Lactation Biology II

369 Milk fat synthesis in thyroid hormone responsive spot 14 null mice is acutely responsive to *trans*-10, *cis*-12 conjugated linoleic acid (CLA). K. J. Harvatine*[1], M. Tanino[2], Y. R. Boisclair[2], and D. E. Bauman[2], *[1]Penn State University, University Park, [2]Cornell University, Ithaca, NY.*

Trans-10, *cis*-12 conjugated linoleic acid (CLA) reduces milk fat concentration and markedly reduces mammary de novo lipogenesis in both cows and mice. Thyroid hormone responsive spot 14 (S14) is decreased in mammary tissue of both the cow and mouse during CLA treatment. We have previously reported a functional role of S14 in CLA-induced inhibition of fat synthesis using the S14 null mouse. Mammary de novo lipogenesis of S14 null dams is hyper-responsive to CLA treatment. After 5 d of CLA treatment milk fatty acids (FA) less than 16 carbons are reduced about 2-fold more in S14 null mice compared with wild-type (WT) mice. Three experiments were conducted to further characterize the response to CLA in S14 null mice. Dams were bred to males of the opposite genotype and fed a low fat chow diet (Teklad 8640). First, recovery after termination of CLA treatment was tested in S14 null mice in a randomized block design. Starting at 8 to 9 d of lactation S14 null received oral doses of water (control) or 18 mg/d of CLA for 3 d. Two pups were euthanized for collection of stomach milk clots and the remaining pups were euthanized 5 d after termination of CLA treatment. CLA decreased pup growth and milk FA less than 16 carbons by approximately 60%, but both responses recovered by 5 d after termination of CLA (CLA × Day interaction $P < 0.01$). Second, the short-term response to CLA was investigated using WT and S14 null mice in a randomized block design with a 2 × 2 factorial arrangement of treatments (genotype × CLA). Starting on 10–12 d of lactation, S14 null and WT dams received water (control) or 20 mg/d of CLA for 24 h. The effect of genotype, CLA, and genotype by CLA interaction was tested. CLA reduced pup weight gain and there was no genotype x CLA interaction. However, there was genotype x CLA interaction for milk FA less than 16 carbons ($P < 0.001$; WT −17% vs S14 null −67%). Lastly, the sensitivity to CLA was tested using a similar randomized block design with WT and S14 null dams. Starting on 6 to 8 d of lactation, S14 null and WT dams received water (control) or 3.5 mg/d of CLA for 5 d. Dose selected was half the lowest dose previously used in a WT dose titration experiment that partially reduced milk fat. There was a genotype x CLA interaction for milk concentration of FA less than 16 carbons with an 8.9% decrease in WT and a 28.7% decrease S14 null. Overall, results demonstrate that the absence of S14 increases the responsiveness of mammary de novo lipogenesis to CLA without affecting sensitivity.

Key Words: CLA, spot 14 (S14), lipogenesis

370 Increased milk production by Holstein cows consuming endophyte-infected fescue seed during the dry period. R. L. Baldwin VI*[1], A. V. Capuco[1], C. M. Evock-Clover[1], P. Grossi[2], R. K. Choudhary[3], T. H. Elsasser[1], G. Bertoni[2], E. Trevisi[2], D. L. Harmon[4], and K. R. McLeod[4], *[1]Bovine Functional Genomics Lab, USDA-ARS, Beltsville, MD, [2]Istituto di Zootecnica, Università Cattolica del Sacro Cuore, Piacenza, Italy, [3]Department of Animal and Avian Sciences, University of Maryland, College Park, [4]Department of Animal Sciences, University of Kentucky, Lexington.*

