

Lactation Biology II

T134 Effects of feed restriction and prolactin-release inhibition at drying-off on milk production, metabolism and mammary gland involution. S. Ollier*¹, X. Zhao², and P. Lacasse¹, ¹*AAFC-Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada*, ²*Department of Animal Science, McGill University, Sainte-Anne-de-Bellevue, QC, Canada*.

The risk of acquiring a new intramammary infection during the dry period increases with milk production at drying-off and decreases as mammary gland involution progresses. A method commonly used to reduce milk production is a drastic reduction in feed supply in the days that precede drying-off. Our recent work has shown that milk production can also be reduced by an inhibition of the lactogenic signal driven by prolactin (PRL). To compare these 2 drying-off procedures, 24 Holstein cows in late lactation were assigned to 3 treatments based on milk yield, SCC and parity. They were fed 1) a lactation diet until drying-off (Control), 2) only dry hay the last 5 d before drying-off (Hay), or 3) as Control cows, but received twice daily i.m. injection of quinagolide (4 mg per injection), a specific inhibitor of PRL-release, from 5 d before drying-off to 13 d after (Quin). Cows from Control and Hay treatments received water injections. Blood and mammary secretion samples were collected on the last 7 d before and 1, 3, 5, 7, 10, and 14 d after the last milking. Quinagolide induced a sharp decrease ($P < 0.001$) in basal serum PRL concentrations on all the injection days. Interestingly, PRL was also depressed in Hay cows before drying-off. Both Quin and Hay treatments induced a drop ($P < 0.001$) in milk production, which averaged 17.9 and 10.1 kg/d, respectively, at drying-off compared with 24.8 kg/d for Control cows. Feeding dry hay decreased blood concentrations of glucose ($P < 0.001$) and most aminoacids (12 / 19; $P < 0.05$), and increased blood concentrations of β -hydroxybutyrate and Nonesterified fatty acids significantly ($P < 0.001$), whereas quinagolide did not affect these metabolites. Mammary secretion SCC was greater at d 1 of the dry period in the Hay ($P < 0.001$) and at d 5 in the Quin ($P < 0.05$) than in the Control cows. The BSA concentration increased faster ($P < 0.05$) in the mammary secretion of both Hay and Quin than that of Control cows, whereas the citrate:lactoferrin ratio, another indicator of involution rate, decreased faster ($P < 0.001$). In conclusion, this experiment shows that PRL-release inhibition could be a new alternative to reduce milk production before the drying-off and to hasten mammary gland involution without disturbing the metabolism of the cow. This strategy may reduced the incidence of new intramammary infection at drying-off.

Key Words: quinagolide, dry period, prolactin

T135 Effects of recombinant bovine somatotropin on blood flow to the mammary gland in early lactating Holstein cows. H. L. Sánchez-Rodríguez*¹, R. C. Youngblood¹, J. E. Curbelo¹, C. Steadman¹, R. C. Vann², E. Baravik-Munsell³, S. T. Willard^{1,5}, and P. L. Ryan^{1,4}, ¹*Department of Animal and Dairy Sciences, Mississippi State University*, ²*Brown Loam Branch Experimental Station, Mississippi State University, Raymond*, ³*Department of Clinical Sciences, Mississippi State University*, ⁴*Department of Pathobiology and Population Medicine, Mississippi State University*, ⁵*Department of Biochemistry and Molecular Biology, Mississippi State University*.

Doppler and B-mode ultrasound were used to evaluate the effects of recombinant bovine somatotropin (rBST) administration on mammary gland (MG) blood flow volume (BFV), Resistance Index (RI) and vessel diameter in cycling, lactating Holstein cows. The rBST ($n = 4$; 500

