
Dairy cows have a daily rhythm of milk synthesis that appears to be driven by the molecular clock of the mammary gland and is modified by the time of feed availability. Protein metabolism is intimately linked to circadian rhythms in other species, but the effect of amino acids on the mammary clock is unknown. The objective was to determine the effects of the timing of protein absorption on the daily rhythms of milk synthesis in dairy cows. Nine cannulated lactating Holstein cows (128 ± 46 d postpartum; mean ± SD) were randomly assigned to 1 of 3 treatment sequences in a 3 × 3 Latin square. Treatments were abomasal infusions of 500 g/d of sodium caseinate either 24 h/d (CON) or for 8 h/d from 0900 to 1700 h (DAY) or from 2100 to 0500 (NGT). Treatment periods were 15 d with a 6-d washout. During the final 8 d of each period, cows were milked every 6 h. A 24-h rhythm was fit to time-course production data using cosine analysis and the amplitude and acrophase (time at peak) were determined. Daily milk and protein yield were decreased by NGT compared with CON, while fat yield was increased by DAY (P < 0.05). Daily fat and protein concentration were not affected by treatment. Milk yield failed to fit a 24 h rhythm in CON or DAY, but a rhythm was induced by NGT (P = 0.03). Neither fat yield nor protein yield fit a rhythm in either treatment. Fat concentration fit a daily rhythm in all treatments (P < 0.05), with the amplitude decreased 57% by DAY and 26% by NGT (P < 0.05). The rhythm of milk fat concentration was phase advanced ~2 h by DAY and phase delayed ~1 h by NGT (P < 0.05). Protein concentration fit a daily rhythm in CON and DAY but not NGT. The phase of protein concentration was delayed ~1.25 h by DAY and the amplitude was increased 2-fold relative to CON (P < 0.05). The time of protein infusion influenced daily rhythms of milk and milk protein synthesis. Night infusion abolished rhythms of protein concentration and induced rhythms of milk yield while day infusion increased the amplitude of protein concentration. Data analysis and results from this study suggest a role of amino acids in entraining the molecular clock of the mammary gland.

Key Words: daily rhythm, milk synthesis, nutrient entrainment

312 Effects of circadian rhythm disruption during the dry period on tissue mobilization in multiparous dairy cattle. R. Klopp*1, T. S. Steckler1, A. Suarez-Trujillo1, M. Grott2, J. R. Townsend3, K. Plaut3, T. M. Casey1, and J. P. Boerma1, 1Department of Animal Sciences, Purdue University, West Lafayette, IN; 2Animal Science Research and Education Center, Purdue University, West Lafayette, IN; 3Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN.

During the transition period, dairy cattle go through intense physical and metabolic changes. The circadian timing system (CTS), functions to coordinate internal physiology including metabolic processes, therefore, disruption of the CTS, is associated with changes in metabolism. Our objective was to determine if disrupting the CTS from 35 d before expected calving (BEC) until calving affected muscle and adipose tissue mobilization in dairy cattle. In a completely randomized design, 32 multiparous cows were blocked by lactation number, disease incidence from previous lactation and assigned to 1 of 2 treatments, control (C) or phase-shift (PS). C were exposed to 16 h light:8 h dark. PS were exposed to the same amount of light and dark however, every 3 d there was a 6-h shift in the light-dark cycle. BW, BCS, back fat thickness, and muscle depth were measured on 35, 21, and 7 d BEC and 0, 10, 30 and 60 d postpartum (PP). Blood samples were collected on 35, 21, and 7 d BEC and 0, 2, 5, 9, 15, and 22 d PP and analyzed for NEFA, 3-methyl-histidine (3MH), and creatinine. Data were analyzed using the Mixed Procedure of SAS v.9.4 with the fixed effects of treatment, time point, and their interaction and the random effect of cow nested within treatment. There were no treatment differences for BW, BCS, or NEFA (all P > 0.25). There was a significant effect of time point on BCS, BW, and NEFA (P < 0.0001), with a mean loss of 97.6 kg of BW and 0.69 points of BCS from calving to 60 DIM. Cows mobilized 19.1% of their muscle depth and 50% of their subcutaneous body fat from 7 d BEC to 60 DIM. Cows had greater muscle depth (P = 0.07, 4.25 vs. 3.97 cm), also reflected in higher creatinine (P < 0.0001, 3.127 vs. 2.775 ng/mL), which is used to estimate muscle mass. However, 3MH, which is an indicator of muscle mobilization, was also greater (P = 0.03, 431 vs. 356 ng/mL). Disruption of the circadian rhythm during the dry period did not cause increased tissue mobilization and could potentially reduce the amount of muscle mobilization. Regardless of treatment, the extent of both muscle and adipose tissue mobilized during the transition period is substantial.

