T94 Effect of fatty acid profile shifts on bovine primary hepatocyte gluconeogenic and oxidative gene expression. K. Weld*, S. Erb, and H. M. White, University of Wisconsin-Madison, Madison, WI.

During the peripartum period, dairy cows diagnosed with hyperketonemia experience both an increase in circulating FA, and a change in circulating FA profile, compared with non-hyperketonemic cows. Supplementation of dietary lipids can also result in subtle shifts in the circulating FA profile. As FA are known regulators of hepatic genes, these shifts could differentially influence gene expression. The objective was to determine the expression of gluconeogenic and oxidative genes in primary hepatocytes when exposed to an in vivo relevant FA profile and that profile enriched with additional C16:0, C18:0, or C18:1. Primary hepatocytes were isolated from 4 Holstein bull calves (<7 d) and cultured for 24 h. Treatments applied to cells for 24 h were no FA (1% BSA); 0.75 mM FA cocktail (3% C14:0, 27% C16:0, 23% C18:0, 31% C18:1, 8% C18:2, and 8% C18:3; to mimic the serum FA profile of dairy cattle at calving); 0.90 mM FA cocktail; 0.75 mM FA cocktail + 0.15 mM C16:0; 0.75 mM FA cocktail + 0.15 mM C18:0; and 0.75 FA cocktail mM + 0.15 mM C18:1. After harvest in TRIzol, samples were stored at −80°C until RNA extraction, purification, reverse transcription, and quantitative real-time PCR. Expression of genes of interest (carnitine palmitoyltransferase 1A, pyruvate carboxylase, cytosolic and mitochondrial phosphoenolpyruvate carboxylase relative to the geometric mean of 2 reference genes chosen by geNorm (ribosomal protein L32 and GAPDH). Data were analyzed using Proc Mixed (SAS 9.4) with the fixed effect of treatment and calf in the random statement. The addition of FA compared with no FA increased the expression of carnitine palmitoyltransferase 1A (2.22 vs. 3.96 ± 1.59 arbitrary units [AU]; P < 0.05) and PEPCk (0.51 vs. 1.03 ± 0.28 AU; P = 0.03). Enrichment with individual FA did not affect the expression of the genes tested when compared with the 0.90 mM FA cocktail treatment (P ≥ 0.40). These results suggest additional shifts in circulating FA profile within a biological range have minimal additional effects on hepatic gluconeogenic and oxidative gene expression.

Key Words: oxidation, pyruvate carboxylase, liver

T95 Coordinated responses of hepatic lipid-associated proteins in cows with high or low liver lipid content peripartum. H. T. Holdorf*, R. Caputo Oliveira, R. S. Pralle, and H. M. White, University of Wisconsin-Madison, Madison, WI.

Lipid associated proteins may allow for dynamic storage or utilization of liver triglyceride (lvTG). The objective of this study was to examine the coordinated response of liver lipases during the transition to lactation. Multiparous cows, representing a subset of a larger nutritional study (n = 40), were retrospectively grouped by maximum lvTG into a high (>15% DM; n = 6) or low (<15% DM; n = 10) group with no effect (= 1.0) of original dietary treatment. Liver biopsies were collected at −28, −14, = 1, +14, and +28 DRTC and lvTG was quantified. Protein abundance of abhydrolase domain containing 5 (ABHD5), hormone sensitive lipase (HSL), perilipin 1 (PLIN), patatin-like phospholipase domain containing 2 and 3 (PNPLA2, PNPLA3) was determined by Western blot analysis. Data were transformed as log(10)(abundance + 1), to achieve normal residuals. Data were analyzed for main effects of lvTG group, DRTC, and lvTG group x DRTC, and random effect of cow(lvTG group), dietary treatment, and repeated measures within cow using PROC GLIMMIX (SAS 9.4). Main effects were considered significant at P < 0.05 or marginal at P < 0.1. Means were separated by Tukey’s adjustment when interactions were P < 0.05. Abundance of ABHD5 tended to be greater (P = 0.09) at +28 compared with +1 DRTC and PNPLA2 was greater (P < 0.03) at +28 compared with −14 DRTC. An interaction of lvTG group x DRTC was detected (P < 0.05) for HSL and PLIN, but means could not be separated by Tukey’s post hoc analysis. There was an interaction (P < 0.01) of lvTG group x DRTC on the proportion of phosphorylated PLIN, relative to total PLIN (%PPLIN), with reduced (P < 0.03) abundance at +14 and +28 compared with prepartum time points in high lvTG cows. Additionally, %PPLIN tended to be greater (P = 0.07) in low lvTG cows, compared with high lvTG cows, at +28 DRTC. These data indicate that some hepatic proteins, namely ABHD5 and PNPLA2, are increased postpartum regardless of lvTG group while abundance of PLIN and HSL may reflect lvTG content. Shifts in the %PPLIN coincide with changes in lvTG and should be further examined for potential to mediate remobilization of stored lvTG.

