

## Lactation Biology 2

**W194 Effect of feeding level and milking frequency in early lactation on milk production in dairy cattle.** A. G. Rius\*, J. K. Kay, C. V. C. Phyn, S. R. Morgan, and J. R. Roche, *DairyNZ, Hamilton, New Zealand*.

The objective was to evaluate the effects of feeding level (FL) and milking frequency (MF) in early lactation on milk production in grazing dairy cattle. Multiparous Holstein-Friesian cows ( $n = 120$ ;  $\sim 31$  DIM) offered an unrestricted allowance of fresh pasture (UnRes) and milked twice daily (2X) were randomly assigned to one of 4 treatments for 21 d in a 2 by 2 factorial arrangement - 2 FL of pasture (UnRes or a 50% restriction: Res) and 2 MF (once daily: 1X or 2X). After the treatment period, all animals received a generous allowance of pasture and were milked 2X for the remainder of lactation. Body weights (BW) and body condition scores (BCS) were recorded and milk samples collected once weekly. Main effects and interactions during treatment, and for 8 wk post-treatment, were tested using mixed models (GenStat 12.1). Interactions between FL and MF were detected ( $P < 0.01$ ) for milk and protein yields during the treatment period. Decreases due to 1X were greater ( $P < 0.01$ ) in UnRes than in Res cows for milk (4.8 vs. 2.2 kg/d), and protein (0.17 vs. 0.06 kg/d) yields. Upon cessation of treatments milk production was greater ( $P < 0.01$ ) in UnRes cows compared with Res cows (18.4 vs. 17.1 kg/d) and in 2X compared with 1X (18.3 vs. 17.2 kg/d), but there was no interaction. Similarly, UnRes cows had greater ( $P < 0.01$ ) fat (0.79 vs. 0.74 kg/d) and protein (0.67 vs. 0.62 kg/d) yields and 2X cows had greater ( $P < 0.01$ ) fat (0.78 vs. 0.75 kg/d) and protein (0.66 vs. 0.63 kg/d) yields. Relative to their UnRes comparison, Res cows were lighter and thinner ( $P < 0.01$ ; BW: 440 vs. 484 kg and BCS: 3.86 vs. 4.03; 10-point scale) and relative to 1X, 2X cows were lighter ( $P < 0.01$ ; 460 vs. 468 kg) by the end of the treatment period. In summary, reducing milking frequency and the level of nutrition for 21 d in early lactation impaired milk production.

**Key Words:** milking frequency, grazing, milk production

**W195 Expression of key metabolic indicators of energy metabolism across mammary gland development and lactation in dairy cows.** L. J. Ren, H. L. Tong, Q. Z. Li, and X. J. Gao\*, *Key Laboratory of Dairy Science of Education Ministry, Northeast Agricultural University, Harbin, China*.

Appropriate energy supply is critical to normal growth and development of the mammary gland. To understand how key indicators of mammary gland metabolism change with physiological state, 39 primiparous dairy cows were allotted in 13 groups according to stage of mammary development (mo 2, 12, 14 of virgin, mo 2, 4, 6 of pregnancy, d 7, 50, 140, 280 of lactation and d 3, 30 of involution, and 3 animals per group). Mammary gland tissue samples were collected by paracentesis and the contents of important indicators in different periods including hexokinase(HK), glucose-6-phosphatase dehydrogenase (G-6-PDH), isocitrate dehydrogenase(ICDH),  $\text{Na}^+\text{K}^+$ -ATPase,  $\text{Ca}^{2+}\text{Mg}^{2+}$ -ATPase, triglyceride(TG), glucose(Glc), lactose(LAC), energy charge and NADPH were detected. LAC, energy charge and NADPH were detected by HPLC. Others were detected by the testing kits. In this test, qRT-PCR was used to detect those important genes which were related to lactation, including  $\beta$ -actin, sterol regulatory element binding protein-1(SREBP-1), acetyl CoA carboxylase(ACC), D-glucose transport 1(Glut1), AMP-activated protein kinase(AMPK), phosphoenolpyruvate carboxykinase(PEPCK),  $\beta$ -casein and signal transducers and activators

of transcription 5(Stat5) in different mammary gland development periods of dairy cow. The results showed the different indicators reached the highest at different periods. For example, the activities of ICDH and G-6-PD both reached the highest at d 7 of lactation, but the activity of  $\text{Na}^+\text{K}^+$ -ATPase reached the highest at d 280 of lactation. qRT-PCR showed that the expression of these genes was significantly higher at the period of lactation than other periods. In conclusion, according to the changes of metabolism indexes and the expression of these important genes, we can conclude energy metabolism in the course of mammary gland development and lactation of dairy cows.

