76 Effects of milking frequency on integrin signaling in mammary glands of dairy cows. R. Murney*[1], K. Stelwagen[2], T. T. Wheeler[1], J. K. Margerison[3], and K. Singh[1], *[1]AgReserach Limited, Ruakura Research Centre, Hamilton, New Zealand, [2]SciLactis Limited, Waikato Innovation Park, Hamilton, New Zealand, [3]Massey University, Palmerston North, New Zealand.*

In dairy cows, short-term changes of milking frequency (MF) in early lactation can have an immediate and a long-term effect on milk yield. The effect is controlled locally within mammary glands and is thought to be a function of secretory mammary epithelial cell (MEC) number and/or activity. Attachment to the extracellular matrix (ECM) via β_1-integrin is required for both MEC survival and function. Focal adhesion kinase (FAK) mediates the intracellular signaling of integrins. In this study we investigated if ECM/β_1-integrin signaling in MEC is affected by changes in MF. Ten multiparous cows (5 ± 2 DIM) were unilaterally milked for 14 d; udder halves were either milked once a day (1×) or 4 times a day (4×). On d 14, biopsy samples were collected from both rear quarters and mRNA and protein were analyzed by real time RT-PCR and Western blot, respectively. The cows were then milked twice daily (2×) for the remainder of lactation. By the end of the treatment period the 4×-milked udder halves were producing 2-fold ($P < 0.001$) more milk than the 1×-milked udder halves and continued to produce 15% ($P < 0.01$) more milk than 1×-udder halves once returned to 2× milking. Relative secretory activity was estimated by determining mRNA levels of the major milk proteins, αS_1-casein, β-casein and α-lactalbumin, which were increased in the 4×-samples, 8-, 9-, and 12-fold, respectively ($P < 0.01$). Signal transducer and activator of transcription 5 (STAT5) phosphorylation was dramatically ($P < 0.001$) higher in the 4×-tissues, while protein levels for β_1-integrin and FAK were 5- ($P < 0.001$) and 40-fold ($P < 0.001$) higher in 4×-tissues, respectively. Furthermore, STAT5-phosphorylation correlated strongly with the protein abundance of both β_1-integrin ($r = 0.78$, $P < 0.001$) and FAK ($r = 0.86$, $P < 0.001$). The data indicate that β_1-integrin and FAK proteins may be involved in the regulation of milk production in differentially milked bovine mammary glands.

Key Words: milking frequency, cell signaling, β_1-integrin

77 Prolactin-inhibitor cabergoline enhanced the mammary remodeling during drying-off in dairy cows. M. Boutinaud*[1], N. Isaka[4], A. Deflandre[4], E. Gandemer[1,2], P.-G. Marnet[2,3], F. Dessauge[1,2], and V. Lollivier[2,3], *[1]INRA, UMR 1348 PEGASE, Saint Gilles, France, [2]AGROCAMPUS UMR 1348 PEGASE, Rennes, France, [3]Université Européenne de Bretagne, Rennes, France, [4]CEVA Santé Animale, Libourne, France.*

In ruminants, the early phase of drying-off is a period of intense mammary gland remodeling that has great consequences on the next lactation and that is marked by the cessation of prolactin (PRL) release. To assess the effect of PRL inhibition on mammary remodeling, 14 Holstein dairy cows were injected with a single i.m administration of 5.6 mg cabergoline (n = 7) or placebo (n = 7) just after the last milking before drying-off. Mammary biopsy samples were collected one week before drying-off (D-6), at D1 and D8 and used for zymography analyses. Mammary secretion samples (290 mL) were collected using a teat-cannula once during lactation (D-6) and at D1, D2, D3, D4, D8 and D14 after the drying-off. The mammary secretion samples were used for SCC, Na^+, K^+ and BSA determinations and zymography analyses. Mammary epithelial cells (MEC) were purified from mammary secretions after centrifugation and immunocytochemical binding. Blood samples were collected before and after the morning mammary secretion collection for PRL determination. Cabergoline treatment decreased the blood PRL level from D1 to D8 compared with control treatment ($P < 0.001$). SCC was 2.4 fold higher in cabergoline treated cows than in control cows ($P < 0.01$). In addition, cabergoline induced an increase in MEC count ($P = 0.04$) with a reduction of their viability on D3 and D4. No significant cabergoline effect was observed on Na^+ and BSA except a tendency for a higher K^+ content in the mammary secretions of cabergoline treated cows ($P = 0.06$ at D1), suggesting a small increase in the tight junction opening in the mammary gland. In mammary tissue, cabergoline increased the activity of MMP-2 (matrix metalloproteinases) after drying-off (1.4 fold, $P \leq 0.01$). In mammary secretion, cabergoline increased the activity of MMP9 (1.7 fold, $P < 0.05$). Cabergoline treatment was efficient to enhance the extracellular matrix mammary remodeling, the MEC exfoliation and the migration of somatic cells responsible for the mammary gland remodeling. The mammary gland remodeling induced by the lower plasmatic PRL concentration may only be in part explained by a higher tight junction opening.