Key Words: lipolysis, transition cow, fatty liver

T96 Actions of recombinant bovine somatotropin revisited: Characterization of the plasma metabolome and lipidome. A. N. Davis*, W. A. Myers, C. Chang, B. N. Tate, J. E. Rico, and J. W. McFadden, Cornell University, Ithaca, NY.

Recombinant bovine somatotropin (rbST) restores homeorhetic mechanisms to support lactation; however, the effects of rbST on the bovine metabolome and lipidome are undefined. Therefore, 8 multiparous lactating Holstein cows (195 ± 34 DM) were enrolled in a 2 × 2 replicated Latin square design with 14 d periods. Cows received a single injection of rbST (Posilac; Elanco Animal Health, Indianapolis, IN; 0.062 mg/kg BW) or no injection (CON) at period start. On d 8, 9 and 10, an epinephrine challenge (EC; 1.6 µg/kg BW), insulin tolerance test (ITT; 0.1 IU/kg BW), and liver biopsy were performed, respectively. Plasma glucose and total fatty acids (FA) were measured. Plasma was also processed for metabolomics or lipidomics using mass spec. Univariate and multivariate ANOVA were performed. Omic data were generalized log-transformed. Somatotropin elevated milk yields compared with CON (P < 0.01) despite lower DMI (P < 0.05). Milk fat yield and percent, and milk protein and lactose yields were higher in rbST cows (P < 0.01). Plasma total FA levels were increased with rbST (peaking at d 8; P < 0.01). At the start of the EC, rbST cows had 75% higher circulating total FA compared with CON (478 vs 119 µmol/L; P < 0.01) which increased post epinephrine (866 vs. 218 µmol/L for rbST and CON by 15 min, respectively; P < 0.01). Epinephrine responsiveness (change from baseline), and insulin-stimulated plasma glucose clearance and reductions in total FA were not modified by treatment. Plasma levels of oxoproline, glutamic acid, threonine, and fatty acids and their derivatives (e.g., linoleic acid, arachidonic acid, ethyl myristate, and eicosatrienoic acid) were higher in rbST cows (P < 0.05). The plasma lipidome was dynamically modified with treatment (218 out of 385 features; P < 0.05). Treatment with rbST decreased plasma mono-, di-, and triacylglycerols levels (e.g., MG-18:0, DG-18:0/16:0, and TG-16:0/14:0/16:1; P < 0.05). Similarly, many phosphatidylycholines and sphingomyelins were significantly lower in rbST cows by d 8 (P < 0.05). We conclude
that rhST modifies the bovine plasma metabolome and lipidome with increased milk production.

**Key Words:** metabolome, lipidome, somatotropin

T97  **Body condition score in late pregnancy is associated with abundance of hepatic microRNA involved in energy metabolism.**

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The main objective was to evaluate associations between body condition score (BCS) in late-pregnancy, plasma biomarkers, and hepatic abundance of microRNA linked to energy metabolism during in silico and in vivo analyses. At 4 weeks before calving, 26 cows were divided into 2 groups based on BCS, BCS ≥3.50 (n = 13; HiBCS) and BCS ≤ 3.25 (n = 13; LoBCS). Dry matter intake (DMI) from −4 wk of pregnancy through 30 d in milk (DIM) and milk production during the first 30 DIM were recorded. Blood was sampled at −30, −10, 7, 15, and 30 DIM. Liver biopsies were performed at −15, 7, and 30 DIM for analysis of miR-369 5p, miR-186, and miR-200b abundance via RT-PCR after normalization with 3 internal controls. Pathway analysis using the dynamic impact approach revealed these miRNAs play a role in relation to energy metabolism by means of fatty acid metabolism, oxidative phosphorylation, gluconeogenesis, PPAR signaling, and insulin signaling. Data were subjected to repeated measures ANOVA in SAS using PROC MIXED. Main effects were BCS, time, and their interaction, while cow was the random effect. Although daily DMI did not differ prepartum, HiBCS cows averaged 1.54 kg/d (P ≤ 0.05) more DMI/d postpartum. Overall milk production was 5.34 kg/d greater (P = 0.02) in HiBCS compared with LoBCS. Cows in HiBCS also had greater (P = 0.03) overall concentrations of fatty acids, myeloperoxidase (P = 0.03), and β-hydroxybutyrate (BHB) (P = 0.06) compared with LoBCS. In contrast, LoBCS had greater plasma concentrations of alkaline phosphatase (P ≤ 0.01), tocopherol (P = 0.03), and carotene (P = 0.03). miR-186 was the most abundant of the target miRNA evaluated, but had no detectable changes in relation to BCS or time. Abundance of miR-369 5p was lower (P = 0.01) overall in cows with HiBCS. In contrast, abundance of miR-200b had a BCS x time effect (P = 0.03) due to a marked upregulation between −15 and 7 d followed by increased abundance at 30 d in cows with LoBCS. Overall, the responses in miR-369 5p and miR-200b underscore a potentially important physiological role during the periparturient period as it relates to BCS.