Key Words: tissue mobilization, multiparous, transition cow

313 Effect of fatty acid absorption on the daily rhythms of milk synthesis and plasma hormones and metabolites in dairy cows. I. Salfer* and K. Harvatine, The Pennsylvania State University, University Park, PA.

Dairy cows display daily rhythms of milk synthesis that are altered by the time of feed availability and appear to be driven by the circadian clock of the mammary gland. Fatty acids have been shown to entrain circadian rhythms in tissues such as the liver and adipose tissue in experimental models, but their role in the mammary gland is unknown. The objective was to determine the effects of the timing of fatty acid absorption on the daily rhythms of milk synthesis. Nine cannulated lactating Holstein cows (132 ± 90 d postpartum; mean ± SD) were randomly assigned to 1 of 3 treatment sequences in a 3 × 3 Latin square. Treatments were abomasal infusions (132 ± 90 d postpartum; mean ± SD) of 350 g/d of a high C18:1 oil either 24 h/d (CON) or for 8 h/d from 0900 to 1700 h (DAY) or from 2100 to 0500 (NGT). Treatment periods were 12 d with a 6-d washout. During the final 7 d of each period, cows were milked every 6 h to determine the daily patterns milk synthesis across the day. A 24-h rhythm was fit to time-course data using cosine analysis and the amplitude and acrophase (time at peak) were determined. Daily milk and milk protein yield were decreased by NGT compared with CON, while fat yield was increased by DAY (P < 0.05). Daily fat and protein concentration were not affected by treatment. Milk yield failed to fit a 24 h rhythm in CON or DAY, but a rhythm was induced by NGT (P = 0.03). Neither fat yield nor protein yield fit a rhythm in either treatment. Fat concentration fit a daily rhythm in all treatments (P < 0.05), with the amplitude decreased 57% by DAY and 26% by NGT (P < 0.05). The rhythm of milk fat concentration was phase advanced ~2 h by DAY and phase delayed ~1 h by NGT (P < 0.05). Protein concentration fit a daily rhythm in CON and DAY but not NGT. The phase of protein concentration was delayed ~1.25 h by DAY and the amplitude was increased 2-fold relative to CON (P < 0.05). The time of protein infusion influenced daily rhythms of milk and milk protein synthesis. Night infusion abolished rhythms of protein concentration and induced rhythms of milk yield while day infusion increased the amplitude of protein concentration. Data analysis and results from this study suggest a role of amino acids in entraining the molecular clock of the mammary gland.

Key Words: daily rhythm, milk synthesis, nutrient entrainment
ing the amplitude of fat and protein concentration, whereas infusion at night had little effect.

Key Words: daily rhythm, milk synthesis, nutrient entrainment

314 Nitrogen metabolism and insulin signaling targets in adipose tissue of Holstein cows during the periparturient period differ by body condition score. Y. Liang\(^1\), E. Trevisi\(^2\), and J. Loor\(^3\), \(^1\)University of Illinois at Urbana-Champaign, Urbana, IL, \(^2\)Università Cattolica del Sacro Cuore, Piacenza, Italy.