Ergot alkaloids in endophyte-infected grasses inhibit prolactin (PRL) secretion and may reduce milk production of cows consuming endophyte-infected grasses. We hypothesized that consumption of endophyte-infected fescue during the dry period inhibits mammary differentiation and subsequent milk production. Twenty-five multiparous Holstein cows were randomly assigned to 3 treatment groups. Starting at 90-d prepartum, cows were fed endophyte-free fescue seed (control, CON; n = 9), endophyte-free fescue seed and 3x/wk subcutaneous injections of bromocryptine (0.11 mg/kg BW; positive control, BROMO; n = 8), or endophyte-infected fescue seed as 10% of the as-fed diet (INF; n = 8). Although milk yield of groups did not differ at −90 d prepartum, at dry-off (−60 d) INF and BROMO cows produced less milk ($P < 0.05$) than CON (averaging 20, 11 and 14 kg/d for CON, INF and BROMO cows). Throughout the treatment period, concentrations of PRL in the circulation were lower in INF and BROMO cows than CON cows ($P < 0.05$). Basal concentrations of PRL in venous plasma averaged 25.3, 2.8 and 3.7 ng/ml for CON, INF and BROMO cows, respectively. Prepartum release of PRL was also reduced by ergot alkaloids, averaging 19.5, 9.2 and 1.1 μg PRL/ml*h (area under curve) for CON, INF and BROMO cows, respectively. At 10 d of lactation, when treatments were terminated, basal concentrations of PRL in plasma averaged 22.5, 1.6 and 1.4 ng/ml for CON, INF and BROMO cows, respectively. Three wk after the end of treatment, circulating concentrations of PRL were equivalent across groups ($P > 0.05$). Gestation length did not differ between groups. Although treatment 4 wk before dry-off reduced milk yield in INF and BROMO cows, milk production in the ensuing lactation was increased 8% and 9% in INF and BROMO cows relative to CON ($P < 0.05$). We reject our initial hypothesis, as data show that consumption of ergot alkaloids during the dry period increases milk production in the ensuing lactation. We propose that this effect is due to a reduction in PRL during the dry period, analogous to the production effect realized by exposing cows to reduced photoperiod (low PRL) during the dry period.

Key Words: endophyte-infected fescue, prolactin, milk production

371 Association between plasma insulin and progesterone concentrations and the composition of milk fatty acids and lipids. N. Argov-Argaman,* H. Malka, and R. Mesilati-Stahy, *Animal Science Department, Hebrew University, Rehovot, Israel.*

This study examined the association between plasma insulin and/or progesterone concentration and milk fatty acid and lipid composition. Holstein dairy cows, 60 DIM, were synchronized (estrus = d 0) and held as controls (n = 20) or drenched for 14 d (d −5 to d 11) with 500 mL/d liquid propylene glycol to increase plasma insulin level (treatment, n = 20). Milk and blood samples were collected on d −5, 1 and 8 of the cycle. Insulin and progesterone concentrations were determined in the plasma, and fatty acid, polar and neutral lipid compositions were determined in the milk. While not significant, plasma insulin concentration was higher in the treated vs. control group on d 1 (6.09 and 3.8 ng/mL, respectively) and on d 8 (6.2 and 4.5 ng/mL, respectively). In both groups, plasma progesterone concentration increased ($P < 0.001$) through the estrous cycle with an average concentration of 0.18 and 3.7 ng/mL on d 1 and 8, respectively. However, on d 8, peak progesterone level was higher ($P < 0.04$) in the treatment group than in the controls (4.33 vs. 3.09 ng/mL, respectively). Milk fat concentration tended ($P < 0.08$) to negatively interact with plasma insulin and positively interact ($P < 0.07$) with plasma progesterone concentrations. Milk fatty acid composition was associated primarily with the day of the cycle. For instance, saturated fatty acid concentration decreased from 65.8% on d 1 to 63.5% on d 8 ($P < 0.04$). Treatment was the major factor associated with long-chain saturated fatty acid content in the milk, with

higher concentrations in the control vs. treatment groups on both d 1 ($P < 0.05$; 9.3 and 8.0%, respectively) and d 8 ($P < 0.009$; 10.3 and 8.4%, respectively). Treatment was also associated with stearoyl CoA desaturase activity, as reflected by a 15% increase on d 1 ($P < 0.03$) and 10% increase on d 8 ($P < 0.08$). The results suggest that milk fatty acid composition changes during the estrous cycle in association with plasma progesterone concentrations. Propylene glycol administration increased progesterone concentration and to some extent that of insulin, which in turn might affect fatty acid composition.

Key Words: lipids, estrous, propylene glycol

372 Ontogeny of nuclear and cytoplasmic myoepithelial cell markers in pre-weaning Holstein heifers. S. Safayi[1], N. Korn[1], A. DiMascio[2], R. M. Akers[3], A. V. Capuco[4], and S. Ellis*[1], *[1]Clemson University, Clemson, SC, [2]University of Georgia, Athens, [3]Virginia Polytechnic Institute and State University, Blacksburg, [4]USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD.*

