T133  Transcriptional changes in the early lactation mammary gland involved immune signaling pathways but were not affected by NSAID treatment.

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Previous studies have shown that non-steroidal anti-inflammatory drug (NSAID) treatment in early lactation can have a positive impact on whole-lactation milk production in older cows. Our objective was to evaluate transcriptional changes in the mammary gland that could explain increased production responses. Sodium salicylate (SS; 125 g/d) or water (CON) were administered via oral drench to multiparous cows (n = 8/treatment) once daily for 3 d beginning approximately 24 h after parturition, and mammary tissue was collected on d 1, 4, and 45 postpartum. Day 1 tissue was collected immediately preceding the initial drench, and d 4 tissue was collected the day following the final drench. Total RNA extracted from tissue was deep sequenced and analyzed for differential gene expression using DESeq2. Only 16 of 18,286 genes were differentially expressed (false discovery rate <0.1) on d 45 due to NSAID treatment. Given the lack of milk yield and a low mammary transcriptome response to SS, additional analyses focused on time-dependent effects. Of the >8500 genes that were differentially expressed (DE) over time, those meeting cutoff values of 1.5-fold change and adjusted p-value of <0.05 were used for functional analysis across time points in Ingenuity Pathway Analysis software. Analysis of transcriptional differences over time showed downregulation of pathways related to immune cell recruitment and differentiation, including cytokine signaling, TLR activation, inflammasome signaling, and interferon signaling, as well as cell growth and differentiation between wk 1 and 6 of lactation. STAT3 and PPAR signaling were upregulated on d 45 compared with the earlier time points. Additionally, DE genes in our data set showed extensive overlap with pathways related to cholesterol synthesis and retinoid X receptor signaling. Despite the low overall transcriptional effects of SS, transcriptome analysis emphasizes the extensive involvement of immune-related signaling pathways in the switch from lactogenesis to galactopoiesis.

Key Words: NSAID, transcriptome, immune function

T134  Peroxisome proliferator-activated receptor gamma (PPARγ) agonist does not overcome the effect of trans-10,cis-12 conjugated linoleic acid (CLA) but stimulate lipogenic gene expression in mammary explants cultured in vitro. W. B. Junior, P. C. Carraro, E. D. Silva, and D. E. Oliveira*, Santa Catarina State University, Lages, SC, Brazil.

The PPARγ is a ligand-dependent transcription factor coordinating lipogenic genes in the mammary gland and can be modified by conjugated linoleic acid (CLA) and/or chemical agonists. This study used the PPARγ agonist Tiazolinediona (TZD) to evaluate the effect on lipogenic gene expression. Mammary explants were cultured in vitro for 24 h with the following treatments: (1) Control: 400µL of mammary epithelial growth medium; (2) TZD: Control + 40 µL of TZD (10 µmol/L); (3) CLA: Control + 30 µmol CLA (315 µmol/L); and (4) TZDCLA: Control + TZD (10 µmol/L) + 30 µmol CLA (315 µmol/L). The CLA used was a mixture 50:50 of cis-9,trans-11 and trans-10,cis-12. The RNA was extracted, complementary DNA (cDNA) synthesized and qRT-PCR carried out, measuring the gene expression of acetyl-CoA-carboxylase α (ACCα transcript from promoter III), fatty acid synthase (FASN), peroxisome proliferator-activated receptor gamma (PPARγ), sterol regulatory element binding protein-1 (SREBP1), sterol regulatory element-binding protein cleavage-activating protein (SCAP), stearoyl CoA desaturase (SCD), insulin-induced gene 1 (INSIG1), insulin-induced gene 2 (INSIG2). The data were analyzed using the PROC MIXED using treatment and sample as fixed effects and the geometric mean of ribosomal protein 18 (S18) and β-actin as a covariate. Compared with Control, TZD treatment increased the gene expression of SREBP1 (P = 0.0001), INSIG1 (P = 0.0001), INSIG2 (P = 0.005), FASN (P = 0.0001), ACCα (P = 0.0001), SCD1 (P = 0.0001) e PPARγ (P = 0.0001) in 10.1-, 7.9-, 8.5-, 78.3-, 87.5-, 62.7-, and 6.2-fold, respectively. The CLA compared with Control, decreased the expression of FASN (P = 0.04), SCD (P = 0.01) e ACCα (P = 0.05), in 2.8-, 2.1- and 0.4-fold, respectively. Comparing TZDCLA and CLA treatments, TZDCLA decreased the expression of FASN (P = 0.01) and INSIG2 (P = 0.0005) in 2.7 and 15.7 fold, respectively. Overall, our results showed a positive and consistent effect of TZD increasing the gene expression of PPARγ and its targeted genes and CLA reducing the expression of genes involved in milk fat depression.