The periparturient period is characterized by increased insulin resistance status in dairy cows. In non-ruminants, excessive fat deposition is associated with decreased insulin sensitivity and alterations in amino acid (AA) metabolism in adipose tissue. The hypothesis was that abundance of AA transporters, components of the urea cycle, and insulin signaling targets differs by prepartal body condition score (BCS) in dairy cows. Twenty 2 multiparous Holstein cows were divided by BCS before parturition (≥30 d prepartum) into a BCS ≤3.25 (LoBCS, n = 11) or BCS ≥3.50 (HiBCS, n = 11) group. Adipose tissue obtained on d −15, 7, and 30 relative to calving date was used for RT-PCR analysis. Blood was obtained at −30, −15, 7, 10, and 30 d relative to calving date for energy balance biomarker analysis. The statistical model in SAS included the fixed effect of treatment, time, and its interaction. There was no difference in prepartal DMI and milk yield between groups (P > 0.05). A treatment × time interaction was observed for postpartal DMI due to greater responses in LoBCS cows (P < 0.05). A treatment × time was observed for the plasma concentrations of fatty acids (NEFA) due to greater (P < 0.05) responses in HiBCS cows. Compared with LoBCS, HiBCS cows had greater (P < 0.05) plasma concentrations of glucose and hydroxybutyrate (BHBA). Among 6 AA transporters measured, HiBCS cows had greater overall (P < 0.05) mRNA abundance of high-affinity cationic (SLC7A1) and sodium-coupled AA transporters (SLC38A1). Abundance of arginino-succinate lyase (ASL) and arginase 1 (ARG1) (P < 0.05) also was greater overall in HiBCS cows, suggesting that adipose tissue in those cows had greater urea cycle activity. Abundance of protein kinase B1 (AKT1) was upregulated (P < 0.05) in HiBCS cows suggesting enhanced insulin signaling. Overall, the data indicate that BCS is associated with unique profiles of targets related to insulin sensitivity and nitrogen utilization in adipose tissue of dairy cows. The significance of these findings in the context of physiologic adaptations during the periparturient period merit further research.

Key Words: insulin resistance, amino acid transporter, transition cow

315 Plasma metabolome profiles in heifer calves at birth and during the preweaning period are altered by supply of methionine during late-pregnancy. A. Elolimy\(^1\), A. Alharthi\(^2\), E. Abdel-Hamied\(^1\), C. Parys\(^4\), and J. Loor\(^3\), \(^1\)Mammalian NutriPhysiogenomics, Department of Animal Sciences, University of Illinois, Urbana, IL, \(^2\)Department of Animal Sciences, University of Illinois, Urbana, IL, \(^3\)Animal Medicine Department, Beni-Suef University, Beni-Suef, Egypt, \(^4\)Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.

Maternal methionine supplementation in pregnant sows increases amino acid content (methionine, tryptophan, and valine) in plasma of piglets which likely helps support better growth performance during the preweaning period. However, the influence of maternal methionine in dairy cows on plasma metabolome of neonatal calves is unknown. The objective of the current study was to evaluate the impact of maternal methionine supply during late-pregnancy in dairy cows on plasma metabolome in neonatal calves, and its association with growth performance during the preweaning period. Heifer calves born to Holstein cows receiving either a control (CON) diet (n = 10) or CON plus rumen-protected methionine (MET, Evonik Nutrition & Care GmbH) (n = 10) during the last 28 d of pregnancy were selected. Plasma samples collected at d 0 (i.e., at birth before colostrum feeding), 2, and 42 of age were used for LC-MS untargeted metabolomics. Multivariate analysis of metabolome data was performed with MetaboAnalyst 4.0. No differences between groups were detected in daily starter intake (P = 0.77) during the preweaning period. However, MET heifers had greater body weight, hip height, wither height, body length, and average daily gain (P < 0.05). Plasma metabolomics revealed a clear separation between MET and CON heifers during the preweaning period. Among enriched metabolic pathways upregulated (≥2-fold) in MET heifers were several associated with amino acid metabolism (tryptophan, valine, tyrosine), thiamin metabolism, and peroxisomal oxidation. In contrast, downregulated pathways in MET calves (≥2-fold) were primarily associated with b-oxidation of fatty acid, TCA cycle flux and CoA synthesis. Overall, in the absence of differences in feed intake, the responses in calf plasma metabolome profiles as a result of enhanced maternal methionine supply suggest that those calves experienced alterations in metabolism. Whether the metabolome profiles reflect provision of extra nutrients and how those profiles are associated with greater body mass and growth performance remain to be established.

