LACTATION BIOLOGY II

0412 Intramammary glucocorticoid during a mammary immune response to lipopolysaccharide modulates the blood-milk barrier. O. Wellnitz*, S. K. Wall, M. Saudenova, and R. M. Bruckmaier, *Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Glucocorticoids such as prednisolone are frequently used in addition to intramammary antibiotic therapy to increase the cure rate of mastitis in dairy cows. This study aimed to investigate the effects of intramammary administered prednisolone during the mammary immune response to lipopolysaccharide (LPS). Five healthy mid-lactation Holstein dairy cows received 1 of 4 intramammary treatments in each of their 4 quarters: prednisolone (10 mg), LPS (100 μg), LPS (100 μg), and prednisolone (10 mg), or saline control. Milk samples were taken 0, 3, 6, 9, 12, 24, and 36 h after challenge. Somatic cell count (SCC), and concentrations of lactate dehydrogenase (LDH), serum albumin (SA), and tumor necrosis factor-α (TNFα) in milk and mRNA abundance of TNFα, Interleukin (IL)-8, and IL-1β in milk somatic cells were compared at each time point. Differences between quarters were tested by analysis of variance using a MIXED procedure and were considered significant if $P < 0.05$. Control and prednisolone infused quarters did not show changes of SCC, LDH, SA, and TNFα concentrations in milk and mRNA expression of TNFα, IL-1β, and IL-8 in milk somatic cells. Concentrations of SCC and TNFα in milk increased similarly in LPS challenged quarters independent of additional prednisolone application. However, the increase of LDH activity and SA concentration in LPS challenged quarters was diminished by prednisolone ($P = 0.028$ and $P < 0.001$, respectively) from 1352 ± 845 to 264 ± 107 U/L for LDH, and 8.17 ± 0.29 to 2.68 ± 0.30 mg/L for SA at 6 h after challenge, respectively. The mRNA abundance of TNFα, IL-8, and IL-1β in milk somatic cells increased in response to LPS challenge unaffected by prednisolone. In conclusion, the intramammary administration of the glucocorticoid prednisolone does not induce an increase of SCC, changes in concentrations of blood components in milk, and does not change the production of TNFα, IL-8, and IL-1β in milk cells in response to intramammary LPS challenge. However, the intramammary administration of prednisolone clearly reduces the disruption of the blood-milk barrier induced by endotoxin challenge shown by a reduced appearance of blood constituents like SA or LDH in milk. This effect could have an important influence on the severity and on the cure rate of mastitis.

Key Words: prednisolone, blood-milk barrier, mastitis

0413 Milk prolactin response after experimental infection with different coagulase-negative staphylococci in dairy heifers. K. Piccart*[1], S. Piepers[1], J. Verbeke[1], N. Melo de Sousa[2], J. F. Beckers[2], and S. De Vliegher[1], *[1]Ghent University, Ghent, Belgium, [2]University of Liège, Liège, Belgium.*

Coagulase-negative staphylococci (CNS) are the most common group of bacteria involved in subclinical bovine mastitis. Dairy heifers infected with CNS seem to produce more milk than uninfected heifers, but the underlying mechanism is yet unclear. This study investigates the response of prolactin (PRL) in milk as a potential mediator of milk yield (MY) after experimental challenge with different CNS species. Eight Holstein-Friesian heifers in mid-lactation (126 d in milk ± 66) were challenged in a split-udder design with 3 different CNS isolates: 1 *S. fleurettii* isolate from sawdust and 2 phenotypically dissimilar *S. chromogenes* isolates. The first *S. chromogenes* isolate originates from a chronic intramammary infection, while the other is cultured from a teat apex. Three quarters were simultaneously inoculated with 1.0×10^6 colony forming units. The remaining quarter was infused with sterile phosphate-buffered saline and served as a control. Milk samples were obtained for measuring PRL (by radioimmunoassay) at various time points starting 24 h preinoculation until 72 h after challenge. Furthermore, quarter MY was recorded. Milk samples were cultured to evaluate bacterial clearance. A linear mixed regression model, using heifer and quarter as random effects, was built to evaluate the PRL response after infection with sampling time and inoculation type as fixed effects. Preinoculation data were not included in the analysis. None of the quarters developed clinical symptoms and none of the heifers showed signs of illness. Milk culture results revealed that all CNS were eliminated before the end of the trial. Even though this study did not focus on MY, a decreased production was observed in all quarters. The infection status did not have a demonstrable effect on milk PRL concentration: no significant difference was found between infected and control quarters, or between different CNS-isolates ($P = 0.40$). However, milk PRL generally changed over time ($P < 0.05$). These findings suggest that milk PRL is not a likely candidate to explain any potential increase in milk production after a subclinical infection caused by *S. chromogenes* or *S. fleurettii*.