Key Words: milk fat depression, milk fat synthesis, nuclear receptor

T135  Effects of feed restriction on synthetic capacity of the bovine mammary gland. D. J. Seymour*, J. J. M. Kim1, J. Doelman2, and J. P. Cant1, 1Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, 2Nutreco Nederland BV, Boxmeer, the Netherlands.

Mechanisms that regulate the milk synthetic response to nutrient supply in lactating dairy cows remain largely unexplained. The objective of this study was to evaluate short- and long-term changes in expression of mammary genes related to secretory cell turnover and milk-synthesizing activity per cell in lactating Holstein dairy cattle subjected to restricted feeding. Pairs of cows (234 ± 27 DIM, n = 7) were blocked by date and average daily milk yield and fed either 100 or 60% of ad libitum intake for 14 d. The feed restriction treatment commenced with 16 h of no access to feed. Milk production dropped 4.8 kg/d by d 1 of restriction and remained at that level until d 14 (P < 0.01). On d 1, plasma glucose and BHBA concentrations did not differ between treatment groups (P > 0.67), but FA concentrations were 2 times higher (P < 0.01) in restricted cows. There were no differences in these metabolite concentrations between treatment groups on d 13 (P > 0.18). Mammary mRNA expression of milk protein genes and genes related to protein synthesis and secretion were not affected after 16 h of feed withdrawal (P > 0.10), but expression and protein abundance of cyclin D1 were downregulated 56 and 42% (P ≤ 0.04), respectively. After 14 d, cyclin D1 expression in mammary tissue was no longer low (P = 0.32) but expression of the pro-apoptotic DNA damage-inducible transcript 3 (aka CHOP) was elevated 69% (P = 0.04). There were no differences between treatments in mammary parenchymal DNA mass or proportions of proliferating and apoptotic tissue was no longer low (P > 0.67), but FA concentrations were 2 times higher (P < 0.01) in restricted cows. There were no differences in these metabolite concentrations between treatment groups on d 13 (P > 0.18). Mammary mRNA expression of milk protein genes and genes related to protein synthesis and secretion were not affected after 16 h of feed withdrawal (P > 0.10), but expression and protein abundance of cyclin D1 were downregulated 56 and 42% (P ≤ 0.04), respectively. After 14 d, cyclin D1 expression in mammary tissue was no longer low (P = 0.32) but expression of the pro-apoptotic DNA damage-inducible transcript 3 (aka CHOP) was elevated 69% (P = 0.04). There were no differences between treatments in mammary parenchymal DNA mass or proportions of proliferating and apoptotic cells on d 14 (P > 0.37). However, parenchymal tissue and protein mass were 24 and 29% lower, respectively, in restricted versus unrestricted cows (P = 0.03) and the glands produced 45% less milk daily per gram of parenchymal DNA. Results suggest that both mammary cell number and activity per cell are acutely regulated within 16 h of a change in total

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T136  Comparison of metabolites and hormones involved in the control of energy partitioning during the lactation of dairy ewes and goats. M. F. Lunesu1, A. Prandi2, A. Comin2, G. C. Bombol1, P. Sechi1, P. Nicolussi2, M. Decandia, and A. Cannas*.1, 1University of Sassari, Sassari, Italy; 2University of Udine, Udine, Italy; 2Istituto Zooprofittico Sperimentale della Sardegna, Sassari, Italy; 4Dipartimento di Ricerca nelle Produzioni Animali, Agris, Olmedo, Italy.

This research studied the evolution of metabolites and hormones involved in the control of energy partitioning during early and mid-lactation of dairy ewes and goats and assessed in mid-lactation interactions with the type of carbohydrates used in the diet. Twenty Sarda ewes and 20 Saanen goats were compared from 15 ± 5 d in milk (DIM; mean ± st.dev.) to 134 ± 5 DIM in the same feeding conditions. Since parturition, each species was fed a high starch diet (20.4% starch, 35.5% NDF, DM basis), whereas from 92 ± 11 DIM each species was allocated to 2 dietary treatments: high starch (HS; 20.0% starch, 36.7% NDF, DM basis) and low starch-high digestible fiber (LS: 7.8% starch, 48.8% NDF, DM basis) diets. The LS diet was obtained by substituting cereal grains with soyhulls. Blood samples were collected monthly and analyzed for plasma glucose, NEFA, growth hormone (GH), IGF-1 and leptin. Data were studied by using the PROC MIXED procedure of SAS for repeated measurements. From early to mid-lactation, glucose concentration was higher in ewes than in goats (54.6 vs. 48.4 mg/dl ± 1.2 (mean±SEM); P < 0.0001). NEFA concentration was lower in ewes than in goats (0.25 vs. 0.31 mmol/L ± 0.03; P = 0.036). IGF-1 concentration did not differ (108.8 vs. 94.2 ng/mL ± 11.64; P > 0.1). Goats had higher plasma GH (4.47 vs. 2.28 ng/mL ± 0.57; P < 0.001), with a marked peak in early lactation not observed in ewes, higher leptin concentration (26.3 vs. 11.4 ng/mL ± 2.1; P < 0.0002), and lower plasma insulin content (0.11 vs. 0.26 μg/L ± 0.02; P < 0.0001) than the ewes. In mid-lactation, metabolites and hormones were not affected by the diets in both species. In conclusion, this experiment found that (1) the ewes had a hormonal profile more directed to the partitioning of dietary energy in favor of body reserve accumulation, rather to milk production, than the goats; (2) in mid lactation the hormonal status was not affected by the prevalent type of carbohydrate (starch or digestible fiber) of the diets; iii) blood leptin was much higher in goats than in ewes, despite the latter accumulated much more body reserves than the former.

