Cows experiencing heat stress in utero produce less milk in their first lactation relative to their normothermic herdmates. However, it is unclear if heat stress in utero affects development of the mammary gland (MG), which may affect future milk yield. We hypothesized that exposure of the fetus to heat stress in utero alters mammary microstructure and cell processes that determine mammary cell number later in life. Heifers (in-utero CL [IUCL], n = 10; in-utero HT [IUHT], n = 9), were born to dams housed in shaded barns either with fans and soakers or without cooling devices during late gestation (dry period, ~46 d), respectively. During their first lactation, heifer milk yield was recorded from calving to 84 d in milk (DIM). MG were biopsied at 21 and 42 DIM. Sectioned tissues were stained with Masson’s trichrome to visualize morphology. Apoptosis and proliferation of mammary cells were determined through immunohistochemistry (TUNEL and Ki67, respectively). Alveoli luminal area was measured, alveoli were counted, MG connective tissue was quantified, and TUNEL and Ki67 positive and negative mammary epithelial and stromal cells were counted in Image J. Data were analyzed by repeated measures ANOVA or generalized linear mixed models using SAS. IUCL had lower colostrum yield (3.7 vs. 5.5 ± 0.4 kg/d, P = 0.01) but higher milk yield (31.5 vs. 30.2 ± 0.4 kg/d; P = 0.05) relative to IUHT. Alveoli number was similar between groups, but MG of IUCL tended to have larger alveoli than IUHT (4,390 vs. 3,577 ± 348 μm², P = 0.11). The MG of IUCL tended to have less connective tissue in the stromal compartment relative to IUHT (86,727 vs. 15,839 ± 27,616 μm², P = 0.08). IUCL tended to have a greater percent of proliferating cells in MG, driven by a significant difference in percent stromal cells proliferating (2.0 vs. 1.0 ± 0.4%, P = 0.05). There was no difference between groups in percent mammary epithelial cells or stromal cells undergoing apoptosis (P > 0.05). These results suggest that fetal exposure to heat stress in utero adversely affects mammary development in the first lactation, with consequences for milk yield.

Key Words: intrauterine environment, fetal programming, mammary microstructure

Recently the effects of methionine (Met) supplementation in milk performance of dairy cows through gene expression regulation have become more evident. Histone methylation (HM) can affect gene expression and consequently milk biosynthesis. Therefore, we evaluated the effect of Met on histone methylation in bovine mammary epithelial alveolar cells (MacT) incubated at increased concentrations of l-methionine. Prior to transfection cells were cultivated in high glucose Dulbecco modified Eagle’s medium (DMEM) with sodium pyruvate and supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin and Fungizone antimycotic. The plasmids used in this study were the pcDNA3-K9 and pcDNA3-K27 (Addgene) for analysis of HM through fluorescence resonance energy transfer (FRET) technology. Cells were seeded 24 h before transfection at 30,000 cells/well in a 96-well plate. Cells were transfected with Lipofectamine 3000 as the transfection reagent at 0.3 uL/well and at 50 ng/well of plasmid in a reduced serum medium (OptiMEM) deprived of FBS. Transfected cells were treated for 24h in triplicates with 0, 125, 250, and 500 μM l-methionine. An inverted fluorescent microscope for live imaging (EVOS FL Auto) equipped with a motorized scanning stage, and an environment-controlled chamber at 37°C and 5.0% of CO₂ was used to take 4 pictures/well at 4x magnification 0, 12, and 24h post-treatment. Transfection efficiency, viability, and quantification of HM were assessed using the CellProfiler software. Data were analyzed using the PROC MIXED of SAS and significance was declared at P ≤ 0.05. Least squares means separation was corrected using Tukey’s test. Overall HM in K9 increased (P ≤ 0.05) in cells incubated with Met as early as 12h post-treatment, and this effect remained until 24h. The HM in K27 by Met seemed to be less effective, in fact, HM tended (P = 0.09) to be lower in cells incubated with 500 uM of Met than control. To expand on these effects, global DNA methylation and gene expression analysis will be performed. Our results indicate that Met treatment can affect the HM status of histone tail residues differently, and this can result in profound changes in gene expression regulation. The extent of dietary Met in HM at the mammary gland level and consequently milk synthesis remains unknown.