Key Words: cow, drying-off, prolactin

78 Efficacy of cabergoline to reduce udder pressure and milk leakage after dry-off in dairy cows. S. Bertulat*[1], N. Isaka[2], A. Deflandre[2], A. Lopez[2], T. Hetreau[3], and W. Heuwieser[1], *[1]Clinic for Animal Reproduction, Freie Universität Berlin, Berlin, Germany, [2]CEVA Santé Animale, Libourne, France, [3]Centre d'élevage Lucien Biset, Poisy, France.*

A recent study demonstrated a relationship between high milk yield, high udder pressures (PRE) and elevated stress levels after dry-off and revealed an animal welfare concern regarding current dry-off strategies. Cabergoline inhibits prolactin release and is approved to treat false pregnancies in bitches. The objective of this study was to evaluate the efficacy of cabergoline to reduce PRE and milk leakage after dry-off. Two hundred sixty-three high-yielding (≥16 kg milk/d) dairy cows were enrolled 7 d before (d −7) and followed up until 14 d (d 14) after dry-off. Cows were milked twice daily until dry-off (d 0) and treated with a single i.m. injection of 5.6 mg cabergoline (CAB; n = 130) or placebo (PLA; n = 133) after last milking (controlled, randomized and blinded study design). PRE was measured 4 d before (i.e., before and after milking) and 1, 2, 3, 7, 10 and 14 d after dry-off using a hand-held dynamometer (Penefel DFT 14; Bertulat et al., 2012). Udder firmness was assessed manually using a 4-point firmness score (0 = flabby, 3 = very hard; Gleeson et al., 2007). Milk leakage was recorded on d −4, d 1, d 2, d 3 and d 7, respectively. Data were analyzed using Chi^2 or Fisher exact test to compare qualitative variables and two-sample *t*-test for quantitative variables. After dry-off PRE increased in both groups, but was lower ($P < 0.05$) on d 1 and d 2 in CAB (d 1 = 0.79 ± 0.35 kg; d 2 = 0.93 ± 0.45 kg) than PLA cows (d 1 = 1.16 ± 0.6 kg; d 2 = 1.07 ± 0.49 kg). PRE on d 1 exceeded values measured on d -−4 before milking in 53.2% of PLA, but only 26.2% of CAB cows ($P < 0.001$). Moreover, cows in PLA group were more likely ($P < 0.05$) to have an udder firmness score of 3 than CAB cows on d 1 (27.9% vs. 67.2%), d 2 (41.5% vs. 54.9%) and d 3 (27.1% vs. 42.1%), respectively. The percentage of cows with milk leakage during the 1st wk after dry-off was also lower in CAB (10.2%) compared to PLA (19.8%) cows (P = 0.03). Our data provide evidence that a single injection of cabergoline

reduced PRE and firmness after dry-off and decreased the prevalence of milk leakage. Further research is warranted to evaluate, if cabergoline is able to reduce stress and alleviate pain after dry-off.