Key Words: methionine, calf, metabolomics

316 Effects of hindgut acidosis on metabolism, inflammation and production in dairy cows consuming a standard lactation diet. M. A. Abeyta\(^1\), E. A. Horst\(^1\), E. J. Mayorga\(^1\), B. M. Goetz\(^1\), M. Al-Qaisi\(^1\), C. S. McCarthy\(^1\), M. R. O’Neil\(^1\), B. C. Dooley\(^1\), P. Piantoni\(^2\), G. F. Schroeder\(^2\), H. A. Ramirez-Ramirez\(^1\), and L. H. Baumgard\(^1\), \(^1\)Department of Animal Science, Iowa State University, Ames, IA, \(^2\)Cargill Animal Nutrition Innovation Center, Elk River, MN.

Impaired intestinal integrity caused by excessive hindgut starch fermentation is a potential source of peripheral inflammation in dairy cattle. Study objectives were to evaluate effects of hindgut acidosis in lactating dairy cows. Five rumen-cannulated Holstein (244 ± 49 DIM, 33 ± 3 kg milk/d) cows were enrolled in a study with 2 experimental periods (P) during which cows were fed a diet with 26% starch ad libitum. During P1 (4d), baseline data were collected. During P2 (7d), cows were abomasally infused with 4 kg/d of starch (1 kg pure corn starch + 1.25 L H2O/infusion at 0000, 0600, 1200, and 1800 h). Milk, feces, and blood samples were collected daily. Effects of time were assessed using PROC MIXED (SAS Inst. Inc., Cary, NC). Compared with P1, starch infusion markedly reduced fecal pH (5.98 vs. 6.76; P < 0.01) and increased fecal starch content (3-fold; P = 0.01). Milk yield and DMI were reduced by starch infusion (4 and 5%, respectively; P < 0.05). Rectal temperature remained unchanged following infusions. Milk urea nitrogen decreased (10%; P < 0.01) relative to P1; however, starch infusions did not affect milk fat, protein or lactose content. Milk SCC increased 11-fold (P = 0.05) during P2. Circulating insulin and β-hydroxybutyrate increased (141 and 53%, respectively; P < 0.01), while plasma glucose tended to decrease (9%; P = 0.06) at 14h relative to the first infusion (RFI). Starch infusion increased blood urea nitrogen 2h RFI (22%) before it promptly decreased (12%; P = 0.03) for the remainder of P2. Additionally, P2 NEFA concentrations decreased (32%; P < 0.01) relative to P1. Monocytes decreased (38%; P = 0.03) at 2h RFI, but there were only minor increases in white blood cells, neutrophils, lymphocytes, eosinophils and basophils relative to P1 (10, 13, 7, 30, and 20%, respectively; P < 0.05). Circulating serum amyloid A and lipopolysaccharide binding protein...
concentrations were unaffected by starch infusion. Although abomasal starch infusion markedly reduced fecal pH and altered bioenergetics, it only had minor effects on metabolism, inflammation, and production in cows consuming a moderately high starch diet.

**Key Words:** gut permeability, gut health, endotoxin

### 317 Hepatic one-carbon metabolism, methionine cycle, and transsulfuration pathways are altered by prepartum adiposity and feed intake in peripartal grazing cows. M. Vailati-Riboni*1, Y. Lian1, D. Coleman1, S. Meier2, C. Burke2, J. K. Kay2, M. D. Mitchell3, C. G. Walker2, M. A. Crookenden2, A. Heiser3, J. R. Roche3, and J. J. Loor1, 1Department of Animal Sciences, University of Illinois, Urbana, IL, 2DairyNZ, Hamilton, New Zealand, 3University of Queensland, Queensland, Australia, 4AgResearch, Palmerston North, New Zealand.