Myoepithelial cells (MC) have roles in cell proliferation and differentiation and hence may affect mammary parenchymal morphogenesis. Because MC can limit parenchymal growth in other species, it is important to understand MC-related mechanisms involved in early bovine mammary development. We previously saw changes in expression of the nuclear marker transformation-related protein 63 (P63) and the cytoplasmic marker common acute lymphoblastic leukemia antigen (CD10), corresponding with changes in the pattern of MC development in prepubertal heifers between 40 and 160d of age. In this study, we investigated the ontogeny of MC development during the pre-weaning period by tracking P63 and CD10 expression with immunofluorescent staining. Holstein heifers (n = 4/age) were sacrificed and sampled at 0 (<12 h of birth), 7, 14, 21, 28, 35, and 42 d of age. The basal epithelium was traced and MC marker expression within the outlined region was quantified using multispectral imaging of stained paraffin sections from each parenchymal sample. Fluorescent intensity (FI) of the markers in traced regions on each slide was normalized against a reference sample and then evaluated statistically using Mixed procedures in SAS. Samples were also used for subjective histologic assessment of MC marker distribution. Our analysis showed increased FI of CD10 and P63 at d 42, relative to d 0 ($P \leq 0.02$). The ratio of CD10 to P63 remained constant ($P = 0.94$) from d 0 to d 42. The increased intensity but consistent ratio values could be explained by our observation that P63+ nuclei were more closely spaced in the older calves. These results highlight a need to analyze expression within individual nuclei rather than quantifying expression within the entire basal layer. Double positive (P63+/CD10+) and double negative (P63-/CD10-) cells and single positive cells expressing either marker (P63-/CD10+ or P63+/CD10-) were present in both basal and supra-basal layers. As a result, our data does not yet define a sequential progression of bovine mammary MC differentiation. Further studies with additional MC markers are therefore required to define the sequence of bovine MC ontogeny.

Key Words: myoepithelial, mammary development, heifer

373 Ultrasonographic monitoring of mammary parenchyma growth in preweaned Holstein heifers. K. M. Esselburn*[1], T. M. Hill[2], K. M. O'Diam[1], V. A. Swank[1], H. G. Bateman II[2], R. L. Schlotterbeck[2], and K. M. Daniels[1], *[1]Department of Animal Sciences, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, [2]Nurture Research Center, Provimi North America, Brookville, OH.*

Mammary parenchyma (PAR) is present at birth in negligible quantities and undergoes extensive postnatal growth. Researchers have long been interested in nutritional effects on the mammary gland. However, monitoring in vivo growth of PAR has historically been difficult, necessitating slaughter studies to measure PAR quantity. Advances in ultrasound (US) technology warrant revisiting its use as a non-invasive tool to monitor PAR growth in vivo. Holstein heifers (n = 24; 41 ± 1kg initial BW) from a single farm were randomly assigned to 1 of 3 milk replacers (MR) at 2–3 d of age. Heifers were fed MR at 660 g/d until weaning at 42 d. MR contained 27% CP and was formulated with 3 fat and fatty acid compositions. MR treatments were A) only lard, 17% fat, B) animal fat supplemented with 1.25% NeoTect4 (MR, Provimi North America, Brookville, OH), 17% fat, and C) milk fat, 33% fat. Starter (20%CP) and water were fed ad lib for 56d. A real time B-mode US with a 7.5-MHz convex probe was used to examine 2-dimensional (2D) PAR area in all 4 glands of heifers once weekly from 2 to 3 d of age to 52 d. Individual digital images of each gland were saved for further analysis. At 52 d of age, heifers were slaughtered to validate final US measurements. At slaughter, the left half of the mammary gland was removed and examined by US 24 h later. Also at that time, left front and left rear glands were bisected to produce a sagittal plane view of PAR for comparison to US images. In all cases 2D areas of PAR were determined using ImageJ software (NIH). Data were analyzed using mixed procedure of SAS. Additionally, 8wk paired data were analyzed using the correlation procedure. There were no differences in PAR area due to diet. Regardless of dietary treatment, PAR grew over time (0.049cm^2 initially; 0.420cm^2 at 52 d ante-mortem; SEM = 0.020), as expected. Positive correlations existed between all paired variables analyzed (PAR area of bisected sagittal plane view; PAR area ante-mortem from US; PAR area postmortem from US; all r >0.60). Methodology used here demonstrates that US is an effective tool for measuring weekly changes in PAR area in vivo.