Key Words: prolactin, mastitis, coagulase-negative staphylococci

0414 Regulation of nuclear IGFBP-3 in response to intrinsic apoptotic stress in bovine mammary epithelial cells. A. Agostini-Dreyer, A. E. Jetzt, and W. S. Cohick*, *Rutgers, the State University of New Jersey, New Brunswick.*

Following peak lactation, the number of secretory mammary epithelial cells (MEC) in the bovine gland gradually

decreases due to increased apoptosis, leading to a decrease in lactation persistency. However, the mechanisms that govern apoptosis in the bovine MEC are under-investigated. We have previously shown that anisomycin (ANS), an activator of the intrinsic apoptotic pathway, is a potent inducer of IGFBP-3 production in MAC-T cells, and that knock-down of IGFBP-3 with siRNA attenuates the ability of ANS to activate apoptosis. Interestingly, IGFBP-3 is found in both the nucleus and the conditioned media in response to ANS, indicating a potential for both intra- and extracellular functions. Whether nuclear IGFBP-3 arises from secreted IGFBP-3 is controversial. In the present work, MAC-T cells were transfected with a plasmid expressing GFP-tagged IGFBP-3. Analysis using fluorescent microscopy indicated that IGFBP-3 resided basally in the cytosol and translocated to the nucleus in response to ANS. Since IGFBP-3-GFP is too large to passively diffuse through nuclear pores, this supports a role for active nuclear import. Chemical inhibition of the nuclear transport protein importin-β with importazole reduced ANS-induced nuclear IGFBP-3-GFP, indicating that IGFBP-3 utilizes importin-β for nuclear import. Endoglycosidase-H digestion of nuclear fractions showed that intracellular IGFBP-3 was glycosylated, indicating it had been transported through the secretory pathway. However, inhibition of ER to Golgi transport with Brefeldin A inhibited secretion of IGFBP-3 but increased its nuclear accumulation, indicating that secretion is not required for nuclear localization. In support of these data, inhibition of clathrin-mediated endocytosis with the chemical inhibitor Pitstop2 did not impact nuclear localization of IGFBP-3. In summary, these data show that secretion is not required for ANS-induced nuclear localization of IGFBP-3 and that its nuclear import is a regulated event.

Key Words: mammary gland, lactation, IGFBP-3

0415 Cellular composition of water buffalo mammary gland and its proliferation status during dry and mastitis.

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Mammary alveoli, composed of mammary epithelial cells, are the structural and functional units of a mammary gland, which secrete milk into the alveolar lumen. Milk production is directly related to secretory activity and number of alveolar cells. Alternation in cellular composition, in particular alveolar cells, thus has a direct role in volume of milk secretion. We collected mammary tissues of milking water buffalo from a slaughterhouse for the purpose of evaluation of its cellular composition, proliferation status, and identification of myoepithelial cells. Out of 21 buffaloes, ~80% of mammary samples were from the dry animals (evidenced by presence of small nonsecretory epithelial cells and virgin-like state of the gland) and ~20% of samples were from the lactating animals. All the lactating animals were affected with mastitis (suggested by the presence of fibrin clots, cellular debris, and loss of alveolar epithelium), which were also confirmed by a veterinary pathologist. The fraction of total mammary epithelium (large secretory and small nonsecretory cells; mean % ± SE) did not differ in dry vs. lactation period (22.6 ± 1.66 vs. 18.7 ± 3.59; $P > 0.05$). However, number of small nonsecretory epithelium were greater in dry period (22.6 ± 1.66 vs. 10.1 ± 2.97; $P = 0.006$) than the lactation period, likely due to differentiation of nonsecretory cells into secretory cells. Number of myoepithelial cells (identified by vimentin expression in basal layer of epithelium) were greater (23.38 ± 2.89 vs. 5.55 ± 1.10; $P = 0.004$) in mastitis-afflicted than the dry animals. Increased expression of vimentin (also a marker of migrating cells) in the stroma, suggested the role of cell migration in inflammation, tissue regeneration and immune response during mastitis. Thus, mastitis-infected animals had increased number of myoepithelial cells and stromal migratory cells in their mammary glands. A greater number of myoepithelial cells during mastitis was concomitant with increased (5×) expression of Ki-67, a marker of cell proliferation. This study demonstrates cellular composition of buffalo mammary glands as well as supports the idea that infection of mammary gland enhances proliferation of mammary epithelial cells.