mg) was administered once at 42 d postpartum, while Control cows ($n = 4$) received 1.75 mL of saline solution. Ultrasound and temperature recordings were taken at d -7, -5, -2, 0, 1, 2, 5, 7, 9, 12, 14, 16, and 19 and the periods before and after treatment (d 0) were compared. The MG perfusion was characterized by the BFV and RI of the left and right pudendoepigastric trunk arteries (PETA). Additionally, both PETA diameters, posterior skin temperatures (ST) of the MG, and rectal temperatures (RT) were recorded. No differences were observed between the right and left PETA ($P > 0.05$); therefore, both values were combined for further analysis. Administration of rBST increased BFV ($P < 0.001$; 2837.73 ± 99 and 3107 ± 73 mL/min before and after, respectively) and decreased RI ($P < 0.001$; 0.46 ± 0.02 and 0.40 ± 0.01 before and after, respectively). In Control cows, a general decrease in BFV ($P < 0.001$; 3032.47 ± 95 and 2660.60 ± 56 mL/min before and after, respectively) and increase in RI ($P < 0.001$; 0.44 ± 0.02 and 0.51 ± 0.01 before and after, respectively) were observed through the sampling period. The PETA diameters were greatest in rBST treated than in Control cows ($P < 0.01$; 20.29 ± 0.02 and 19.71 ± 0.02 mm, respectively). A positive correlation was observed between the PETA diameter and BFV in Control ($r = 0.18$; $P < 0.01$) and rBST ($r = 0.29$; $P < 0.001$) cows, respectively. The ST variability of the MG was increased ($P < 0.05$) by rBST administration. However, RT and milk yield were not affected ($P > 0.05$) by treatment. These preliminary findings suggest that Doppler ultrasound may represent a feasible technique for the study of the vascular perfusion changes in the MG associated with rBST administration. Further studies in later stages of lactation are required to clarify the trends observed in RT and milk yield.

Key Words: mammary gland blood flow, Doppler ultrasound, dairy cows

T136 Effects of colostrum versus formula feeding on hepatic glucocorticoid and α_1 - and β_2 -adrenergic receptors in neonatal calves. D. Rohrbeck, J. Steinhoff-Wagner, E. Kanitz, and H. M. Hammon,* *Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany*.

Colostrum feeding affects glucose metabolism and the endocrine status in neonatal calves. Cortisol and catecholamines stimulate glycogen degradation and gluconeogenic enzyme activities in liver via glucocorticoid receptors (GR) and α_1 - and β_2 -adrenergic receptors (AR). We have tested the hypothesis that the number of GR as well as α_1 - and β_2 -AR in neonatal calves depends on colostrum feeding. We used 14 male German Holstein calves, fed either colostrum or formula for 4 d (group C and F, respectively) twice daily. Nutrients in formula based on milk protein (lactalbumin, casein), lactose, and coconut fat in same amounts as in colostrum milking, but formula contained no biologically active substances such as hormones and growth factors. Amounts (per meal) fed were 4% of BW on d 1 and 5% of BW on d 2 to d 4. On d 4 blood samples were taken to measure plasma concentrations of glucose and cortisol and liver samples were harvested 2 h after feeding. For AR measurements, liver membrane suspensions (2 mg protein/ml) were prepared and saturation binding assays were performed with increasing concentrations of (³H)-prazosin and (³H)-CGP-12177 for determination of α_1 - and β_2 -AR, respectively. For GR measurements the cytosol fraction (8 mg protein /ml) was incubated with increasing concentrations of (³H)-dexamethasone. Maximal binding capacity (B_{max}) and binding affinity (K_D) were calculated by computer program. Data were analyzed by General Linear Model of SAS with feeding as fixed effect. Plasma

glucose concentrations were higher ($P < 0.05$) in C than F, but plasma cortisol concentrations did not differ between groups. For GR, B_{\max} was higher ($P < 0.05$) in C than F, whereas K_D indicated no differences. B_{\max} of α_1 -AR tended to be higher ($P < 0.1$) in C than F, but K_D of α_1 -AR as well as B_{\max} and K_D of β_2 -AR were not affected by feeding in a significant manner. Results indicated dependence of GR and α_1 -AR on milk feeding immediately after birth and pointed at possible involvement of GR and α_1 -AR in regulation of neonatal glucose metabolism in calves. Supported by DFG, Germany.

Key Words: calves, colostrum feeding, glucocorticoid and adrenergic receptors

T137 Fitness of lactation curve functions to daily and monthly test-day milk data in an Ethiopian dairy cattle population. G. Gebreyohannes¹, S. Koonawootrittriron¹, M. A. Elzo^{*2}, and T. Suwanasopee¹, ¹*Kasetsart University, Bangkok, Thailand*, ²*University of Florida, Gainesville*.