Key Words: ultrasound, mammary, dairy calf

374 Proteomic analysis of the nuclear phosphorylated proteins in dairy cow mammary epithelial cells treated with prolactin. J.-G. Huang, X.-J. Gao,* Q.-Z. Li, L. Zhang, F. Zhao, N. Zhang, Y. Lin, and Z. Sun, *Key Lab of Dairy Science, Ministry of Education, Northeast Agriculture University, Harbin, Heilongjiang, China.*

Prolactin (PRL) is a versatile signaling molecule and regulates a variety of physiological processes, including mammary gland growth and differentiation and the synthesis of milk proteins. While PRL is known to be necessary for high levels of milk protein expression, the mechanism by which the synthesis of milk proteins is stimulated at the transcript level is less known. A major modification important in the transcript level is protein phosphorylation. To gain additional insights into the molecular mechanisms at the transcript level underlying PRL action on the dairy cow mammary epithelial cells (DCMECs), nuclear phosphoproteins whose expression distinguishes proliferating regulated by PRL in DCMECs were identified. A phosphoprotein-enriched fraction from nuclear proteins was obtained by affinity chromatography, and a 2-dimensional gel electrophoresis (2-DE) and matrix assisted laser desorption/ionization time of matrix-assisted laser desorption/ionization/time of flight mass spectrometry (MALDI-TOF MS) were used to identify the changes of nuclear phosphoproteins in DCMECs treated with prolactin. Results: Seven proteins displaying ≥ 2-fold difference in abundance upon PRL treatment in DCMECs were identified by MALDI-TOF MS. The protein–GARS (GlyRS), which belongs to the class-II aminoacyl-tRNA synthetase family, plays a global role in the milk protein synthesis. SERPINH1 (Heat shock protein 47), which is the first heat shock protein found to be a member of the serpin superfamily, regulates physiologic functions such as complement activation, programmed

cell death, and inflammatory processes. PRDX3, which belongs to a family of antioxidant enzymes, plays an important role in scavenging intracellular reactive oxygen species (ROS). ACTR1A, belongs to the actin family, which is associated with transport of p53 to the nucleus. Annexin A2, a Ca^{2+}-dependent phospholipid-binding protein, maintains the viability and cell cycle regulation of DCMECs. PSMB2, PSMD10, which belong to ubiquitin-proteasome system, are involved in several cellular processes, including cell cycle control, cellular stress responses, intracellular signaling. This screening reveals that prolactin influences the levels of nuclear phospho-proteins in DCMECs. This result opens new avenues for the study of the molecular mechanisms linked to the synthesis of milk proteins.

Key Words: nuclear phosphorylated protein, prolactin, 2-DE

375 Analysis of differentially expressed miRNA in dairy cow mammary gland identifies HK2-regulating miRNAs. Z. Li,* H. Y. Liu, and J. X. Liu, *Institute of Dairy Science, MOE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou, China.*

MicroRNAs (miRNAs) are non-coding RNAs of about 22 nucleotides in length. Functional studies have demonstrated that miRNAs play critical roles in a wide series of biological processes including development and disease pathogenesis. To investigate the functional roles that miRNA play in the mammary gland (MG) of lactating cows, the bovine MG miRNA transcriptomes were profiled using microarray. A custom-designed microarray assay was performed to analyze miRNA expression patterns in the MG of lactating and nonlactating dairy cows. Compared with non-lactation MG, a total of 226 miRNAs in the lactating MG showed significant differences in expression ($P < 0.01$). There were 120 miRNAs including bta-miR-199b and bta-miR-125b downregulated, whereas 106 miRNAs such as bta-miR-133a and bta-miR-500 were upregulated. Meanwhile other 73 new miRNAs in bovine MG were detected. Target prediction and network construction speculated that 5 differentially expressed miRNAs (bta-miR-125b, bta-miR-199b, bta-miR-181a, bta-miR-484 and bta-miR-500) could contribute to the lactation by targeting the hexokinase 2 (HK2) gene, the key enzymes in glucose metabolism. The potential target sites for all these 5 miRNAs were identified in predicted bovine HK2 3′UTR. These miRNA mimics were introduced into MAC-T cells to evaluate their effects on the HK2 at the translation level. Of these miRNAs, bta-miR-484 and bta-miR-500 showed the most inhibitory effect on production of HK2 protein, while this was not the case for other 3 miRNAs. These results indicated that miR-484 and miR-500 may exert their effect on lactation function via targeting HK2. The present study provides a systematic transcriptome profiling differentially expressed between lactating and nonlactating bovine MG.

Key Words: bovine mammary gland, hexokinase 2, microRNAs