Key Words: cabergoline, dry-off, udder pressure

79 The effects of continuous light on milk yield, milk composition, IGF-1 and prolactin in dairy cows. S. Ferneborg*[1], E. Ternman[1], A. A. K. Salama[2], G. Caja[2], and S. Agenäs[1], *[1]Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]Group of Ruminant Research (G2R), Universitat Autonoma de Barcelona, Bellaterra, Spain.*

Light improves the lactational performance (feed intake and milk yield) of dairy cows. When long (16h) and short (8h) photoperiods are compared the higher milk yield in the 16h photoperiod seems to be mediated by higher secretion of IGF-1 and prolactin (PRL). Continuous lighting, on the other hand, is not recommended since no positive effect is seen on milk yield. However, it is often used as an on-farm management tool to keep cow activity high in intensive production systems with automatic milking. In calves, decreased levels of PRL have been seen in 24h light compared with 16h light, but the effect of 24h light on IGF-1 is unknown. In this pilot study, the short-term effects of 2 different light treatments on milk yield, milk composition, IGF-1 and PRL secretion were examined. Five lactating Swedish Red dairy cows were daily subjected to 24–0 (continuous light) or 4–20 (light 0930 to 1330) light-dark treatments in a changeover design. Cows were adapted to the treatments for 60h before recording and sampling started. Milking was performed at 12h intervals, and milk samples were collected at each milking for milk composition analysis. Blood samples were taken through a permanent jugular cannula hourly over a 36h sampling period, frozen and analyzed for IGF-1 (IMMULITE immunoassay) and PRL (ELISA). Neither milk yield (26.8 ± 5.2 L/d) nor milk composition differed between treatments, but plasmatic IGF-1 concentrations were greater ($P < 0.05$) in the 4–20 vs. 24–0 cows (133 ± 2 vs. 124 ± 2 ng/mL). Moreover, IGF-1 levels were greater ($P < 0.05$) during the circadian night than during the circadian day in the 4–20 cows and tended to be greater ($P < 0.10$) in the 24–0 cows. The secretory pattern of PRL differed between treatments, with an increase in secretion 1 min after the start of milking for 4–20 vs. 24–0 cows (124 ± 13 vs. 78 ± 8 ng/mL; $P < 0.05$), while basal concentrations did not differ between treatments. In conclusion, short-term continuous light did not change basal PRL, but decreased IGF-1 levels in plasma without changing milk yield or milk composition. Further research will explore the effects of continuous light on milk production and welfare of dairy cows.

Key Words: photoperiod

80 Timing of first milking and colostrum feeding affect serotonin (5-HT) concentrations in cows and calves. J. J. Gross[2], J. Laporta[1], R. M. Bruckmaier[2], and L. L. Hernandez*[1], *[1]University of Wisconsin, Madison, [2]University of Bern, Bern, Switzerland.*

Hormonal signals differentially regulate the timing of parturition, as well lactogenesis and potentially colostrum formation in the mammary gland. Serotonin (5-HT) has been determined to be a homeostatic regulator of lactation. To this end, we performed an experiment in which we manipulated the timing of first milking to investigate the effects on 5-HT concentrations in the maternal and calf circulation, as well as colostrum composition. Twenty cows were randomly assigned to 2 groups: control (CON) milked for the first time 4 h post-calving, and a treatment (TRT) group that were milked for the first time approximately 1 d before calving, and were milked 4 h post-calving. Maternal blood samples were collected for 4 d pre-partum, 3 times daily, and one blood sample was taken 4 h after parturition. Calves were fed 2 L of colostrum from their respective dam that was collected at their first milking, either 1 d before parturition (TRT) or 4 h post-calving (CON) 4 h after birth. Calf blood samples were collected 4 h after birth (before colostrum feeding), 12 h after birth, and at 3 wk of age. Colostrum samples were collected from the entire udder. 5-HT was analyzed in serum and colostrum samples. Circulating 5-HT concentrations were significantly higher in CON cows when compared with TRT cows ($P < 0.0001$). Colostrum 5-HT concentrations were increased in TRT cows compared with the CON ($P = 0.0165$). Finally, calves born to TRT cows had increased circulating 5-HT concentrations compared with the CON on all dates evaluated, with the greatest increase being at 3 wk of age. These data suggest that 5-HT plays a role in regulating colostrum composition, and may be of importance to the calf. Further research should be conducted in an attempt to separate the roles of circulating 5-HT from that produced within the mammary gland.