**Key Words:** dairy cow, mammary gland, energy metabolism

**W196 Insulin stimulates glucose uptake by regulating cell viability and expression of glucose transporter 8 gene in bovine mammary epithelial cells.** K. Zhao, H. Y. Liu\*, and J. X. Liu, *Institute of Dairy Science, MOE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou 310029, P.R. China*.

Glucose transporter 8 (GLUT8) is expressed at high levels in the bovine mammary gland. Because the classical insulin responsive glucose transporter GLUT4 is not detected in the mammary gland of dairy cows, GLUT8 is postulated to be an alternative insulin responsive transporter. In this study, bovine mammary epithelial cells (BMEC) were used to examine the effect of insulin on cell viability and glucose uptake, and to verify the possible role of GLUT8 in insulin-regulated glucose uptake. The BMEC were cultured in DMEM/F12 medium containing 10% FCS, and treated with different levels of insulin (0, 5, 50, and 500 ng/ml) for 48 h after 24 h starvation without FCS. Viability of the cells was determined by MTT method. Glucose uptake and mRNA expression were determined by enzymatic coloring glucose oxidase/peroxidase assay, and by SYBR green method of real-time PCR, respectively. The viability of the cells was enhanced with the increasing level of insulin ( $P < 0.05$ ), with highest value at 500 ng/ml. Compared with control, insulin (500 ng/ml) increased glucose uptake ( $P < 0.05$ ), while expression of GLUT8 gene was elevated in all insulin-treated groups ( $P < 0.05$ ). As predicted, expression of the GLUT1 gene, the predominant glucose transporter, was not affected by insulin ( $P > 0.05$ ). In addition, insulin-induced glucose uptake was totally suppressed by the protein synthesis inhibitor, cycloheximide ( $P < 0.05$ ). Pretreatment with LY294002, a specific inhibitor of PI3-K, for 30 min, significantly reduced the insulin-stimulated glucose uptake ( $P < 0.05$ ). In contrast, SB203580, an inhibitor of p-38 MAPK, did not influence the insulin-induced glucose uptake ( $P > 0.05$ ). These results indicate that GLUT8 is an insulin responsive transporter in BMEC. Insulin may stimulate glucose uptake primarily via regulating cell viability and thus expression of GLUT8 in BMEC. This effect may be mediated through PI3-K- linked signaling pathways.

**Key Words:** insulin, glucose transporter 8, bovine mammary epithelial cell

**W197 Pathogen-specific and dose-dependent response of the bovine mammary gland to lipopolysaccharide from *E. coli* and lipoteichoic acid from *S. aureus*.** R. M. Bruckmaier\*, E. T. Arnold, and O. Wellnitz, *Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstr. 109a, 3001 Bern, Switzerland*.

Lipoteichoic acid (LTA) and lipopolysaccharide (LPS) are proinflammatory cell wall components expressed by gram-positive or gram-negative

bacteria, resp. This study was performed to investigate if intramammary challenge with LTA from *S. aureus* or LPS from *E. coli* elicits a different immune response. Five cows per group with somatic cell counts (SCC) below  $100 \times 10^3/\text{ml}$  were intramammarily challenged in one quarter with 10 or 20  $\mu\text{g}$  of LTA or 0.2, 1, or 10  $\mu\text{g}$  LPS in 10 mL saline solution (0.9%). At 0, 6, and 12 h after challenge biopsy samples of the mammary gland or milk cells from 1 L of milk were taken for mRNA expression measurements of immune factors by quantitative RT-PCR. Additionally, from cows that received no biopsy small milk samples (~5mL) were taken hourly. Differences between treatments and time points were considered as significant if  $P \leq 0.05$ . SCC increased in all treatments within 4 h. The increase of SCC, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), and lactate dehydrogenase (LDH) in milk was dose dependent. TNF $\alpha$  concentration in milk did not increase in LTA-treated quarters. mRNA expression of TNF $\alpha$ , interleukin (IL)-8 and IL-1 $\beta$  increased dose dependently in mammary tissue and in milk cells. This effect was more pronounced in milk cells compared with the tissue within the same dosage. The results show that LPS induced a stronger response of the measured factors than LTA in dosages with the induction of a similar SCC response. A low dosage of LTA induced a SCC increase but hardly any response of the measured factors. In addition, milk cells showed a stronger immune response to the same concentration of LPS than the mammary tissue. This reflects the situation during an infection of the mammary gland where the first reaction of the immune system is realized by the somatic cells which are already present in the milk before challenge. Then the bacteria or endotoxins must exceed a certain level to induce an immune response of milk cells as well as of mammary tissue. In conclusion, the immune response of the mammary gland is dependent on toxin type and dosage.