**Key Words:** BCS, microRNA (miRNA), transition period

T98  **Effects of butyrate supplementation on blood glucagon-like peptide-2 concentration and gastrointestinal function in lactating dairy cows fed diets differing in starch content.**

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The objective of this study was to evaluate effects of butyrate supplementation on blood glucagon-like peptide-2 (GLP-2) concentration, nutrient digestibility and responses to SARA challenge of cows fed diets differing in starch content. Eight Holstein cows were blocked by parity and assigned to one of 4 × 4 Latin squares balanced for carryover effects with a 2 × 2 factorial arrangement of treatments. Treatments were dietary starch content [low starch = 20% (LS) vs. high starch = 29% (HS)] and butyrate supplementation (butyrate vs. control) with 21-d periods. Butyrate was provided as Gustor BP70 WS (NOREL, S.A., Madrid, Spain), containing 70% sodium butyrate and 30% fatty acid mixture, at 2% of dietary DM, and control premix contained 70% wheat bran and 30% fatty acid mixture. Feeds,orts, and fecal samples were collected from d 17 to 19 to determine apparent total-tract nutrient digestibility. Blood samples were collected on d 19. Cows were feed-restricted at 60% of daily intake on d 20, and SARA challenge was conducted by providing 0.6%BW of steam flake corn grain in addition to each treatment diet on d 21, and blood and ruminal fluid samples were collected. Data were analyzed using Fit model procedure of JMP. The model included fixed effects of dietary starch content, butyrate supplement, their interaction, period, and square, and random effects of cow nested in squares. Cows fed butyrate increased serum BHB concentration (P < 0.01), tended to increase plasma GLP-2 concentration (P = 0.06), and increased DM digestibility (P < 0.05) compared with control. During SARA challenge, rumen endotoxin concentration was higher for cows fed HS + butyrate compared with cows fed the other diets (P < 0.05), but cows fed butyrate tended to decrease plasma/rumen endotoxin ratio than control (P = 0.08). Serum haptoglobin concentration was not affected by treatment. These results indicate that butyrate supplementation may increase plasma GLP-2 concentration and total-tract DM digestibility, and keep plasma endotoxin concentration low relative to its ruminal concentration.

**Key Words:** butyrate, glucagon-like peptide-2 (GLP-2), gut function

T99  **Characterization of metabolic and oxidative status in Italian Mediterranean water buffalos during the peripartum period.**

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During the transition from pregnancy to lactation, markers of oxidative stress in blood commonly increase and peak in the first weeks of lactation in dairy cows. Our objective was to characterize the oxidative status in water buffalos, in context with indicators for metabolic stress during that phase. Blood samples were collected weekly from 10 buffalo cows (lactation number 4.6 ± 1.6; daily milk yield 9.0 ± 1.9 kg; means ± SD) from 6 weeks (wk) ante partum (ap) until wk 8 postpartum (pp). Beside nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHB), the following variables were determined in serum using photometric assays: derivatives of reactive oxygen metabolites (dROM), ferric reducing ability (FRAP), thiobarbituric acid reactive substances (TBARS), and advanced oxidation protein products (AOPP). Linear mixed models with time (wk) as repeated effect considering the nested periods ap and pp and cow as random effect were used to evaluate the time courses using SPSS. The dROM values declined with time (P < 0.001) from peak values in wk 2 and 1 ap toward lowest values from wk 3 to 8 pp. FRAP was not affected by time. The oxidative stress index (OSI), i.e., the calculated ratio (dROM/FRAP), also decreased with time showing greater values ap than pp (P < 0.001). The TBARS values did not change with time, whereas AOPP concentrations were greater ap than pp (<0.001); BHB concentrations were not affected by time. Greater NEFA values were observed ap than pp (P < 0.001). Both BHB and NEFA values remained below the thresholds applied for dairy cows to define subclinical or clinical ketosis, thus indicating that the buffaloes studied herein were not under particular metabolic stress. The greater
OSi values before calving resulted from increased concentrations of pro-oxidants rather than from decreased anti-oxidants. The profiles of indicators for oxidative stress reported in the literature for dairy cows indicate that oxidative stress occurs in early lactation; in contrast, the most oxidative stress in water buffaloes was observed in late pregnancy rather than lactation in our study.