Twenty 8 mid-lactation grazing dairy cows of mixed age and breed were randomly allocated to 1 of 4 treatment groups in a 2 × 2 factorial design: 2 prepartum body condition score (BCS) categories [4.0 (thin) and 5.0 (optimal); 10-point scale] and 2 levels of energy intake during the 3 wk preceding calving (75% and 125% of estimated requirements). Liver tissue was harvested by biopsy at −7, 7, and 28 d relative to calving. Samples were homogenized and used for 14C assays to investigate betaine-homocysteine S-methyltransferase (BHMT), methionine synthase (MS), and cystathionine-β-synthase (CBS) activity. Metabolites relative concentration was analyzed using a targeted GC/MS profiling approach. Data were normalized with tissue protein concentration, and subjected to repeated measures ANOVA via PROC MIXED in SAS, with BCS, feeding, and Time as fixed effects, and cow as random effect. All enzymes were affected by time, with BHMT activity peaking (P < 0.05) at d 7, while CBS and MS activity decreased postpartum (P < 0.05).

Overall, thin cows had greater (P < 0.05) MS activity, while cows fed 125% requirements had greater (P < 0.05) CBS activity. An interaction was detected for CBS activity, as thin cows fed 125% of requirements had greater (P < 0.05) overall activity. BCS 5 cows had overall greater betaine, glycine, acetylcholine, serine, and taurine concentrations. The same metabolites, plus choline and, N-Ndimethyl glycine were overall greater in liver of cows fed 75% of requirements. An interaction was detected for the aforementioned metabolites and also methionine, cystathionine, cysteinesulfinylate, and hypotaurine due to overall greater levels in BCS 5 cows fed at 75% of requirements compared with other groups. Data indicate activity of enzymes involved in hepatic choline and methionine metabolism in pasture-based cows follow a different trend to those reported for high yielding cows in confinement. Furthermore, metabolomics underscores the need to restrict BCS 5 cows prepartum to ensure an optimal transition into lactation.

**Key Words:** branched-chain AA (BCAA) transporters, tissue abundance, body condition

### 318 Plasma branched-chain amino acids (BCAA) and mRNA abundance of 3 different BCAA transporters in adipose tissue, muscle and liver of dairy cows with high or normal body condition score. L. A. Webb*1, H. Sadri2, K. Schuh1,3, S. Egert4,5, P. Stehle5, C. Koch6, G. Dusel3, and H. Sauerwein1, 1Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Bonn, Germany, 2Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran, 3Department of Life Sciences and Engineering, Animal Nutrition and Hygiene Unit, University of Applied Sciences Bingen, Bingen, Germany, 4Institute of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany, 5Department of Nutrition and Food Sciences, Nutritional Physiology, University of Bonn, Bonn, Germany, 6Educational and Research Center for Animal Husbandry, Hofgut Neumühle, Münchweiler a.d. Alsenz, Germany.

Branched-chain amino acid transporters (BCAAT) are crucial to sensing AA availability and regulating BCAA homeostasis, e.g., via interaction with the mechanistic target of rapamycin. Except for mammary gland and placenta, their abundance in metabolically active tissues in dairy cows is mostly unknown. Thus, we aimed to 1) compare the mRNA abundance of the most relevant BCAAT, i.e., solute carrier family 1 member 5 (SLC1A5), SLC7A5, and SLC38A2 in muscle, liver and subcutaneous adipose tissue (AT) as well as the plasma BCAA profiles and 2) test whether high and normal BCS cows may differ in these variables.