The objective of this research was to identify a lactation curve function that best fit daily (DD) and monthly test-day milk (MD) data in an Ethiopian dairy cattle population. Three functions were compared: an incomplete gamma (IG), a modified incomplete gamma (MIG; $b = 1$) and an inverse polynomial (IP). Analysis used 6,707 lactation milk records of 2,066 cows collected from 1979 to 2010 in the Bako, Holetta and Debre Zeit research centers. Breed groups were Horro (H), Boran (B), B-Friesian, H-Friesian, B-Simmental, H-Simmental, B-Jersey and H-Jersey. The MIG and IG were first log-transformed to linear form before fitting. Goodness of fit of IG, MIG, and IP were compared using R-squared values. Factors affecting R-squared values were analyzed using a model that contained herd-year-season, parity, data type (DD and MD), breed group, lactation curve function and the interaction between data type and lactation curve function as fixed effects, and residual as a random effect. All factors in the model affected R-squared values ($P < 0.001$). Least squares means of R-squared values (LSMR2) were compared among subclasses of each factor. Milk data from cows in later parities (>4 parities; range 0.84 to 0.85) showed significantly higher LSMR2 ($P < 0.001$) than first parity cows (0.80 ± 0.002). The LSMR2 of functions fitted to MD data (0.88 ± 0.001) was higher ($P < 0.001$) than the one for DD data (0.79 ± 0.001). Horro cows had higher LSMR2 (0.86 ± 0.003) than other breed groups (0.77 to 0.85 ; $P < 0.001$). The MIG (0.90 ± 0.002) and IP (0.90 ± 0.002) functions had similar LSMR2, but both MIG and IP were significantly different from IG (0.71 ± 0.002 ; $P < 0.001$). The MIG function had the highest LSMR2 for DD data (0.88 ± 0.002), while the IP function had the highest LSMR2 for MD data (0.94 ± 0.002). The MIG and IP functions can be recommended for describing lactation patterns of Ethiopian dairy cattle using daily and test-day milk data, respectively.

Key Words: cattle, lactation curve function, test-day

T138 Effect of rearing intensity on growth performance and on mammary tissue in Holstein yearling heifers. V. Lollivier^{*2,1}, F. Dessauge^{1,2}, M. Boutinaud^{1,2}, and Y. le Cozler^{2,1}, ¹*INRA, UMR1348 Pegase, Saint-Gilles, France*, ²*Agrocampus Ouest, UMR1348 Pegase, Rennes, France*.

Intensive growth during the first months of life influence the shaping animal's final body weight, but if excessive, it may alter the mammary gland development and subsequent milk yield, at least during lactation one. A long-term experiment aimed at studying the effect of growth intensity from birth to insemination in Holstein dairy heifers, combined

to a targeted age at first calving of 22 or 24 mo, is being conducted and includes each year 60 to 70 heifers. Preliminary results indicate that the effect of intensive growth on lactation performance could be limited. Moreover, the effect on mammary development remains to be studied. In October 2011, 3 heifers which followed the routine rearing procedure (SP) and 4 heifers which were reared according to the intensive growth program (HP) were slaughtered at one year of age. Body composition and mammary gland weight were measured and mammary tissue morphology was investigated. Average daily gain from birth to slaughter was 874 and 968 g/d for SP and HP heifers, respectively. This corresponded to an average body weight of 358 and 389 kg at 360 and 355 d of age respectively. Mammary gland weight was not significantly affected by the treatment (2.22 vs 2.02 kg for SP and HP heifers, respectively). Impact of growth intensity on lobulo-alveolar mammogenesis was studied through histological analysis of mammary tissue. Results may have implications for understanding control mechanisms that regulate parenchymal development in Holstein heifers.

Key Words: mammogenesis, growth intensity, heifer

T139 Obesity and parity affect the mammary gland serotonin (5-HT) system. K. E. Merriman,^{*} J. LaPorta, and L. L. Hernandez, *University of Wisconsin, Madison*.