Key Words: oxidative stress, water buffalo, transition period


The study objective was to evaluate the effect of grazing in early lactation on changes in triglyceride content and expression of genes related to lipid metabolism in the liver of dairy cows. Primiparous Holstein cows (n = 18, 528 ± 40 kg BW and 3.2 ± 0.2 BCS; fall calving) were used in a randomized block design and assigned, at calving, to 2 nutritional treatments during the first 65 d postpartum (DPP) of lactation: [i] TMR ad libitum (58% forage, 42% concentrate; TMR) or [ii] grazing of Medicago sativa (6-h am grazing in 3-d strips; pasture allowance = 20 kgDM/d) plus TMR (70% of ad libitum TMR; PAS). All cows consumed at each milking, 2.0 kgDM/d of a grain-soybean meal concentrate. Plasma and liver biopsies were collected pre and postpartum (−7 and +42 DPP) to measure plasma NEFA concentrations and hepatic triglyceride (TAG) content and mRNA abundance of genes related to lipid metabolism (SYBR-Green real time RT-PCR). Data were analyzed as repeated measures with a mixed model that included DPP and treatment within DPP as fixed effects. Milk energy output was greater (P = 0.04) for TMR than PAS cows (21.6 vs. 20.0 ± 0.41 Mcal/d) while cow BW did not differ between nutritional treatments. Expression of ACADVL and PPARG mRNA were not affected by nutritional treatments. However, CPT1A mRNA was greater in TMR than PAS cows (2.63 vs. 1.99 ± 0.2; P = 0.04), consistent with the greater plasma NEFA concentration in former cows during postpartum (0.63 vs. 0.42 ± 0.03 mmol/L; P < 0.01). In addition, although liver TAG content did not differ between TMR and PAS cows (14 vs. 11 ± 2%), hepatic TAG and plasma NEFA concentrations were negatively correlated only in TMR cows (r = −0.95, P < 0.01).

Results indicated that TMR cows adapted their hepatic metabolism to increase fatty acid oxidation parallel to the increased energy demands of their greater milk production.

Key Words: choline, fatty liver, gene expression, grazing

T101  Rumen-protected choline acts directly in the liver regulating expression of genes involved in reduction of fatty liver in dairy cattle. M. Zenobi1, P. Tribulo2*, B. Barton3, J. Santos1, P. Hansen1, and C. Staples1, 1University of Florida, Gainesville, FL, 2Instituto de Reproduccion Animal Cordoba, Cordoba, Argentina, 3Balchem Corp., New Hampton, NY.

Supplementation of rumen-protected choline (RPC; ReaShure, Balchem Corp., New Hampton, NY) reduced liver fat accumulation in feed restricted pregnant, nonlactating dairy cows. In these same cows, we evaluated if RPC concurrently altered hepatic gene expression. Pregnant, nonlactating multiparous Holstein cows (n = 77) were enrolled at 64 ± 10 d before expected calving date. Dietary treatments were 0, 30, 60, 90, and 120 g/d of ReaShure. Experimental periods were 14 d with 5 d of ad libitum intake (AL), and 9 d of feed restriction to consume 31% of caloric requirement (FR) to induce negative nutrient balance. Methionine was supplemented during the FR period to maintain the same daily intake of the AL period. Liver tissue was collected on d 5 and 14. Transcript abundance was determined by RT-qPCR using the Fluidigm assay for 93 target genes involved in assembly and secretion of VLDL, CDP-pathway, gluconeogenesis, inflammation, oxidative stress, transcription factors, metabolism of lipid, lipoprotein, and phosphatidylcholine; and 3 reference genes. The ACt values were calculated relative to the geometric mean of the reference genes and used for statistical analyses using the Mixed procedure of SAS. Birth weight of the calves and number of days prepartum at enrollment were used as covariates. Expression of genes was affected (P < 0.05) by RPC during both periods. After cows developed fatty liver (d14) there were 20 differentially expressed genes (DEG), whereas there wer 13 DEG on d5. Changes in expression of genes due to RPC suggest increase in phosphatidylcholine metabolism, and reduction of TAG synthesis, among others. In conclusion, RPC regulates expression of genes in the liver, more extensively under fatty liver conditions to reduce TAG contents. Results demonstrate that regulation of hepatic gene expression is one mechanism by which RPC supplementation leads to the optimal performance of transition cows.