Key Words: methionine, milk genomics

In vitro histone manipulation of bovine mammary epithelial cells through methionine supplementation. F. Rosa* and J. S. Osorio, Dairy and Food Science Department, South Dakota State University, Brookings, SD.

Although metabolic pathways for milk synthesis are well understood, less is known about the mechanisms that control these processes. The mechanistic target of rapamycin complex 1 (mTORC1) is an evolutionary conserved nutrient-sensing pathway that plays a central role in the regulation of nutrient metabolism. Previous work has implicated mTORC1 in the control of protein synthesis in bovine mammary epithelial cells (BMEC), but whether it is involved in regulation of other milk components like lipids is not known. The objective of this study was to assess the role of mTORC1 on de novo lipid synthesis in BMEC. Primary BMEC were isolated from lactating mammary tissue of 3 independent cows and induced to differentiate by addition of lactogenic hormones (5 μg/mL insulin, 5 μg/mL prolactin and 5 μg/mL hydrocortisone). Data were analyzed using a randomized complete block design using PROC MIXED in SAS. Treatment differences were considered significant when P < 0.05. To assess the role of mTORC1 on lipid synthesis, BMEC were treated with a vehicle (control) or 100 nM rapamycin, a specific inhibitor of mTORC1, for 16 h. Phosphorylation of the mTORC1 targets eukaryotic initiation factor 4E binding-protein 1 (4EBP1) Thr70, ribosomal protein S6 (rpS6) Ser240/44, and S6 kinase 1 (S6K1) Thr389 were reduced (P < 0.05) in BMEC treated with rapamycin, compared with control cells, confirming mTORC1 inhibition. To determine the effect on mTORC1 activity on de novo lipid synthesis, we measured 3H-acetate incorporation into total cellular lipids by liquid scintillation counting. We found that rapamycin-treated cells showed a decrease (P < 0.05) in total lipid synthesis which was reduced by 24% in treated BMEC. To identify potential mechanisms by which mTORC1 regulates lipogenesis in BMEC, we measured the mRNA abundance of enzymes involved in lipogenic pathways by real-time qPCR. SREBP1 gene expression that encodes for sterol regulatory element binding protein 1, a master lipogenic transcription factor, was decreased (P < 0.05) by 20% in rapamycin treated BMEC. Rapamycin also reduced...
expression of the key lipogenic genes FASN, FABP3 and DGAT1, but not of SCD1 or ACACA. In conclusion, these results demonstrate an important role for mTORC1 in regulation of de novo lipid synthesis in BMEC. Funded by grants from NSERC to S.A.B. and a scholarship from Op+Lait-FRQNT to M.-A.G.

Key Words: bovine, mechanistic target of rapamycin complex 1 (mTORC1), lipid

479 Citrate and choline in milk are biomarkers of mammary inflammation in heat stressed and LPS challenged dairy goats. A. Contreras-Jodar*1, S. Love1, N. Mehaba1, G. Caja1, and A. A. K. Salama1,2, 1Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain, 2South Dakota State University, Brookings, SD.

As a follow up of a previous experiment done in dairy goats (Love et al., 2016; ADSA Annual Meeting) to elucidate the response to an E. coli endotoxin (LPS) challenge, milk data by nuclear magnetic resonance (1H-NMR) from Murciano-Granadina dairy does in mid-lactation (n = 8; 2.2 ± 0.1 L/d) and submitted to 12–12 h photoperiod and thermoneutral (TN; THI_NRC = 65–59; n = 4) or heat stress (HS; THI_NRC = 83–75; n = 4) conditions, were run in an experiment according to NRC (1971) on d-12 of experiment, TN and HS does were infused with 2 mL of LPS (10 μg) in one udder-half, at random, whereas the other udder-half was with saline. Milk samples were collected post-LPS challenge (h 0, 6, 12 and 24) and analyzed by 1H-NMR spectroscopy. Data analyses were done by R v.3.2.3 and included PCA (principal component analysis) and PLS-DA (partial least square-discriminant analysis) assessment, with cross validation leave-one-out, to detect milk biomarkers. Biomarkers were identified by cow’s milk data (Sundekilde et al., 2013).