Key Words: water buffalo mammary gland, cellular composition, vimentin, proliferation

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0416 Use of the RatLoft in laboratory conditions decreases pup mortality in lactating mice.

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Mice in laboratory conditions are under considerable stress. Lactating dams may manifest this psychological distress through a decrease in milk yield or increase in pup mortality. The RatLoft (Research Animal Welfare Equipment, LLC, Madison, WI) is a stainless steel tube that hangs over the side of the cage with access to food, allowing the dam time away from her pups. Here, we examined the effect of the RatLoft on milk yield, circulating serotonin (5-HT), pup mortality, and behavioral distress as measured by the Porsolt Forced Swim Test (FST). Pregnant mice deficient for tryptophan hydroxylase-1 ($TPH1^{-/-}$, $n = 10$) and wild-type mice (WT, $n = 10$) were randomly assigned to loft (L; $n = 5$) and no loft (NL; $n = 5$) treatment groups. Milk yield was measured daily for 21 d. The FST was performed on d 10 and pup mortality was recorded throughout the experiment. Blood was collected on d 1, 9, and 21. Data was analyzed using a 2-way ANOVA. Milk yield increased over time in all animals ($P < 0.0001$). Presence or absence of the RatLoft did not affect milk yield ($P > 0.05$). Overall, WT mice had increased milk yield compared with $TPH1^{-/-}$ mice, regardless of the presence of RatLoft ($P < 0.05$).

The FST used to evaluate behavioral distress indicated that the presence or absence of RatLoft was not significant ($P > 0.05$). Serum 5-HT concentrations were increased in WT compared with TPH1$^{-/-}$ mice. Presence or absence of the RatLoft did not affect circulating 5-HT concentrations ($P > 0.05$, 373 vs. 309 ± 55 ng/mL), but 5-HT concentrations decreased throughout lactation (455 vs. 245 ± 65 ng/mL on d 1 and 21, respectively). Serotonin concentrations were increased in TPH1$^{-/-}$ mice with the L ($P < 0.01$; 33 ± 13 ng/mL vs. 13 ± 1.4 ng/mL). The TPH1$^{-/-}$ mice with L had less 5-HT on d 1 and 9 compared with d 21 ($P = 0.005$; 21 and 17 vs. 51 ± 6.5 ng/mL, respectively). The 5-HT levels in TPH1$^{-/-}$ mice with NL did not change over time ($P > 0.05$). Pup mortality was significantly less for dams with L as compared with mice with NL ($P = 0.047$, 0.49 ± 0.16 pups/dam vs. 0.195 ± 0.06). Mortality rates were not different between WT and TPH1$^{-/-}$ mice ($P > 0.05$). These results demonstrate that access to RatLoft during lactation decreases pup mortality rates in all animals, as well as increased 5-HT concentrations in TPH1$^{-/-}$ mice. In conclusion, use of the RatLoft could prove beneficial to researchers working with lactating mouse models to decrease pup mortality rates.

Key Words: serotonin, lactation, RatLoft

0417 Addition of glycerol to lactating cow diets stimulates milk protein yield to a greater extent than addition of corn grain. D. L. Bajramaj*[1], R. V. Curtis[2], J. J. M. Kim[2], V. R. Osborne[1], T. Wright[3], and J. P. Cant[1], *[1]University of Guelph, Guelph, ON, Canada, [2]Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada, [3]University of Guelph/OMAF, Guelph, ON, Canada.*

