

# Lactation Biology I

**125 Serotonin (5-HT) regulates calcium mobilization at the onset of lactation in rats.** J. LaPorta, T. L. Peters, K. E. Merriman, and L. L. Hernandez,\* *University of Wisconsin, Madison.*

Serotonin is a known homeostatic regulator of lactation and was recently demonstrated to be a regulator of bone turnover. Circulating calcium ( $\text{Ca}^{2+}$ ) is known to decrease at the onset of lactation, and often results in milk fever in dairy cattle. Serotonin is synthesized in a 2-step reaction from the amino acid L-tryptophan (L-TRP). The rate-limiting step is catalyzed by tryptophan hydroxylase (TPH1) isoform to form 5-hydroxytryptophan (5-HTP). To explore 5-HT's role on  $\text{Ca}^{2+}$  homeostasis in the transition period (10 d pre and postpartum) rats were fed 3 diets ( $n = 15$  per treatment): control (CON), 5-HTP (0.2% total diet) and L-TRP (1.35% total diet) to increase endogenous 5-HT production. Milk samples were collected on d 1, 5 and 9 of lactation to measure  $\text{Ca}^{2+}$  concentrations. Serum and plasma samples were obtained on d 20 of gestation and d 9 of lactation to measure circulating 5-HT,  $\text{Ca}^{2+}$  and parathyroid hormone-related protein (PTHrP) levels. Total mRNA was isolated from mammary gland tissue from d 9 lactating animals and analyzed for PTHrP, TPH1, plasma membrane  $\text{Ca}^{2+}$  ATPases 1 and 2 (PMCA1, 2), sodium- $\text{Ca}^{2+}$  exchanger 1 (NCX1), secretory  $\text{Ca}^{2+}$  ATPase 1 and 2 (SPCA1, 2), and sarco(endo)plasmic reticulum  $\text{Ca}^{2+}$  ATPase 2 (SERCA2). The 5-HTP and L-TRP treatments effectively increased serum 5-HT over time ( $P < 0.001$ ), with a greater increase seen in the 5-HTP cohort. Plasma PTHrP was significantly increased ( $P < 0.05$ ) on d 9 of lactation in the 5-HTP cohort. There was a significant increase of milk  $\text{Ca}^{2+}$  in the 5-HTP and L-TRP cohorts ( $P < 0.05$ ), and decreased serum  $\text{Ca}^{2+}$  on d 9 lactation in the L-TRP group ( $P < 0.05$ ). The mRNAs for PTHrP, TPH1, NCX1, PMCA2, SPCA2, and SERCA2 were increased in the mammary glands of the 5-HTP cohort ( $P < 0.05$ ) and in the L-TRP cohort, except for PMCA2 and PTHrP ( $P < 0.05$ ). The SPCA1 mRNA was decreased in both the 5-HTP and L-TRP cohorts ( $P < 0.05$ ). These results suggest that feeding 5-HTP increases PTHrP induction in the plasma and mammary glands of transition rats, and increases  $\text{Ca}^{2+}$  transport within the mammary gland. It is possible that the L-TRP cohort could be working through a different mechanism to increase  $\text{Ca}^{2+}$  transport.

**Key Words:** 5-hydroxytryptophan (5-HT),  $\text{Ca}^{2+}$ , parathyroid hormone-related protein (PTHrP)

**126 Genes and functions associated with photoperiodic effects on the mammary gland.** T. B. McFadden\*<sup>1</sup> and E. H. Wall<sup>2</sup>, <sup>1</sup>*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada,* <sup>2</sup>*Department of Medicine, University of Vermont, Burlington.*

Pubertal heifers exposed to short day photoperiod during induction of lactation produced more milk in the ensuing lactation than heifers exposed to long day photoperiod, but the mechanisms behind this effect are unknown. To gain insight into potential mechanisms, we used microarray analysis to identify genes differentially expressed in mammary gland of heifers hormonally induced into lactation while exposed to different photoperiods. Holstein heifers ( $n = 6$ /treatment; 14 mo old) were assigned to long day (LD; 16 h light:8 h dark), or short day (SD; 8 h light:16 h dark) photoperiod and fed melengesterol acetate for 14 d to synchronize estrous. Heifers were then treated with estrogen and progesterone (E + P; 0.1 and 0.25 mg/kg per d) for 7 d to induce lactation. Mammary tissue was obtained by biopsy at 0, 5, and 10 d relative