Milk citrate increased in HS does (R2 = 0.96; Q2 = 0.24) indicating a shift in macropathways mitochondrial function (i.e., transporting mitochondrial citrate to cytosol to produce inflammatory mediators such as PGE2, ROS and NO). When HS does were challenged with LPS, there were dramatic increases of choline, N-acetylcarnitohydrates (i.e., N-acetylcarnitohydras and N-acetylcarnitohydras) and 1-lactate, as well as, a strong decrease of lactose in milk (R2 = 0.79; Q2 = 0.49). Expected benefits of choline (mainly synthesized from Met) were the modulation of the immune function by mean the neurotransmitter acetylcholine and the protective role of N-acetylearnitohydrates against the adherence of pathogenic bacteria to the mammary epithelium. 1-lactate reflected the activation of the immune system by mitochondrial-oxidative shift to cytosolic (glycolytic) pathway. In conclusion, the metabolism profile of goat’s milk was markedly affected by the environmental conditions and the udder health status. Milk biomarkers indicated the occurrence of inflammatory stages in the mammary gland under HS and LPS stressing conditions, being citrate and choline, respectively, the most affected metabolites. Supported by MINECO Spain (Projects AGL-2013-44061-R and RTI2015-0035-C03-02).

Key Words: circadian rhythm, lactation, food entrainment


Subclinical hypocalcemia (SCH) predisposes cows to other periparturient disorders. Mitigation strategies are crucial to prevent dairy cows from succumbing to SCH. Our lab has previously demonstrated that pre-partum infusions of 5-hydroxytryptophan (5-HTP), the precursor to serotonin, modulates calcium metabolism in transition period dairy cows, yet the precise mechanism in the mammary gland is unknown. The objective of this study was to determine if intravenous (IV) infusion of 5-HTP in mid-lactation dairy cows regulated calcium metabolism in the mammary gland. This study utilized a randomized complete block design with 12 multiparous Holstein cows, blocked by parity, receiving either IV 1.5 mg/kg 5-HTP (n = 6) or saline (n = 6) for 3 consecutive days. Baseline blood samples were taken before treatment and blood and mammary tissue was collected at 0, 8, and 24 h post final 5-HTP administration. Mammary tissues were analyzed using qPCR to evaluate mRNA expression of calcium sensing receptor (CasR), parathyroid hormone related-protein (PTHrP), plasma membrane calcium ATPase2 (PMCA2), calcium release-activated calcium channel protein 1 (ORAI1), and secretory pathways calcium-ATPase 1 (SPCA1), and whole blood samples were analyzed for serotonin and total calcium concentrations. Milk yield was recorded daily. Serotonin concentrations were increased (P < 0.0001) in 5-HTP treated cows. Interestingly, there was no difference in CasR, PTHrP, PMCA2, ORAI1, and SPCA1 expression between 5-HTP and saline treated cows (P = 0.51, P = 0.92, P = 0.43, P = 0.16, and P = 0.08, respectively). However, total blood calcium decreased in 5-HTP compared with saline treated cows after each IV infusion (P = 0.0074, P = 0.0901, P = 0.0088, respectively).

Key Words: metabolism, goat mastitis, heat stress

482 The effect of night restricted feeding on the molecular circadian clock of the mammary gland. I. J. Salfer* and K. J. Harvatine, The Pennsylvania State University, University Park, PA.

Circadian rhythms are generated within tissues through a molecular clock made of a set of transcription factors that oscillate in a 24 h manner. These rhythms can be entrained both by photoperiod and feeding time. Dairy cows display daily rhythms of milk synthesis that are altered by feeding time, but the role of molecular clocks in these rhythms is poorly understood. To determine the impact of feeding on the mammary molecular clock, 11 mid-lactation (168 ± 50 DIM) multiparous Holstein cows were used in a crossover design (5 or 6 cows per treatment) with two 25-d periods. Treatments included (1) ad libitum feed available for the entire day (AL), or (2) night-restricted feeding, where feed availability was limited to 16 h/d from 2000 h to 1200 h (NR). All cows were housed in the same 19:5 light:dark cycle. Milk samples were collected at 0700 h and 1900 h on d 11 and d 17 of each period and analyzed for fat and protein concentration. Mammary tissue was collected from all cows via needle biopsy to represent 4 times across the day (0400, 1000, 1600, and 2200 h). The expression of clock genes Bmal1, Clock, Cry1, Per1, Per2, and Rev-erba was determined at each time point using Real-Time RT-PCR. Cosinor rhythmometry was performed using SAS Proc Mixed to determine if expression of clock genes fit a 24 h rhythm and if the amplitude and acrophase (time at peak) differed between treatments. The NR treatment reduced dry matter intake (1.9 kg), yield of milk (3.0 kg), fat (160 g) and protein (113 g), and milk protein concentration (0.06%); all P < 0.05, while milk fat concentration was not affected (P = 0.91). Daily rhythms of Bmal1, Clock, and Per1 were not observed in either treatment. Cry1 fit a 24 h rhythm in both treatments (P < 0.05), with an increased amplitude and an acrophase occurring 8.5 h later in NR compared with AL. Rev-erba fit a daily rhythm in the NR treatment (P = 0.03) and tended to fit a rhythm in the AL treatment (P = 0.05). The acrophase of Rev-erba was shifted 6.5 h later in NR compared with AL, but the amplitude did not differ by treatment. Per2 did not express a daily rhythm in the AL group, but a rhythm was induced in NR cows (P = 0.02). These results indicate that key components of the mammary molecular clock are influenced by feeding time.