As the biofuel industry grows and expands, there is an increased availability of the byproduct glycerol, which could be a glucogenic feedstuff for dairy cows. The objective of this study was to determine if the addition of glycerol to the diet of dairy cows would stimulate milk protein yield in the same manner as the addition of corn grain. Twelve lactating dairy cows were assigned at 81 ± 5 d in milk to 3 diets in a repeated 3 × 3 Latin square design. The diets were a 70%-forage diet considered the base diet, the base diet with 19% ground and high-moisture corn replacing forages, and the base diet with 15% refined glycerol and 4% added protein supplements to be isocaloric and isonitrogenous with the corn diet. Diets contained 17.2, 17.9, and 17.3% CP, respectively, and 34, 28, and 30% NDF, respectively. The diets were fed for periods of 28 d each, and milk, feed, and blood samples were collected during the last week of each period for compositional analysis. Treatment differences were evaluated by ANOVA using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) with cow as a random effect. Dry matter intake increased from 23.7 kg/d on the base diet to 25.8 kg/d on the corn diet ($P = 0.007$) and to 27.2 kg/d on the glycerol diet ($P < 0.001$). There was a tendency for DMI to be higher with glycerol than corn ($P = 0.06$). Milk production increased from 39.2 kg/d on the base diet to 43.8 kg/d on the corn diet ($P < 0.001$) and to 44.2 kg/d on the glycerol diet ($P < 0.001$). There was no difference in milk yield between corn and glycerol diets. Milk protein content was 3.19, 3.33, and 3.44% on the base, corn, and glycerol diets, respectively, and the stimulation by glycerol was greater than the stimulation by corn ($P = 0.037$). Protein yield was increased 197 g/d by the addition of corn and 263 g/d by the addition of glycerol, and the glycerol effect was larger than the corn effect ($P = 0.054$). Efficiency of capture of dietary protein in milk protein, however, was lower on the glycerol diet at 29.5% compared with 32.6% on the corn diet ($P = 0.017$). It was concluded that glycerol stimulated milk protein yield to a greater extent than corn grain.

Key Words: glycerol, milk protein synthesis, dairy cow

0418 Glucose does not stimulate milk protein yield of dairy cows when essential amino acids are in excess supply. K. Nichols*[1], M. Carson[2], J. J. M. Kim[1], J. A. Metcalf[2], J. P. Cant[1], and J. Doelman[2], *[1]Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada, [2]Nutreco Canada Agresearch, Guelph, ON, Canada.*

To determine if glucose stimulates milk protein yield when essential amino acids (EAA) are supplied in excess, 5 early-lactation, rumen-fistulated dairy cows (78 ± 13 d in milk) were abomasally infused for 5 d with EAA and glucose solutions in a 5 × 5 Latin square design. The 5 infusion treatments were saline, 844 g/d EAA in the profile of casein, 1126 g/d EAA, 844 g/d EAA + 1000 g/d glucose, or 1126 g/d EAA + 1000 g/d glucose. Cows were fed a diet containing 6.96 MJ/kg NE_L and 12% crude protein on a dry basis. Milk composition and yield during the last 2 d of each period and plasma metabolite concentrations during d 4 of infusion were subjected to ANOVA using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC), where cow was a random effect. EAA infusion increased essential and branched-chain AA concentrations in plasma 3- to fourfold compared with saline ($P < 0.001$). Non-EAA concentrations decreased 11 to 17% during EAA infusions ($P < 0.001$). Addition of glucose to EAA infusates decreased essential and branched AA concentrations in plasma ($P < 0.031$) and had no effect on non-EAA concentrations. Essential amino acid infusion increased concentrations of NEFA ($P = 0.004$) and urea N ($P < 0.001$) in plasma, but had no effect on glucose concentrations. Addition of glucose to EAA infusates increased glucose concentrations in plasma 13 to 19% ($P < 0.001$), decreased NEFA 26 to 32% ($P < 0.001$) and had no effect on urea N. Dry matter intake was not affected by EAA infusion, while daily milk yield increased 16% and milk protein yield increased 27% (for an average of 262 g/d) at the highest level of EAA infusion compared with saline ($P < 0.001$). Milk protein concentration increased from 2.9% with saline to 3.3%

with EAA ($P < 0.001$). The addition of glucose to EAA infusates caused DMI to decrease 0.65 kg/d ($P = 0.040$), tended to increase milk yield ($P = 0.057$), had no effect on milk protein yields ($P = 0.318$), but tended to decrease milk protein concentration ($P = 0.097$) and decreased milk fat concentration ($P < 0.001$). Thus, increased supply of glucose at high levels of EAA supplementation did not improve milk protein yields, but because of the decline in feed intake there was an increased efficiency of capture of dietary protein into milk protein.

Key Words: milk protein, essential amino acids