Key Words: choline, fatty liver, gene expression


Poor regulation and maintenance of circulating calcium is of concern in dairy cows, particularly during the peri-parturient period as cows adapt to increased demands of colostrum and milk production. When regulation of circulating calcium fails, hypocalcemia ensues, with repercussions of this disease negatively affecting cow health throughout early lactation. The objective of this study was to characterize physiologic responses of a subclinical hypocalcemic challenge in lactating and nonlactating dairy cows. Using a randomized complete block design, 12 dry, non-pregnant multiparous Holstein cows and 12 early lactation (5–20 DIM) multiparous Holstein cows received either (1) a continuous 24-h intravenous solution of 0.9% saline or (2) 5% ethylene glycol tetraacetic acid (EGTA) in 0.9% saline (n = 6 lactating, n = 6 nonlactating/treatment) with the aim of maintaining blood ionized calcium (iCa) less than 1.0 mM. Blood samples were collected immediately before infusion, hourly during infusion, and 4, 8, 12, 24, 48, and 72 h post-infusion to monitor blood iCa concentrations. Groups were compared using a mixed model ANOVA with time included as a repeated measure. Infusion of EGTA effectively decreased circulating iCa concentrations in both lactating and dry, non-pregnant cows compared with saline infusion (0.90 ± 0.01 mM, 0.84 ± 0.01 mM vs 1.25 ± 0.01 mM, 1.23 ± 0.01 mM; P < 0.0001). Lactating-EGTA infused cows had higher iCa during the infusion period compared with dry, non-pregnant EGTA cows (P = 0.003). Lactating-EGTA cows had higher iCa concentrations than all other treatments (1.36 ± 0.026 mM; P < 0.05) 24 h post-infusion. Additionally, dry, non-pregnant, EGTA cows required less EGTA to maintain subclinical hypocalcemia compared with lactating EGTA cows (P < 0.0001). This data suggests that homeostatic response to perturbation of calcium metabolism differs among early lactation and dry non-pregnant cows. Additionally, this demonstrates the early lactation dairy cow is capable of adapting to calcium challenges more rapidly and has improved feedback mechanisms to maintain circulating calcium concentrations than a dry, non-pregnant cow.

Key Words: calcium, hypocalcemia
Hepatic gene expression during the transition period is dynamic and responsive to changes in hormones, nutrients, and fatty acids (FA). Concentration and profile of FA differs postpartum between cows diagnosed with hyperketonemia (HYK) or not (nonHYK). The objectives were to determine hepatic gene expression and the relationship between FA profile and gene expression in HYK and nonHYK cows. Cows were enrolled 28 d before calving. Plasma samples were collected at d 1, 3, 7, 9, 11, and 14 d and liver biopsies at 1, 14 and 28 d. Postpartum plasma samples were analyzed for BHB and nonesterified FA. Fatty acid profile (−3, 1, 14 d) was determined via acid methylation and GC. Cows were classified as HYK (BHB ≥ 1.2 mM postpartum; average concentration ± SD, 9 ± 5 d; n = 13) or nonHYK (BHB < 1.2 mM; n = 15). Liver was analyzed for FA profile and expression of pyruvate carboxylase (PC) and cytosolic phosphoenolpyruvate carboxylkinase (PEPCKc) quantified by real time PCR, analyzed by standard curve method, and normalized to ribosomal protein L32 (6 reference genes explored with geNORM). Data were analyzed in PROC MIXED containing the fixed effects of time [repeated cow(HYK)], HYK, and time × HYK. Time × HYK protein L32 (6 reference genes explored with geNORM). Data were analyzed by standard curve method, and normalized to ribosomal protein L32 (6 reference genes explored with geNORM). Data were analyzed in SAS 9.4 using PROC MIXED containing the fixed effects of time [repeated cow(HYK)], HYK, and time × HYK. Time × HYK affected PC (P < 0.01) with HYK cows having decreased PC on d 1 (0.94 ± 0.33 vs. 0.33 ± 0.08 arbitrary units [AU]; P < 0.01) but not d 14 (0.18 vs. 0.13 ± 0.08 AU; P = 0.74) or 28 (0.28 ± 0.08 vs. 0.08 ± 0.08 AU; P = 0.12). Abundance of PEPCKc was not affected by HYK (P = 0.28) or time × HYK (P = 0.87). This resulted in decreased PC:PEPCKc on d 1 (P = 0.01), but not d 14 or 28 (P > 0.10), suggesting decreased complete oxidative capacity at parturition in cows that later develop HYK. The decreased PC in HYK cows at d 1 was not correlated with differences in nonesterified FA concentration or FA profile (P > 0.10). These data support that there are differences in PC:PEPCKc at calving in cows with subsequent HYK; however, shifts in FA profile do not appear to be related to downregulation of PC at d 1 in cows that subsequently develop HYK. Ultimately, the decreased PC:PEPCKc at parturition may contribute to onset of HYK and the regulatory mechanism should be further examined.