Key Words: circadian rhythm, lactation, food entrainment

J. Dairy Sci. Vol. 101, Suppl. 2 405
Milk yield was not different \((P = 0.57)\) between treatments. This data suggests serotonin’s modulation of calcium homeostasis may be dependent upon physiological stage of lactation in the dairy cow. Further experiments should be conducted to determine the impact of stage of lactation on serotonin’s manipulation of calcium metabolism.

**Key Words:** serotonin, lactation, calcium

**482**  
Dry period heat stress impacts mammary protein metabolism in the subsequent lactation. B. Dado-Senn*1, A. L. Skibiel1, E. Meyer2, S. I. Arriola Apelo2, and J. Laporta1, 1Department of Animal Sciences, University of Florida, Gainesville, FL, 2Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.

Dry period (DP) heat stress impairs milk production in the subsequent lactation, decreasing milk protein yields. We hypothesized that DP heat stress will impact milk protein synthesis in the subsequent lactation, potentially through altered amino acid (AA) transport and impaired mechanistic target of rapamycin (mTOR) signaling in the mammary gland. Holstein cows were enrolled into heat-stressed (HT, shade, \(n = 12\)) or cooled (CL, shade, fans and soakers, \(n = 12\)) treatments for a 46-d DP (THI \(\geq 68\)). After calving, all cows were managed together and cooled. Milk yield and components were recorded (AfiFarm) daily until 84 d in milk (DIM). Milk samples were collected at 14, 42, and 84 DIM to analyze milk protein profile. Mammary gland biopsies (\(n = 6/treatment\)) were collected at peak lactation (42 DIM). Western blotting was used to determine expression and phosphorylation of mTOR signaling pathway proteins (ULK1, p70 S6K, rpS6, and 4E-BP1), and qRT-PCR to measure gene expression of mTOR targets and AA transporters (SLC1A1, 1A5, 3A2, 7A1, 7A5, and 36A1). Data were analyzed by general linear mixed models in SAS. Compared with CL cows, milk protein yield and percentage was reduced in HT cows by 0.2 kg/d and 0.11%, respectively (0.95 vs. 1.15 kg/d \(\pm 0.24, P < 0.01; 2.79 \pm 0.02, P < 0.01\)), but milk protein profiles did not differ. At 42 DIM, gene expression of mammary AA transporters SLC1A1 and SLC3A2 was upregulated in HT cows \((P = 0.02, P = 0.05, \text{respectively})\). Expression, rather than phosphorylation, of mTORC1 targets was altered in HT cows. Proteins stimulated by mTORC1 phosphorylation to sustain mRNA translation for milk protein synthesis, p70 S6K and its substrate RPS6, were downregulated in HT cows \((P = 0.01, \text{both})\). Proteins suppressed by mTOR phosphorylation and involved in autophagy (ULK1) or repression of mRNA translation (4E-BP1) were upregulated in HT cows \((P = 0.01)\). Gene expression of RPS6 and p70 S6K did not correlate with protein expression, as they tended to be upregulated in HT cows \((P < 0.10)\). In conclusion, DP heat stress reduced milk protein percentage and yield, possibly through inhibited mTOR signaling and altered AA transporter expression.

**Key Words:** mTOR, amino acid, heat stress