Key Words: serotonin, lactation, colostrum

81 Essential amino acid deficiencies and imbalances regulate milk protein synthesis through mTOR signaling in lactating bovine mammary glands. J. Doelman*[1], R. V. Curtis[2], M. Carson[1], J. J. M. Kim[2], J. P. Cant[2], and J. A. Metcalf[1], *[1]Nutreco Canada Agresearch, Guelph, ON, Canada, [2]Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada.*

Part of the mechanism by which essential amino acids (EAA) stimulate milk protein synthesis may be through effects on mammalian target of rapamycin (mTOR) signaling in the mammary gland. To determine the effect of imbalance and deficiencies of EAA on mammary mTOR signaling, 6 early-lactation, rumen-fistulated dairy cows (102 ± 4 DIM) were abomasally infused for 5 d with either saline, EAA, EAA less methionine, EAA less phenylalanine, EAA less histidine, or EAA less tryptophan in a 6 × 6 Latin square design. The EAA infusion rates were based on the EAA content of 1000 g/d casein. Cows were fed a TMR with a forage to concentrate ratio of 65:35 to provide 100% of the NE_L requirement and 65% of the metabolizable protein requirement. Mammary tissue was collected by biopsy on the final day of each experimental period for quantification of signaling protein activity. Milk protein yield increased 22% during the EAA infusion, compared with saline ($P < 0.001$), while methionine, phenylalanine and histidine deficiencies decreased milk yield by 15.7, 23.6, and 22.6%, respectively, compared with EAA ($P < 0.001$). Additionally, milk protein concentration was 0.36, 0.37 and 0.25 percentage points lower during methionine, phenylalanine and histidine deficiencies, respectively ($P < 0.001$). Immunoblot analysis showed increased phosphorylation of ribosomal protein S6 kinase (S6K1) in response to methionine deficiency ($P < 0.04$), while the phosphorylation state of eukaryotic initiation factor 2B (eIF2B) was decreased in all treatments relative to saline ($P < 0.04$). These results indicate that milk protein synthesis is sensitive to the supply of methionine, histidine, and phenylalanine, specifically. S6K1 and eIF2B appear to be sensitive to the supply of total EAA, yet their activation during EAA deficiency did not stimulate milk protein yields.

Key Words: milk protein, essential AA, mTOR signaling

82 Effects of arginine concentration on the in vitro expression of casein and mTOR pathway related genes in mammary epithelial cells from dairy cattle. M. Z. Wang*[1,2], B. L. Xu[1], H. R. Wang[1], D. P. Bu[2], J. Q. Wang[2], and J. J. Loor[3], *[1]College of Animal Science and*

Technology, Yangzhou University, Yangzhou, Jiangsu, China, [2]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [3]Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana.

Arginine is a conditionally-essential amino acid that is taken up by bovine mammary gland in excess of its output in milk. In this study we evaluated the effects of arginine level on the expression of casein and signaling pathway-related genes in mammary epithelial cells. The treatments (applied for 24 h) were designed to be devoid of Arg 0X (control; 0.00 mg/L), resemble the profile of Arg in casein (Arg 1X; 278.00 mg/L), be deficient, Arg 0.25X (69.50 mg/L) and Arg 0.5X (139.00 mg/L), or be in excess of the amount in casein, Arg 2X (556.00 mg/L), Arg 4X (1,112 mg/L), and Arg 8X (2,224 mg/L). The expression of CSN1S, CSN3 and mTOR in the experimental groups was higher than those of the control group ($P < 0.05$). Except for Arg 0.25X and Arg 8X ($P > 0.05$), the expression of CSN1S2, CSN2 and JAK2 in other experimental groups was higher ($P < 0.05$) than those in the control group. Except for Arg 8X ($P > 0.05$), the expression of STAT5 in the other experimental groups was higher than those of the control group ($P < 0.05$). It was also observed that, except for Arg 0.5X, S6K expression was higher in other experimental groups than the control group ($P < 0.05$). In contrast, except for Arg 0.25X, the other experimental groups resulted in lower 4EBP1 expression than the control group ($P < 0.05$). Among groups, the expression of CSN1S1, CSN1S2, CSN2, CSN3, JAK2, STAT5, mTOR and S6K gene was highest with Arg 2X ($P < 0.05$); the reverse was true for 4EBP1 gene, with the lowest expression in this group ($P < 0.05$). Taken together, arginine appears to play an important role in the transcriptional regulation of casein genes and mTOR related genes in bovine mammary epithelial cells.