Key Words: liver, transition cow, ketosis


Under grazing conditions, daily intake can be estimated as the product of intake rate and grazing time but interactions with cow’s pre-grazing energy state may modify this relationship. A path analysis approach was used to explore relationships between ingestive behavior and cow’s pre-grazing metabolic variables affecting voluntary herbage intake of the first grazing bout in a morning session (VHI) in grazing dairy cows. Data of short-term ingestive behavior and pre-grazing metabolic variables was collected of 18 dairy cows (9 multiparous and 9 primiparous, days in milk = 73 ± 7; BW = 521 ± 32 kg; BCS = 2.75 ± 0.25; milk yield = 26 ± 3 kg) that grazed vegetative oat pasture (8 h of access to pasture from 8:30 to 16:30 h; pasture allowance = 30 kg DM/day; DM = 14%; CP = 23%, NDF = 46%, dry basis) and received supplementation after pm milking (6 kg DM/day of TMR of 70:30 forage to concentrate ratio, as-fed basis) from a short grazing experiment (20 d). Data were analyzed in SAS using the CALIS procedure. The direct and indirect relationships between bite rate (BR) and length of the first grazing bout (LFG, min), incisor arcade breadth (AB, cm) and pre-grazing serum glucose, insulin, glucagon, NEFA and BHB concentrations, with VHI (expressed as DM per 100 kg of metabolic weight) were estimated. The path analysis showed that direct effect of LFG was higher than AB (0.84 vs 0.34, P < 0.05), and BR did not correlate (0.026, P > 0.05) with VHI. In addition, pre-grazing NEFA and BHB concentrations correlates negative and positively to VHI (−0.56, P < 0.005; 0.71, P < 0.005), respectively, as indirect effects, and, pre-grazing NEFA and BHB concentrations correlates negative and positively (−0.74, P < 0.005; 0.71, P < 0.005), respectively, with LFG, as direct effects. The AB also correlates negatively to LFG but with lower standardized path correlation coefficient (−0.32, P < 0.05) than pre-grazing NEFA and BHB concentrations. Results predicted that the duration of the first grazing bout and pre-grazing NEFA and BHB concentrations are the main explanatory variables that affect the short-term herbage intake of dairy cows under temperate pastures.

Key Words: grazing bout, metabolic variable, Holstein cow

Intravenous lipopolysaccharide infusion modifies the bovine metabolome and lipidome. J. W. McFadden*, J. E. Rico1, E. A. Horst2, L. M. van den Brink2, and L. H. Baumgard2, 1Cornell University, Ithaca, NY, 2Iowa State University, Ames, IA.

Endotoxemia is a feature of steatohepatitis and sepsis. To define bovine metabolism in response to lipopolysaccharide (LPS) during a fatty acid (FA) insult, 10 multiparous Holstein mid-lactation cows were treated with a single i.v. bolus of saline (control; n = 5) or endotoxin (LPS E. coli O55:B5 at 0.375 μg/kg of BW; n = 5). Immediately post saline or LPS administration, all cows were i.v. infused a triglyceride (TG) emulsion (Intalipid 20% at 200 mL/h; Frasenius Kabi) for 16 h while fasted. Plasma was collected at h 0, 4, 8, 12, and 16, relative to bolus administration, start of TG infusion, and fasting. Liver was biopsied before (d −5) and after (h 16) these conditions. Plasma and liver metabolites were extracted for untargeted metabolomics or lipidomics using mass spec. Data were generalized log-transformed, auto-scaled, and analyzed using multivariate analyses. For metabolomics, metabolite identification was based on a mzCloud mass spectral score >80%. Mass spec detected 511 compounds. Although plasma FA (e.g., 18:2) levels increased with time (< 0.05), only 50 of 122 hepatic TG decreased (< 0.05). Similar reductions in plasma lyso phosphatidylcholine (LPC) levels developed post LPS (17 of 18; e.g., LPC-16:0; P < 0.05). Pronounced reductions in plasma lysophosphatidylcholine (LPC) levels developed post LPS (17 of 18; e.g., LPC-16:0; P < 0.05). Similar reductions in plasma LPC:phosphatidylcholine ratios were observed (P < 0.05). Endotoxin increased plasma pyruvic and lactic acids, and microbial-derived phenylacetylglutamine (P < 0.05). Also, LPS decreased plasma citric and 5-aminovaleric acids, and leucine, tyrosine, and threonine levels decreased by h 16 of FA insult (P < 0.05). In liver, TG infusion with fasting increased 7 of 8 ceramides (P < 0.05). Plasma salicylic and 2-hydroxyhippuric acids, and leucine, tyrosine, and threonine levels decreased by h 16 of FA insult (P < 0.05). As a result, endotoxin-induced inflammation. Additionally, endotoxin promotes aerobic glycosylation and intestinal permeability, and inhibits intestinal leucine uptake. We conclude that LPS modifies the metabolome and lipidome of the lactating cow experiencing elevated circulating FA.