Key Words: energy partitioning, lactating ewe, lactating goat

T137  Effects of extracellular Zn and G protein-coupled receptor 39 silencing on immortalized bovine mammary epithelial (MAC-T) cells. J. E. Shaffer, L. K. Mamedova*, and B. J. Bradford, Kansas State University, Manhattan, KS.

Both form and concentration of supplemental Zn has been shown to impact milk production and mammary health in dairy cattle. However, the physiological mechanisms by which these effects are produced remain to be fully elucidated. One potential route is by direct effect on mammary epithelial cells (MEC). Zinc is known to act as a ligand for GPR39, a G protein-coupled receptor expressed in a variety of cell lines and tissues, where it promotes cell survival and proliferation by a G_{aq} pathway characterized by intracellular Ca^{2+} release followed by phosphorylation of kinases including ERK and AKT. The objective of this study was to characterize the presence and activity of GPR39 in an immortalized bovine MEC line (MAC-T). Using RT-qPCR, GPR39 was found to be expressed in a variety of bovine tissues as well as in MAC-T cells. Two siRNA constructs (siGPR39a and siGPR39b) were designed and utilized in vitro for the knockdown of GPR39 expression in MAC-T cells. Cells were cultured on 12-well plates, transfected with siGPR39a, siGPR39b, or a universal negative control (siCON) 24 h before subsequent treatments. Cells were then treated for 10 min with either a Zn-free physiological saline solution (0 Zn), or 100 μM Zn (100 Zn), and after another 10 min, cells were harvested for RNA and protein. Transcript abundance was determined by RT-qPCR and protein phosphorylation by Western blot. There was a tendency for 100 Zn to increase GPR39 mRNA abundance compared with 0 Zn in siCON cells (P = 0.096). In 100 Zn cells, transcript abundance of GPR39 was reduced 63% by siGPR39a (P = 0.02) and 57% by siGPR39b (P = 0.04). No effects of GPR39 knockdown, Zn treatment, or their interaction were observed on phosphorylation of AKT or ERK, 2 common intermediates of G_{aq} signaling. In summary, extracellular Zn was not observed to activate G_{aq} signaling in MAC-T cells regardless of GPR39 expression. 

Key Words: zinc, MAC-T, lactation physiology

T138  The bovine milk microbiome and somatic cell count. S. L. Brooker*, K. M. Yahvali, B. A. Casperson, J. E. Williams, B. Shafii, W. Price, J. Tinker, and M. A. McGuire, 1University of Idaho, Moscow, ID, 2Purdue University, West Lafayette, IN, 3Boise State University, Boise, ID.

Efforts to determine causative agents in mammary inflammation in dairy cows are critical to animal welfare and economic viability. Two key questions to address are 1) what factors are important in maintaining a healthy milk microbiota and 2) what factors lead to the manifestation of bacterial infection or inflammation. Quarter milk samples from 103 mostly Holstein cows were obtained from 2 different dairies in Idaho. Characterization of the microbial community was performed by culture independent Illumina sequencing of amplicons from the V1-V3 hypervariable region of the 16S rRNA gene to determine relative abundance of bacteria present. Almost 45% of the reads were unclassified at the genus level, showing one of the limitations of this study. From the cows, 350 quarters had low somatic cell count (SCC) (<200,000 cells/mL), 26 had mid SCC (200,000–400,000 cells/mL), 3 quarters omitted due to missing SCC. Milk microbial communities were characterized with major membership by genera such as Staphylococcus (5–20%), Corynebacterium (5–10%), and Clostridium XI (5%). Higher SCC quarters tended to have elevated amounts of Staphylococcus and Streptococcus. Using the nonnegative matrix factorization (NMF) methodology, the community structure was best described by Caryophanon, Coxiella, Gemella, and Luteipluratus, though the contribution differed greatly between quarters, SCC, and dairy. Overall, diversity (Shannon Diversity) was similar across quarters within an individual (16.22 – 17.68) whereas the diversity markedly decreased as SCC increased (18.04, 12.16, and 11.50 for low SCC, mid SCC, and high SCC, respectively). This pattern was also reflected by dairy. The dairy with a larger number of mid and high SCC quarters and had an overall lower average diversity (12.43) relative to the other dairy (21.87). In summary, quarters, though independent glands, appear to be biological replicates of the system under healthy conditions. More work is needed to determine the various aspects of microbial community structure that may confer health or disease states for mastitis in dairy cows. This work was supported by the Idaho State Board of Education.