Thirty-eight multiparous Holstein cows, 15 wk ante partum (a.p.) were allocated to high (HBCS; BCS > 3.75) or normal BCS groups (NBCS; BCS < 3.75) receiving 7.2 or 6.8 MJ/kg DM, respectively, until dry-off to promote the difference. During the dry period and early lactation, cows were fed the same diets; differences in BCS (P < 0.001) were maintained. Blood and biopsies from M. semitendinosus, liver and AT (tail head) were sampled at d 49 a.p., 3, 21 and 84 postpartum (p.p.). Free BCAA were quantified by HPLC; mRNA abundance was assessed via qPCR.

Data were analyzed as repeated measures with a Linear Mixed Model (SPSS). Concentrations of all BCAA changed with time (P ≤ 0.01), most markedly in HBHCBS cows, with a nadir around calving. Neither individual nor total BCAA differed between groups. For Ile, a group × time interaction was found (P = 0.03): At d 3 p.p. HBCS cows tended to have lower Ile concentrations than NBCS cows (P = 0.08), vice versa at d 21 (P = 0.02). Abundance of BCAAT varied only with tissue and time (P < 0.01). The SLC1A5 and SLC7A5 mRNA were most abundant in AT; liver had 3 and 24% and muscle 11 and 37% of the AT values, respectively. In muscle, SLC1A5 and SLC7A5 mRNA peaked at d 21 p.p. Both AT and muscle had 4-fold greater SLC38A2 abundance than liver, with irregular time courses. Our results indicate that AT could be a major site of BCAA uptake that is rather independent of BCS and time relative to calving in dairy cows.

**Key Words:** branched-chain AA (BCAA) transporters, tissue abundance, body condition

### 319 Abundance of hepatic patatin-like phospholipase domain-containing protein 3 protein was inversely related to peripartum hepatic triglyceride accumulation. R. S. Pralli*, H. T. Holdorf, C. R. Seely, R. Caputo Oliveira, and H. M. White, University of Wisconsin-Madison, Madison, WI.

The objectives of this study were to determine hepatic patatin-like phospholipase domain-containing protein 3 (PNPLA3) protein abundance peripartum and the relationship with hepatic triglyceride (TG) content. Multiparous cows were blocked by expected calving date and randomly assigned to a control (n = 13) or fatty liver induction (FLI; n = 12) treatment. Control cows were fed ad libitum peripartum, while FLI cows were offered a 6 kg cracked corn top-dress prepartum and feed restricted to 80% ad libitum intake at + 14 d relative to calving (DRTC) until blood β-hydroxybutyrate ≥ 3.0 mM. Liver biopsies were collected at −28, −14, +1, +14, +28, +42, and +56 DRTC. Quantification of hepatic PNPLA3 via semiquantitative Western blot was normalized to total sample protein. Statistical analysis was performed using the GLIMMIX procedure (SAS 9.4). Hepatic TG %DM and PNPLA3 abundance were log2 transformed. Models included fixed effects of treatment, DRTC, and treatment × DRTC, as well as random intercepts of cow, block, and repeated measures of cow across DRTC. The PNPLA3 model included the −28 DRTC abundance as a covariate. Hepatic PNPLA3 abundance was also regressed against hepatic TG. All FLI and control cows had blood β-hydroxybutyrate ≥ 3.0 mM. Hepatic TG content did not differ...
between treatments \( P = 0.50 \) but differed over time \( P < 0.001 \). Prepartum hepatic TG was lower than postpartum; the postpartum maximum and nadir TG were at +14 and +56 DRTC \( P < 0.05 \), respectively. No treatment difference was detected for hepatic PNPLA3 abundance \( P = 0.31 \). A time effect was observed \( P < 0.001 \) with increasing PNPLA3 from −14 to +56 DRTC. The greatest PNPLA3 abundance was observed during the postpartum decrease of hepatic TG. Hepatic PNPLA3 had an inverse relationship \( (\beta = −0.31; \ P = 0.03) \) when regressed against hepatic TG content. These results suggest hepatic PNPLA3 protein abundance was not altered by FLI but changed over time inversely compared with postpartum hepatic TG. Furthermore, regression analysis suggests that incremental increases in hepatic PNPLA3 may lessen TG accumulation peripartum.

**Key Words:** fatty liver, lipase, transition cow