Key Words: dairy cow, endotoxin, metabolome

Lipopolysaccharide induces lipolysis and reduces insulin sensitivity in subcutaneous adipose tissue from transition dairy cows. M. Chirivi*, J. Laguna1, L. Worden2, C. Prom2, A. Lock2, and G. Contreras1, 1Department of Large Animal Clinical Sciences,
Adipose tissue (AT) inflammation and excessive lipolysis predispose transition cows to metabolic disorders. In human and rodent AT, lipopolysaccharide (LPS) has been shown to trigger inflammatory responses and lipolysis and reduce insulin sensitivity (IS). The effect of LPS on lipolysis and IS in AT of dairy cows during the transition period has not been determined. We hypothesized that LPS triggers lipolysis and reduces IS in AT of transition dairy cows. Subcutaneous AT (SCAT) explants were collected from 12 Holstein dairy cows at −14 d prepartum and +6 d and +12 d after calving. Explants were incubated in the presence of LPS (CON = 0 μg/mL medium or LPS = 20 μg/mL medium). The effect of LPS on stimulated lipolysis was determined using isoroten- enol (ISO = 1μM) and LPS plus isorotenol (LPSISO) The impact of LPS on the anti-lipolytic responses induced by insulin at high (1µL/L, LPS-IIH) and low (0.2µL/L, LPS-IL) concentrations was determined by comparing it to the effect of insulin on lipolysis during ISO stimulation (ISO-IIH; ISO-IL). Lipolysis was determined by quantification of glycerol release. Statistical analyses were performed using a mixed effect linear model. Compared with CON, LPS increased glycerol release from SCAT by 73 ± 18% across all time points (P < 0.001) and tended (P = 0.09) to be affected by time relative to parturition with higher release of glycerol at −14 d (87 ± 2%) compared with +6 d (70 ± 2%) and +12 d (63 ± 2%). LPSISO increased the lipolytic response by 40 ± 17% compared with ISO (P < 0.05) and 255 ± 37% compared with CON (P < 0.001). Compared with ISO-IIH, LPS-IIH reduced the antilipolytic effect of insulin by 9 ± 2% (P < 0.05). No differences were observed between ISO-II and LPS-II. Our results demonstrate that LPS reduces IS and triggers lipolysis in SCAT. LPS also potentiates SCAT lipolytic response to adrenergic agonists. Collectively our results suggest that in diseases where plasma levels of LPS are increased, the lipolytic response of AT may be exacerbated through activation of lipolytic pathways and inhibition of the anti-lipolytic effects of insulin by LPS.

Key Words: LPS, lipolysis, adipose tissue

T107  Effects of rumen-protected methionine fed to lactating Holstein cows during a heat stress challenge on mammary mechanistic target of rapamycin (mTOR) signaling. D. N. Coleman1*, M. Vailati-Riboni1, R. T. Pate1, D. Luchini2, F. C. Cardoso1, and J. J. Loor1, 1Department of Animal Sciences, University of Illinois, Urbana, IL, 2Adisseo, Alpharetta, GA.

The objective was to investigate the effects of supplementing rumen-protected methionine (RPM) during a heat stress (HS) challenge on abundance and phosphorylation of mTOR (mechanistic target of rapamycin)-related signaling proteins in the mammary gland. Thirty-two multiparous, lactating Holstein cows (DIM 184 ± 59) were housed in tie stalls and randomly assigned to 1 of 2 environmental treatment groups, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc.; 0.105% DM as top dress) or TMR without RPM (CON)]. The study was divided in a crossover design. The study was divided into 2 periods with 2 phases per period. In phase 1 (9d), all cows were in thermoneutral conditions (TN) and fed ad libitum. During phase 2 (9d), group 1 (n = 16) was exposed to HS using electric heat blankets while group 2 (n = 16) remained in TN but were pair-fed to HS counterparts to control for DMI decreases associated with HS. After a washout period (21d), the study was repeated (period 2). Environmental treatments were inverted in period 2 (sequence), while dietary treatments remained the same. Mammary tissue was harvested via biopsy at the end of both periods and a subset of cows (12/treatment) were used for protein analysis. Data were analyzed using PROC MIXED in SAS with the effects of diet environment and their interaction, and period and sequence to account for the crossover design. Compared with TN cows, HS cows had greater vaginal temperatures (P < 0.001) and respiration rates (P < 0.001). No significant environment by diet interactions or sequence effects (P > 0.10) were observed for the proteins measured. The abundance of phosphorylated mTOR (p-mTOR) was greater with RPM supplementation (P = 0.04) and tended to be greater with HS (P = 0.08). No differences were observed in the abundance of AKT or phosphorylated AKT (P > 0.10). Additionally, CON cows had a greater decrease in milk protein (%) during phase 2 (difference from phase 1) compared with RPM cows (P = 0.04). Overall, preliminary evaluation suggests that RPM supplementation during a HS challenge could alter mTOR activation which may support greater milk protein synthesis.

Key Words: amino acid, lactation, mammary gland

T108  Innate immune response of late-lactation dairy cows is affected to a greater extent by heat stress than rumen-protected methionine. M. Vailati-Riboni1, D. Coleman1, R. T. Pate1, D. Luchini2, F. C. Cardoso1, and J. J. Loor1, Department of Animal Sciences, University of Illinois, Urbana, IL, 2Adisseo, Alpharetta, GA.