Key Words: milk, microbiome, diversity
Effects of supplementary folic acid and vitamin B12 feed-restriction on immune cell functions and blood cell population in dairy cows. N. Vanacker*, C. Girard, M. Duplessis, and P. Lacasse, Agriculture and Agri-Food Canada, Sherbrooke Research and Development Center, Sherbrooke, QC, Canada.

Cows undergoing negative energy balance often experience a state of immunosuppression and are at greater risk of infectious diseases. The present study aimed at evaluating the impact of a folic acid and vitamin B12 supplement and feed restriction on some immune parameters.

Sixteen cows at 45 ± 3 DIM were assigned to 8 blocks of 2 cows each according to their milk production during the previous week, 45 ± 6 kg/d, then within each block, they randomly received weekly intramuscular injections of either saline (C) or 260 mg of folic acid and 10 mg of vitamin B12 (V) for 5 wk. On wk 5, the cows were fed 75% of their ad libitum intake, 24 (±2.5) kg of DM/d, during 4 d. Blood sample samples were taken before the beginning of the experiment, just prior feed restriction and after 3 d of feed restriction to evaluate blood cell population, phagocytosis capacity and oxidative burst of polymorphonuclear leukocyte (PMN). The vitamin supplement did not affect any of the tested variables. Feed restriction reduced \((P < 0.05)\) the percentage of PMN positive for phagocytosis. Accordingly, the percentage of PMN that were positive for oxidative burst after being stimulated with PMA was reduced by feed restriction \((P < 0.05)\). Feed restriction did not affect blood cell population. In conclusion, feed restriction affected the functions of PMN, suggesting that the greater risk of infectious diseases in cow experiencing a negative energy balance is related to impaired immune cell functions.

Key Words: energy balance, phagocytosis, oxidative burst

Differential effects of lipopolysaccharide on expression of major milk protein genes in mouse mammary epithelial cells. Q. Tian*, A. Spitzer, and F.-Q. Zhao, Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT.

Mastitis is an endemic disease in the dairy industry and causes large economic losses to dairy farmers due to reduced milk yield and quality. Mastitis is inflammation of one or more mammary glands caused by bacterial infection. Lipopolysaccharide (LPS) is a major outer membrane component of gram-negative bacteria and a major endotoxin that elicits strong mammary inflammation. The major objective of this study was to investigate the effects of LPS on milk protein gene expression in mammary epithelial cells (MEC), using HC11, a mouse MEC line, as a model. HC11 cells were cultured with 0–500 µg of LPS for 3–24 h to assess cell viability through MTT assay and relative gene expression via real-time reverse transcription PCR. LPS reduced HC11 cell viability in a dose- and time-dependent manner as doses of ≥250 ug/mL reduced cell viability significantly at 4 h, but the threshold decreased to ≥100 ug/mL at 24 h \((P < 0.05)\). However, as little as 0.1 µg/mL of LPS dramatically induced mRNA expression of inflammation markers IL-6, IL-1β and TNFα at 3 h and 24 h of treatment \((P < 0.05)\). When HC11 cells were cultured in medium containing lactogenic hormones prolactin and glucocorticoids, treatment of the cells with 0.1–25 µg/mL of LPS for 3 h and 24 h reduced β-casein gene \((CSN2)\) expression by 40–80% \((P < 0.05)\), but surprisingly increased αS1-casein \((CSN1S1)\) expression by 20–226 fold \((P < 0.05)\). Expression of α-lactalbumin gene \((LALBA)\) was also increased at low concentrations of LPS (0.5 and 1 µg/mL) at 3 h, but decreased by 37–72% at all concentrations tested at 24 h \((P < 0.05)\). In summary, our data demonstrated novel differential effects of LPS on expression of 3 major milk protein genes in MECs, suggesting potential functional differences among these proteins during mastitis. Especially, the dramatic rise of αS1-casein expression by LPS raised a possible immune function of this protein in the mammary gland.

Key Words: gene expression, mastitis, milk protein