to initial E+P injections, and Affymetrix GeneChip Bovine Arrays were used to measure gene expression. Ingenuity Pathway Analysis was used to determine functions enriched by differentially expressed genes. Mammary expression of 187 genes was influenced by photoperiod ( $P < 0.05$ ). Enriched functions included cellular assembly and organization, cell morphology, and molecular transport. Those functions are consistent with differences in mammary gland differentiation and activity, which could support the higher milk yield of heifers exposed to SD. Hormonal induction of lactation elicited differential expression over time of 4540 genes ( $P < 0.05$ ), assigned to functional categories including cell cycle, cell death, and protein synthesis, which correspond with the marked development and activation of the mammary gland in preparation for lactation. There was a significant treatment by time interaction such that the temporal pattern of expression of 598 genes differed between LD and SD heifers ( $P < 0.05$ ). Similar to the main effects, enriched functions included cellular development, organ morphology, and lipid metabolism. We conclude that exposure to SD during hormonal induction of lactation markedly influences gene expression in the mammary gland. The genes and functions identified represent potential mechanisms by which photoperiod alters mammary function.

**Key Words:** gene expression, mammary gland, photoperiod

**127 Effect of ovariectomy on milk yield and mammary gland activity in lactating cow.** L. Yart\*<sup>1,2</sup>, F. Dessauge<sup>1,2</sup>, L. Finot<sup>1,2</sup>, S. Wiart<sup>1,2</sup>, A. Mottin<sup>1,2</sup>, A. Eveno<sup>1,2</sup>, P. G. Marnet<sup>2,1</sup>, and V. Lollivier<sup>2,1</sup>, <sup>1</sup>*INRA, UMR1348 Pegase, Saint-Gilles, France,* <sup>2</sup>*Agrocampus Ouest, UMR1348 Pegase, Rennes, France.*

Milk yield (MY) in dairy cows is highly dependent on both mammary tissue remodeling and mammary epithelial cell activity during lactation. Previous studies on lactating cows suggested that ovarian steroids (estradiol and progesterone) have a negative effect on MY after the peak of lactation. However, little is known about the effects of ovarian steroids on mammary tissue. The aims of this study were to investigate the effect of ovariectomy on MY and on mammary gland remodeling and molecular activity in lactating cows. Fourteen multiparous Holstein cows were either ovariectomized (Ovx,  $n = 7$ ) or sham-operated (Sham,  $n = 7$ ) around 60 d in milk. Cows were milked twice daily during 12 mo; MY was recorded daily throughout the study and milk samples were harvested weekly to follow the milk composition. Mammary tissue samples were collected at 1.5 mo in milk (2 weeks before ovariectomy) and at 4, 8 and 12 mo of lactation to perform molecular analysis on the mammary tissue. Ovariectomy at the time of lactation peak improved MY in the end of lactation (+ 17.1% on mo 8 ( $P < 0.1$ ) and + 60.8% on mo 12 of lactation ( $P < 0.1$ ) for Ovx cows in comparison with Sham cows) without significantly modifying milk composition. Ovariectomy also reduced somatic cell release in milk as of mo 8 of lactation. Moreover, zymography performed on milk samples revealed that mammary tissue remodeling (MMP2 activity) was reduced in Ovx cows. Molecular analysis performed on mammary tissue samples showed that mammary cell apoptosis (Bax transcript level) was reduced by 22.2% ( $P < 0.05$ ) in the Ovx group in an advanced stage of lactation. The Western blot quantification of lactoferrin protein showed that lactoferrin increased over time in both Ovx and Sham cows, but it was reduced by ovariectomy on mo 8 (- 27.4%,  $P < 0.1$ ) and 12 (- 53.3%,  $P < 0.1$ ) of lactation. Taken together these suggest that ovariectomy could delay mammary gland involution.

**Key Words:** ovariectomy, mammary gland, involution

**128 Effect of cooling during the dry period on neutrophil gene expression after *Streptococcus uberis* infection.** I. M. Thompson,\* S. Tao, K. C. Jeong, W. W. Thatcher, and G. E. Dahl, *University of Florida*.