Key Words: arginine, casein expression, mammary epithelial cell

83 Enhancing mammary involution during early stages of the dry period by infusing mammary serum amyloid A3. A. Domenech[1], S. Parés*[1], A. Bach[1,2], and A. Arís[1], *[1]Department of Ruminant Production, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Torre Marimon, Caldes de Montbui, Barcelona, Spain, [2]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Barcelona, Spain.*

The aim of this study was to evaluate the potential of recombinant mammary serum amyloid A3 (M-SAA3) in the improvement of the dry period in dairy cows by stimulating the immune system and epithelial regeneration. At dryoff, immediately after the last milking, the 4 quarters of 9 cows were treated as follows: 1 quarter infused after drying with 1 mg of M-SAA3 (MS3), another quarter infused with 80 ng of LPS (LP) to reproduce the possible effect of LPS traces in purified recombinant fraction, and the other 2 contralateral quarters were infused with saline solution as a negative control (CTRL). Mammary secretions (MS) were obtained just before treatment and at 0800 h during the 3 consecutive days. MS was analyzed for somatic cell count (SCC), fat, protein, and the activity of involution promoters such as metalloproteinases (MMP). Also, in vitro experiments were performed to test the effect of M-SAA3 on primary mammary cells challenged with *S. aureus* and on bovine dendritic cells. Cytokine concentrations were evaluated by qPCR. Data were analyzed using a mixed-effects linear model in the in vivo experiment and by ANOVA in the in vitro study. The activity of MMP-9 during the 3 d of the in vivo experiment was greater ($P < 0.0001$) in MS3 than in the other treatments, and there was a numerical increase in SCC at d 2 compared with LP and CTRL. MS fat and protein were increased ($P < 0.01$ and $P < 0.1$, respectively) in the MS3 treatment compared with LP and CTRL. Results for qPCR of the in vitro experiment showed activation and maturation of the dendritic cells and an increase ($P < 0.05$) in the expression of IL-8 ($6 \times 10^{-3} \pm 0.3 \times 10^{-3}$ vs. $3.9 \times 10^{-4} \pm 3.8 \times 10^{-4}$) in primary mammary cells. Moreover, M-SAA3 decreased by 25% the infection of mammary cultures with *S. aureus*. In conclusion the M-SAA3 shows a clear potential to improve the early stages of the dry period, independently of LPS traces, not only stimulating the mammary gland involution and immune system but also reducing bacterial infection.

Key Words: dry period, immune, infection

84 Lack of circulating serotonin (5-HT) in TPH1-deficient mice down-regulates serum calcium and mammary gland gene expression of calcium transporters. J. Laporta*, K. E. Merriman, S. Weaver, C. Cronick, T. L. Peters, and L. L. Hernandez, *University of Wisconsin, Madison.*

