzed by phase using the PROC MIXED procedure in SAS. Cow was used as random effect, while diet, time, and their interaction were used as fixed effects. DMI and MY were not affected by diet (P > 0.05) in P1, but an interaction of diet ´ time was recorded in P2 (MY, P = 0.01; DMI, P = 0.11) indicating a better recovery from the challenge in NTK compared with CON. NTK significantly reduced (P2, P < 0.05) the somatic cell score and temperature of the infected quarter during the challenge, while rectal temperature was significantly reduced (P2, P < 0.05) at the 24 h mark. No effects (P2, P > 0.05) were recorded for circulating neutrophil and monocyte oxidative burst activity; however, NTK reduced (P2, P < 0.05) their activation as indicated by lower phagocytosis response compared with CON at 36 h post challenge. Furthermore, NTK cows had greater (P2, P < 0.05) plasma concentrations of Ca (P2, P < 0.05), suggesting a better systemic inflammatory status, supporting the hypothesis of a better control of the infection at the level of the mammary gland. Overall, results indicate a protective effect of NutriTek supplementation on udder and systemic health during mastitic events.

Key Words: NutriTek, mastitis, udder health