Heat stress in the dry period affects immune status of dairy cows in lactation. We hypothesized that cooling during the dry period improves immune response to postpartum intramammary infection (IMI) to environmental pathogens such as *Streptococcus uberis*. Cows were dried off 46 d before expected calving and assigned to cooling (CL, n = 15) or heat stress (HT, n = 15). CL cows were housed with sprinklers, fans and shade, whereas the HT group had only shade. All cows were cooled postpartum. Rectal temperature (RT) and respiration rate (RR) were recorded during the dry period. From -46 to 42 d relative to calving, DMI, milk yield and composition were recorded daily, and both BW and BCS weekly. *S. uberis* IMI was induced at 5 d postpartum in a subset of cows (CL, n = 5; HT, n = 5). Blood was collected at 0, 12, 18, 24 and 36 h after IMI. Hematology was performed, and neutrophils isolated for RNA extraction. Neutrophil genes (*TLR2*, *IL1-β*, *IL6*, *IL8*, *IL10*, and *TNFα*) were assessed by real time RT-PCR. Relative to HT, CL cows had lower RT and RR ( $P < 0.01$ ). CL cows also consumed more feed ( $P < 0.01$ ) prepartum but not postpartum, gained more ( $P < 0.01$ ) BW prepartum but lost more ( $P = 0.05$ ) BW in lactation, had higher ( $P = 0.01$ ) BCS score prepartum and a lower ( $P < 0.10$ ) BCS postpartum. During 15 wks of lactation, CL produced more milk ( $38.8 > 33.6$  kg/d;  $P < 0.07$ ) but did not affect milk composition. CL cows had greater ( $P = 0.05$ ) white blood cell count and more ( $P = 0.09$ ) neutrophils than HT during IMI. From 0 to 36 h post IMI, *TNFα* mRNA expression decreased ( $P = 0.02$ ) while *IL6* ( $P = 0.02$ ) and *IL8* ( $P < 0.01$ ) mRNA expression increased in both treatments. Additionally, CL cows had lower ( $P = 0.04$ ) *IL10* mRNA expression at 18 h post IMI. *TLR2* mRNA expression decreased ( $P = 0.05$ ) over time in both treatments. However, CL cows had greater ( $P = 0.02$ ) overall *TLR2* mRNA expression than HT. No differences were detected for mRNA expression of *IL1-β*, *IL6*, *IL8*, and *TNFα*. Cooling cows during the dry period alters immune function and neutrophil response to IMI.

**Key Words:** heat stress, neutrophils gene expression, intramammary infection

**129 Short-term increases in milking frequency and a higher plane of nutrition did not increase total milk production in pasture-based dairy cows during an extended lactation.** A. G. Rius,\* C. V. C. Phyn, J. K. Kay, and J. R. Roche, *DairyNZ, Hamilton, New Zealand*.

Extended lactations (EL) of > 600 DIM could benefit seasonal, pasture-based dairy systems by improving animal welfare and reducing breeding-related costs; however, lower milk yields on an annualized basis limits farmer adoption. This study determined if temporary increases in milking frequency (MF) and nutrition increased milk production during an EL. Non-pregnant, multiparous Holstein-Friesian cows (333 DIM, n = 120) were randomly allocated to one of 4 treatments (n = 30 cows) for 68 d in a 2 × 2 factorial arrangement which included 2 MF and 2 diets. Treatments were cows i) milked twice daily (2×) and fed pasture only (PAS), ii) milked 2× and fed pasture plus 6 kg DM/d of concentrate (CON), iii) milked thrice daily (3×) and fed PAS, and iv) milked 3× and fed CON. Cows were offered a generous pasture allowance (23 kg DM/d) throughout and milked 2× post-treatment. Statistical analyses were conducted on milk production and body weight (BW) during treatments and an 84-d carry-over period. There were no interactions between MF and diet for each trait in either period and treatments did not affect

lactation length (DIM = 570). During treatment, cows milked 3× had increased yields of milk (13.3 vs. 12.0 kg/d;  $P < 0.003$ ) and lactose (0.602 vs. 0.565 kg/d;  $P < 0.05$ ), but not energy-corrected milk (14.7 vs. 14.1 kg/d;  $P > 0.18$ ), relative to 2×. Cows fed CON increased yields of milk (13.1 vs. 12.1 kg/d;  $P < 0.01$ ), protein (0.545 vs. 0.503 kg/d;  $P < 0.02$ ), and lactose (0.612 vs. 0.554 kg/d;  $P < 0.002$ ), and tended to increase energy-corrected milk (14.8 vs. 14.0 kg/d;  $P = 0.08$ ), relative to PAS. Diet and MF did not affect production during the carry-over period or cumulative production from d 1 of treatment to dry-off. Cows fed CON gained more BW (544 vs. 523 kg;  $P < 0.001$ ) and the difference remained for 5 weeks post-treatment. In conclusion, short-term 3× milking and CON increased milk production but these responses did not carry-over to improve total EL yields. Imposing treatments longer than 68 d could increase subsequent production responses but this remains to be tested.

**Key Words:** extended lactation, dairy cows

**130 Transcriptome analysis of blood in heat-stressed dairy goats.** A. A. K. Salama\*<sup>1</sup>, S. Hamzaoui<sup>1</sup>, B. Badaoui<sup>2</sup>, A. Zidi<sup>3</sup>, and G. Caja<sup>1</sup>, <sup>1</sup>Grup de Recerca en Remugants (G2R), Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, <sup>2</sup>Integrative Biology Group, Parco Tecnologico Padano-CERSA, Lodi, Italy, <sup>3</sup>Centre de Recerca en Agrigenomica (CRAG), Bellaterra, Barcelona, Spain.