5-Hydroxytryptamine (5-HT) is synthesized and secreted by the mammary gland as well as a homeostatic regulator. 5-HT was recently determined to be involved in the mammary gland's response to consumption of high-fat diet (HFD) in rats. The objective of our study was to determine the effects of HFD and a HFD plus vertical sleeve gastrectomy (HFD+VSG) on the mammary gland serotonergic system compared with low-fat diet animals (LFD). We conducted qPCR analysis for the following mRNA in the mammary gland: tryptophan hydroxylase 1 (TPH1), the rate-limiting enzyme in 5-HT synthesis, serotonin reuptake transporter (SERT), responsible for the 5-HT reuptake into the cell for degradation, and the 5-HT 7 receptor subtype (HTR7), responsible for regulating mammary gland involution. We determined that LFD primiparous rats had similar expression for TPH1, SERT and HTR7 as nulliparous rats, while multiparous rats had significantly increased TPH1 (30-fold), SERT (700-fold) and HTR7 (6-fold) ($P < 0.05$) expression. In nulliparous HFD and HFD+VSG rats, there was a significant increase in mRNA expression for TPH1 (20, 60-fold, respectively), SERT (325, 75-fold, respectively) and HTR7 (6, 2-fold, respectively) compared with LFD rats ($P < 0.05$). In primiparous HFD and HFD+VSG rats there was also a significant increase in mRNA expression for TPH1 (25, 255-fold, respectively), SERT (25, 990-fold, respectively) and HTR7 (70, 12-fold, respectively) compared with LFD rats ($P < 0.05$). There was no difference in the expression of TPH1, SERT and HTR7 compared with LFD rats ($P > 0.05$) in multiparous HFD and HFD+VSG. These results show that LFD rats have increased serotonergic components with increasing parity. Additionally, it is demonstrated that the serotonergic signaling system is over-stimulated in HFD and HFD+VSG rats that are nulliparous and primiparous relative to LFD. This suggests in normal weight animals, as parity increases there is an increase of 5-HT synthesis in the mammary gland that is necessary to maintain mammary gland homeostasis. In obese animals, there is an over-production of 5-HT and signaling within the mammary gland that could lead to issues with mammary gland homeostasis.

Key Words: 5-hydroxytryptamine, obesity, parity

T140 Cooling of heat-stressed cows during the dry period alters lymphocyte but not mammary gland gene expression. S. Tao^{*1}, E. E. Connor², J. W. Bubolz¹, I. M. Thompson¹, B. C. do Amaral¹, M. J. Hayen¹, and G. E. Dahl¹, ¹University of Florida, Gainesville, ²USDA-ARS, Beltsville, MD.

Heat stress (HT) during the dry period compromises mammary gland development, decreases future milk production, and impairs immune status of dairy cows. Our objective was to evaluate the effects of cooling heat-stressed cows during the dry period on gene expression of the mammary gland and lymphocytes. Cows were dried off 46 d before their expected calving date and assigned to 2 treatments, HT or cooling (CL). Average temperature-humidity index during treatment was 76.6 for all cows. CL cows were cooled with sprinklers and fans that came on when ambient temperature exceeded 21.1°C, whereas HT cows were not. Rectal temperature (RT) was measured twice daily and respiration rates (RR) recorded thrice weekly during the dry period. After parturition, all cows were housed in a free-stall barn with cooling. Lymphocytes were isolated at dry-off, -20, 2, and 20 d relative to calving from a subset of cows (HT, n = 9; CL, n = 10) and mammary biopsies were taken at the same intervals (HT, n = 7; CL, n = 6) for RNA extraction. Gene expression was assessed using a custom multiplex expression assay based on traditional reverse transcription-PCR. Genes involved in prolactin (PRL) signaling (*PRLR-L*, *PRLR-S*, *SOCS2* and 3, *IGFII*, *IGFBP5*, and *Cyclin D1*), fatty acid (FA) metabolism (*ACC* and *LPL*) and *IGFI* were evaluated in mammary tissue, and genes related to FA metabolism (*ACC*, *FASN*, and *LPL*), cytokine production (*IL6*, *IL8*, and *TNF α*) and *IGFI* were evaluated in lymphocytes. Data was analyzed by the PROC MIXED procedure of SAS. Compared with HT, CL cows had lower ($P < 0.01$) RT (39.4 vs. 39.0°C) and RR (78 vs. 46 breath/min) in the afternoon before calving. No differences ($P > 0.15$) were observed in PRL signaling or FA metabolism gene expression in the mammary gland. In lymphocytes, HT cows had higher ($P \leq 0.05$) *IGFI* and *TNF α* mRNA expression during the transition period relative to CL and upregulated ($P < 0.05$) *IL8* and downregulated ($P = 0.01$) *FASN* mRNA expression at 2 d relative to calving. We conclude that cooling HT cows during the dry period alters cytokine production and lipid metabolism in lymphocytes.