**Key Words:** mastitis, lipoteichoic acid, lipopolysaccharide

**W198 Greater milk yield is related to increased DNA and RNA content but not to mRNA abundance of selected genes in sow mammary tissue.** C. Farmer<sup>1</sup>, M. F. Palin<sup>1</sup>, J. F. Trott<sup>2</sup>, and R. C. Hovey<sup>2</sup>, <sup>1</sup>*Agriculture and Agri-Food Canada, Dairy and Swine R & D Centre, Sherbrooke, QC, Canada*, <sup>2</sup>*Dept. of Animal Science, University of California, Davis*.

The relationship between greater sow milk yield and mammary development, expression of selected genes in mammary tissue and serum hormone concentrations in lactating sows was studied. Crossbred sows were separated into 2 groups according to weight gains of their piglets up to d 21 of lactation. Groups were: 1) lower milk yield (LOW, n = 14) or 2) higher milk yield (HI, n = 14), representing lactation weight gains of 4.47 and 5.24 kg/pig, respectively. Jugular blood samples were obtained from all sows on d 3 (for prolactin determination) and 23 (for measures of prolactin, leptin, insulin, glucose and free fatty acids) of lactation. Milk samples were collected on d 3 and 22 of lactation for compositional and leptin analyses. At weaning (d 23), sows were killed and their mammary glands collected, dissected and composition determined. Mammary parenchymal tissue was analyzed for the mRNA abundance of selected genes, namely, porcine prolactin (pPRL), pPRL receptor (all isoforms and the long isoform alone), STAT5A, STAT5B, whey acidic protein and leptin. On d 3 of lactation, jugular concentrations of prolactin tended to be greater ( $P = 0.1$ ) while dry matter and leptin in milk were less ( $P < 0.05$ ) in HI than LOW sows. There was a tendency for HI sows to have more parenchymal tissue per teat ( $P < 0.1$ ) than LOW sows. Parenchymal tissue contained less fat ( $P < 0.05$ ) and there was more DNA and RNA per teat in HI than LO sows ( $P < 0.05$ ). On the other hand, the expression of selected genes within mammary

tissue was unaffected ( $P > 0.1$ ) by treatment. Sow milk yield therefore directly reflects mammary gland composition in late lactation.

**Key Words:** genes, milk yield, sow

**W199 5'-untranslated region haplotypes of beta-2-microglobulin exon IV in Chinese Holstein dairy cows and its association with IgG1 concentration and mass in milk.** C. Zhang<sup>1,2</sup>, G. Liu<sup>1</sup>, J. Wang<sup>1</sup>, D. Bu<sup>1</sup>, L. Zhou<sup>1</sup>, S. Zhao<sup>1</sup>, and Y. Yang<sup>1</sup>, <sup>1</sup>*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China*, <sup>2</sup>*College of Animal Science and Technology, Yangzhou University, Yangzhou, China*.

Production and transfer of IgG into milk is an important area of study because of its biological functions including opsonization, complement fixation, prevention of adhesion of pathogenic microbes to endothelial lining, inhibition of bacterial metabolism, agglutination of bacteria, and neutralization of toxins and viruses. In the mammary gland, IgG is transferred selectively from serum into milk by a neonatal Fc receptor (FcRn)-mediated mechanism. Beta-2-microglobulin ( $\beta 2\text{MG}$ ) is an integral component of the FcRn heterodimer, which has prominent roles in IgG transfer across mammary epithelial cells. This study examined mutations in the 5'-untranslated region (5'UTR) of  $\beta 2\text{MG}$  exon IV and its association with variation of IgG1 concentration and mass in milk. One hundred and eighty-nine Holstein dairy cows in lactation were used to determine the genetic diversity of 5'UTR. Two single nucleotide polymorphisms (SNPs) and insertion/deletion (indel) of 2 base pairs were identified by sequencing the 5'UTR of  $\beta 2\text{MG}$  exon IV and were assigned into 4 haplotypes. These haplotypes were evaluated with respect to IgG1 concentration and mass in the milk. These results demonstrated a significant association between  $\beta 2\text{MG}$  genotypes and concentration and mass of the milk IgG1 ( $P < 0.05$ ). Dairy cows homozygous with a double base-pair deletion (H3H3) had similar serum IgG1 concentration with other genotypes, but significantly lower milk IgG1 concentration and mass ( $P < 0.05$ ). These results suggest that  $\beta 2\text{MG}$  genotypes might serve as a marker of IgG1 production and distribution.