With the aim of studying the effects of environmental heat stress on global gene expression, 8 Murciano-Granadina dairy goats (43.3 ± 1.6 kg BW; 81 ± 3 DIM), kept in metabolic cages and allocated in 2 balanced groups, were randomly assigned to 2 climatic treatments according to a crossover design (35-d periods). Treatments were (temperature, °C; humidity, %; THI, Thorn heat index): 1) thermal neutral (TN, 15 to 20°C and 35 to 45%; THI = 59 to 64), and 2) heat stress (HS, 12 h/d at 37°C and 45%, and 12 h/d at 30°C and 45%, THI = 86 and 77, respectively). Feed intake and milk yield were recorded daily. Blood samples were collected at d 35 of the second period for microarray analysis (n = 3/ treatment). Blood RNA was extracted immediately after collection using RiboPure-Blood Kit (Ambion, Life Technologies, Madrid, Spain). The extracted RNA had an integrity number = 8.6 ± 0.2. Affymetrix GeneChip Bovine Genome designed to monitor expression of approximately 23,000 transcripts was used. The signal intensity of globin genes was low and did not affect the detection of gene expression. The arrays were normalized using the robust multi-array average method. After filtration and normalization, 4,259 genes were included in the analysis. We used the Ingenuity Pathways Analysis (IPA) software to examine biological changes related to heat stress. Feed intake (1.75 vs. 2.47 kg DM/d) and milk yield (1.53 vs. 1.68 L/d) were lower ( $P < 0.01$ ) in HS than in TN goats. We identified 39 and 74 genes whose expression was up- and downregulated, respectively, by HS ( $P < 0.05$ ). These genes were mainly related to biological processes and, to a lower extent, to molecular functions and cellular components. Moreover, IPA analysis detected important pathways related to cell proliferation and death, free radical scavenging, inflammatory response, lipid metabolism, and glycolysis/gluconeogenesis. Transcription regulators affected by HS were: SATB1 (global chromatin organizer) and PPARD (might be related to insulin resistance). In conclusion, chronic HS produced a stress response that was detectable in blood. The HS elicited changes in gene expression related to transcriptional regulation and metabolic processes.

**Key Words:** heat stress, gene expression, microarray

**131 Effects of high feeding level on caprine mammary gland development and milk yield potential.** J. M. Aubry<sup>1,2</sup>, L. Finot<sup>1,2</sup>, L. Yart<sup>1,2</sup>, S. Wiart<sup>1,2</sup>, E. Siroux<sup>1</sup>, M. Chorho<sup>1</sup>, J. Lassalas<sup>1</sup>, and F. Des-sauge<sup>\*1,2</sup>, <sup>1</sup>INRA, UMR1348 Pegase, Saint-Gilles, France, <sup>2</sup>Agrocampus Ouest, UMR1348 Pegase, Rennes, France.

The correlation between growth rate during rearing and the subsequent milk yield potential has been extensively studied in dairy cattle and sheep. Many studies highlighted that high feeding level before puberty negatively affected mammary development, but such results are scarce in dairy goats. Present study aimed then to investigate the effect of high feeding level from rearing to kidding on caprine mammary gland development and milk yield potential. A total of 52 Alpine dairy goats were submitted to 2 feeding levels (n = 26 per treatment). From weaning (17 kg, 60 d of age) to kidding (50 kg, 12 mo of age), they received either a controlled standard diet according to commercial recommendations (BD, 92 UFL - 17 MAT) or an ad libitum diet (HD, 85 UFL - 17 MAT). Goats weighed twice monthly and were milked twice daily. Milk yield (MY) and composition were recorded twice a week. At mid-gestation, 6 goats of each group were slaughtered and mammary glands were

collected. Mammary glands were weighed and dissected for molecular analysis. DNA mammary concentration analysis was measured and used to estimate the total number of mammary cells. Expression of proteins involved in mammogenesis was evaluated on mammary tissue by real-time qPCR, Western Blotting and immunohistochemical staining (ER  $\alpha$ , PCNA, MMPs). Before weaning, average daily gain (ADG) were similar between the 2 groups of animals (230 g/d). From weaning to 4 mo of age and from 4 mo of age to conception, HD goats grew faster than BD goats: 180 and 154 g/d vs. 128 and 127 g/d, respectively. At mid-gestation, HD goats had a well developed mammary gland. At slaughter, mammary glands from HD goats already presented a well organized mammary ductal network with the presence of milk. During lactation one, they had a higher milk yield than BD goats (2.92 kg/d vs. 2.73 kg/d on average,  $P < 0.05$ ) during first 2 mo of lactation. Reproduction performance was not affected. In conclusion, high feeding level from weaning to first kidding does not affect mammogenesis and milk yield potential in dairy goats.

**Key Words:** mammary gland, feeding level, goats