Key Words: heat stress, lymphocyte, mammary gland

T141 Identification and quantification of milk synthesis and secretion related proteins in bovine milk using a proteomics approach. J. Lu^{*1,2}, S. Boeren², J. Vervoort², H. van Valenberg¹, S. de Vries², J. van Arendonk³, T. van Hooijdonk^{1,4}, and K. Hettinga¹, ¹Dairy Science and Technology Group, Wageningen University, Wageningen, the Netherlands, ²Laboratory of Biochemistry, Wageningen University, Wageningen, the Netherlands, ³Animal Breeding and Genomics Centre, Wageningen University, Wageningen, the Netherlands, ⁴FrieslandCampina, Amersfoort, the Netherlands.

Lactation physiology is a process that is still partly understood. Proteomics techniques have shown to be useful to help advance the knowledge on lactation physiology in human and rodent species but is rarely used for dairy cows, except for mastitis. In fact, many aspects in bovine milk secretion still need to be investigated in detail and advanced proteomics techniques can help to improve this knowledge. Filter-aided sample preparation (FASP) and NanoLC-Orbitrap-MS/MS were applied to milk fat globule membrane and serum fractions, which were isolated from bovine tank milk samples (65 Holstein cows). This resulted in the identification of 246 proteins. Most proteins were low in abundance. Many of these low abundant proteins were for the first time found in

bovine milk. The identified proteins in milk were similar to previous reports on the proteome of cells from mammary gland biopsies. The identified proteins were categorized according to their function description in Uniprot (www.uniprot.org). Sixty-three proteins were found to be directly related to milk component synthesis and secretion. These proteins are mainly enzymes and transporters. Thirteen enzymes which are part of the central metabolic pathway were identified. Eleven enzymes catalyze steps in lipid synthesis, including acetyl-CoA synthetase, fatty acid synthase, glycerol-3-phosphate acyltransferase. Forty-three transporters were identified. These transporters are involved in intracellular transport of lipids, proteins, minerals and vitamins, as well as secretion of these compounds. Butyrophilin, adipophilin, and XDH, which are essential proteins for lipid droplet secretion from epithelial cell, were all found in milk. Besides identification of proteins, dimethyl-labeling was tested to perform relative quantification of the proteins and was shown to give accurate results. The proteins identified to be involved in milk synthesis and secretion suggests that milk may be used rather than more intrusive tissue biopsies in the study of milk synthesis and secretion. These proteomic techniques can be used to solve different biological questions to increase our knowledge of lactation.

Key Words: proteomics, lactation physiology, milk synthesis and secretion

T142 Physiological state but not gestational photoperiod affects weights of liver and thymus in mice. P. A. Bentley^{*} and T. B. McFadden, University of Alberta, Edmonton, Alberta, Canada.

Exposing dairy cows to short day photoperiod during gestation increased milk yield by 3 kg/d in the subsequent lactation. Although the mechanisms responsible for this effect are unknown, recent studies in other mammals have shown effects of altered photoperiod on peripheral tissues; such changes could influence milk yield. In this experiment, mice were used to investigate the effects of gestational photoperiod on lactation and development of mammary gland and other organs. We hypothesized that altering photoperiod during gestation would elicit physiological changes causing differences in milk production. Mice (n = 6/treatment x time) were exposed to long (16L:8D), normal (12L:12D) or short (8L:16D) day photoperiod treatment during gestation. Two groups of mice/treatment were sacrificed on d 17 and 19 of gestation. The remaining mice were allowed to pup and after parturition were placed on 12L:12D and litter size was standardized. For all mice, organ weights were determined immediately after sacrifice on d 17 and 19 of gestation and d 5 and 10 of lactation. For lactating mice, litter weight gain was measured daily to estimate milk yield. Data were analyzed for main effects and interaction of photoperiod and time using Proc GLM of SAS. There was no significant effect of photoperiod on litter weight gain during the first 10 d of lactation, nor on organ weights. However, litter weights of dams exposed to long or short day photoperiod were numerically greater after d 5 of lactation than those exposed to normal day length. Dam weight did not vary by photoperiod treatment and was used to normalize organ weights. Liver weights were greater ($P < 0.05$) on d 10 of lactation than during gestation, corresponding to the increased metabolic demand of lactation. Thymus weights decreased ($P < 0.01$) from d 17 to d 19 of gestation but increased ($P < 0.0001$) from d 5 to d 10 of lactation. Differences in thymus weight may reflect altered immune activity during the periparturient period. These data indicate some physiological adaptations to pregnancy and lactation in mice that may provide targets for studying molecular mechanisms involved in similar adaptations in dairy cows.

Key Words: photoperiod, gestation, metabolism