**Key Words:** beta-2-microglobulin gene, immunoglobulin G1, path analysis

**W200 How does increased milking frequency stimulate milk production?** M. Dehghan-Banadaky\*, M. Eslamizad, K. Rezaayadi, H. Kohram, and R. Heydari, *University of Tehran, Karaj, Tehran, Iran*.

We tested the hypothesis that frequent milking stimulates milk production through increased prolactin secretion. Multiparous (n = 105) and primiparous (n = 15) Holstein cows were used in a completely randomized design and assigned at calving to 1 of 3 treatments as follows: 1) 6 times a day milking for the entire lactation (6X); 2) 6 times a day milking for 90 DIM, then switched to 3 times subsequently (6X-3X); and 3) 3 times a day milking for the entire lactation (3X). Milk production was recorded every other day for the first 60 DIM and subsequently on 2 consecutive days a week. Blood samples were taken from each cow on 15, 30, 60, 90, 120, 150, 210, and 270 DIM. Plasma prolactin concentrations were determined using a double antibody radioimmunoassay procedure. Data were statistically analyzed using the repeated measures option in Proc Mixed of SAS with cow as a random effect. Milk and fat corrected milk (FCM 3.5%) yield were greater in 6x and 6x-3x cows than in 3x treatments (41.03, 42.3; 40.11, 40.60 VS 37.97, 38.40 kg/d, respectively). Plasma concentrations of prolactin were  $57.73 \pm 1.32$ ,  $59.62 \pm 1.64$ , and  $58.87 \pm 1.73$  ng/ml in 6x, 6x-3x, and 3x cows, respectively. Increased milking frequency did not alter the concentration of plasma prolactin at any sampling time ( $P = 0.53$ ). In

conclusion, results of the present study did not support the hypothesis that increased milking frequency stimulates milk production through increased prolactin.

**Key Words:** milking frequency, prolactin, Holstein cow

**W201 Impact of duration of milk storage in the mammary gland on milk composition throughout milking.** M. Dutreuil<sup>1,2</sup>, C. Cébo<sup>3</sup>, J. Guinard-Flament<sup>2,1</sup>, and C. Hurtaud<sup>\*1,2</sup>, <sup>1</sup>*INRA UMR1080 Production du lait, Saint-Gilles, France*, <sup>2</sup>*Agrocampus Ouest UMR1080 Production du lait, Rennes, France*, <sup>3</sup>*Unité GABI, Jouy-en-Josas, France*.

Our objective was to study the effect of duration of milk storage in the mammary gland on milk composition throughout milking. Three durations of milking storage were studied on 6 dairy cows averaging  $118 \pm 22$  DIM: 13-, 20-, and 24-h. The trial was carried out according a double Latin square 3'3 with 14 d periods. Yield and composition were measured on milk samples collected every min during morning milking on d 10 of each period. Statistical analysis was carried out using

the Proc Mixed procedure in SAS software with repeated statement. Milk yield, lactose content, milk protein yield and content remained unchanged between treatments. Milk fat content and lipolysis did not vary according to the duration of milk storage. In contrast, there is a significant interaction between duration of milk storage and kinetics of milk ejection for 2 parameters: fat yield and specific MFG area. Milk fat yield increased during the whole milking for the 13-h milk storage whereas it started to decrease at the end of milking for 20- and 24-h milk storages. The specific MFG area decreased less during milking for the 24-h milk storage. The lipolysis, measured by method of copper soap, decreased during milking whereas the milk fat content and milk fat globule diameter increased (respectively from 1.45 to 7.55% and from 3.1 to 4.1  $\mu\text{m}$ ). Variations in the profile of milk fatty acids were also noticed as an increase in desaturation activity of the mammary gland (increase of C14:1/C14:0 ratio) during milking. In conclusion, there is a great change in milk composition during milking. But duration of milk storage in the mammary gland induced no important change on the kinetics of milk secretion.

**Key Words:** milk composition, milk fat globule, milking