Heat stress (HS) has been shown to reduce immune functions, however, feeding rumen-protected methionine (RPM), a known immunostimulant, may mitigate its effects. Thirty-two multiparous, lactating Holstein cows (DIM 184 ± 59) were randomly assigned to 1 of 2 environmental groups, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc.; 0.105% DM of TMR as top dress) or without (CON)] in a crossover design, with 2 periods and 2 phases per period. In phase 1 (9d), cows were in thermoneutral conditions (TN; 16.0 ± 2.5°C, 71.4 ± 7.5% humidity, THI = 60 ± 3%) and fed ad libitum. In phase 2 (9d, 16.8 ± 2.5°C, 66.8 ± 8.0% humidity, THI = 61 ± 4%), group 1 (n = 16) was exposed to HS using electric heat blankets (THI = 89 ± 3%). Group 2 (n = 16) remained in TN but was pair-fed to HS counterparts. After a 21d washout period, the study was repeated (period 2). Environmental, but not dietary treatments were inverted (sequence effect). Blood was sampled 6d into period 2 and incubated with PI-labeled E. coli. Cells were stained to segregate neutrophils (CH138A) and monocytes (CD14) via flowcytometry. DHR was used as indicator of oxidative burst. Data were analyzed using PROC MIXED in SAS, with diet, environment, and their interaction as main effect, as well as period and sequence to account for the crossover design. Cow was used as random effect. HS increased (P < 0.001) rectal temperature and respiration rate (+0.3°C, +13.7 breaths/min) compared with CON. RPM did not affect (P > 0.05) functionality of both cell type. HS, instead, decreased neutrophil (P = 0.01, 72 vs 64%) and monocyte (P = 0.001, 27 vs 19%) oxidative burst and monocyte phagocytosis capacity (P = 0.05, 10 vs 8%).) while it only tended to decrease (P = 0.11, 9 vs 7%) neutrophil phagocytosis. A sequence effect was detected for both monocyte (P = 0.01, 19 vs 27%) and neutrophil (P = 0.03, 64 vs 73%) oxidative burst, with overall lower response in animals subjected to HS in period 1. Results highlight the negative effect of HS on dairy cow innate immune function, including a probable carry over chronic effect. RPM was ineffective in counteracting the detrimental effect of HS during late-lactation, probably due to the short feeding period before the imposed stress.

Key Words: methionine, heat stress, immune function

The advance of genetics is continually leading cattle to more efficient utilization of nutrients and improved performance, but also to more metabolic disorders. Environmental changes, i.e., higher ambient temperature and humidity contribute to changes in metabolic requirements. A completely randomized design was used to evaluate the effect of heat stress and cooling while feeding an −11 mEq/kg of dietary cation anion difference (DCAD) diet to characterize thermoregulatory and hematological changes of cows in the dry period (DP). Cows were dried-off ~46 d before parturition and randomly assigned to cooling (shade, fans and soakers; CL) or heat stress (shade; HT). In the DP, vaginal temperature (VT, °C; every 10 min), respiration rate (RR; breaths per min) and temperature humidity index (THI) were recorded to evaluate heat strain. On one day (THI ≥78.8 in CL and HT) blood samples were collected at 0600, 1200, 1800 and 2400 from cows (HT = 27.5 ± 12.8 d dry; CL = 24.3 ± 14.8 d dry; n = 12/group) to assess electrolytes, hematology, and blood gases. The model included the fixed effect of prepartum environment (HT vs. CL) in a completely randomized design with cow as random effect analyzed using repeated measures. Urine pH was recorded weekly and was similar for HT and CL cows (P = 0.36). Cows under HT had higher VT and RR (P < 0.01), lower blood pH (HT = 7.41 ± 0.003, CL = 7.43 ± 0.003, P < 0.05) and had higher iCa at 0600 (HT = 1.30 ± 0.02, CL = 1.25 ± 0.01, P < 0.05) relative to CL. There was a time effect, whereby iCa was lower at 1200 but higher at 2400, regardless of treatment (P < 0.01). CL cows had higher blood glucose at 1200 and 2400 relative to HT cows (66.6 ± 1.08 vs. 63.4 ± 1.06, P < 0.05; 68.3 vs. 64.1 ± 1.08 P < 0.01, respectively). There was also a time effect on K concentration, whereby K was lower at 1200 but higher at 0600, regardless of treatment (P < 0.05). HT cows had higher PCO2 relative to CL cows (P < 0.05). Comparing HT vs CL, no differences were found for Na, Hct, Hgb, HCO3, pO2, SO2 and tCO2. Thus, we observed different physiological responses of cows receiving the same level of negative DCAD, but under different environmental conditions.

**Key Words:** electrolytes, hematology