Serotonin (5-HT) is a homeostatic regulator of lactation and is known to play a role in calcium homeostasis. 5-HT is synthesized in a 2-step reaction from L-tryptophan (L-TRP), with the rate-limiting step catalyzed by tryptophan hydroxylase (TPH1) to form 5-hydroxytryptophan (5-HTP), which is then further converted to 5-HT by aromatic amino acid decarboxylase. We recently demonstrated increasing circulating 5-HT up-regulates mammary gland expression of calcium transporters in rats. To demonstrate that 5-HT is responsible for regulating calcium transport in the mammary gland, we performed an experiment in lactating mice that are genetically deficient for TPH1 (−/−, n = 7) and compared them to wild-type mice (+/+, n = 7). On d 1 and 10 of lactation blood samples were collected to measure serum 5-HT and calcium concentrations. All animals were euthanized on d10 of lactation and mammary gland tissue was harvested. Total RNA was isolated from mammary gland tissue to measure the mRNA expression of TPH1, 5-HT transporter (SERT) and the following calcium transporters: plasma membrane Ca^{2+} ATPases 1 and 2 (PMCA1, 2), sodium-Ca^{2+} exchanger 1 (NCX1), secretory Ca^{2+} ATPase 1 and 2 (SPCA1, 2), and sarco(endo)plasmic reticulum Ca^{2+} ATPase 2 (SERCA2), by real-time RT-PCR. Total protein was also isolated from mammary gland tissue to measure 5-HT content. Serum 5-HT concentration were decreased in the −/− dams compared with +/+ dams on both d1 and 10 of lactation ($P < 0.036$) as was mammary gland 5-HT content on d10 of lactation ($P = 0.0039$). Serum calcium was decreased on both d1 and 10 of lactation in −/− dams compared with +/+ dams ($P < 0.023$). Mammary gland TPH1 and SERT mRNA expression was decreased in −/− dams compared with +/+ dams ($P < 0.011$). Mammary gland mRNA expression of NCX1, SERCA, SPCA1 and 2, PMCA1 and 2, were decreased in −/− dams compared with +/+ dams ($P < 0.038$). These results demonstrate that 5-HT is critical for regulation of circulating calcium concentrations and calcium transport within the mammary gland during lactation.

Key Words: serotonin, calcium, lactation

85 Importance of progesterone and prolactin profiles, and of parturition on the composition of colostrum obtained before and after parturition. J. J. Gross[1], E. C. Kessler[1], V. Bjerre-Harpoth[2], and R. M. Bruckmaier*[1], *[1]Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland, [2]Department of Animal Science, Aarhus University, Foulum, Denmark.*

Progesterone (P4) and prolactin (PRL) are key regulators that mediate the initiation of both parturition and onset of lactation including colostrum formation. The hypothesis was tested that colostrum formation is regulated by changes of P4 and PRL but independent of the actual time

of parturition. To achieve this goal, 23 multiparous cows were randomly assigned to 2 groups: control (PP, postpartum only, n = 11) milked for the first time 4 h post-calving, and a treatment (AP, ante- and postpartum, n = 12) group that was already milked approximately 1 d before calving and again at 4 h after parturition. Colostrum yield before and after parturition was recorded and proportional samples were analyzed for total IgG, fat, protein and lactose. Blood samples for the analyses of P4 and PRL were collected at 8 h intervals for 4 d pre-partum until calving, and another sample was taken at 4 h after parturition. Total IgG mass increased with increasing time span between the P4 drop and first milking ($P < 0.05$). Total IgG mass and milk yield tended to increase with decreasing time span between PRL peak and first milking ($P < 0.1$), and also with increasing time between parturition and first milking ($P < 0.1$). Protein concentration in colostrum decreased with increasing interval between drop of P4 and PRL peak ($P < 0.05$). Milk yield, fat, protein and lactose concentration did not differ between PP and AP at the first milking. However, total IgG mass was higher in AP than in PP ($P < 0.05$) which is obviously due to the low milk yield in AP. In AP cows, milk protein decreased between pre- and postpartum milking, while lactose concentration increased ($P < 0.05$). In colostrum obtained at 4 h postpartum, milk fat was increased in AP compared with PP, while protein, total IgG concentration and mass were decreased ($P < 0.05$). Milk yield and lactose were not different between AP and PP at 4 h postpartum. These data suggest that synthesis and secretion of constituents into first colostrum do not only depend on changes in PRL and P4.

Key Words: colostrum, composition, lactogenesis

86 Feeding entrainment of the mammary circadian rhythm in FVB mice. L. Ma*, Y. Ying, A. Clarke, P. Bartell, and K. J. Harvatine, *Penn State University, University Park.*

Food entrainment of adipose and liver circadian rhythms is well established, but little is known about the regulation of the circadian rhythm of the mammary gland. Food entrainment of circadian rhythm of milk fat synthesis was investigated in wild-type FVB mice. Treatments were ad libitum feed intake and 7 h feed restriction during the day (DR; 1100 to 1800 h) or night (NR; 2300 to 0600 h) from d 7 to 14 of lactation. Dam intake and body weight and litter gain were recorded 2×/d. On d 14 of lactation, dams were euthanized at 0600, 1200, 1800, or 2400 h (n = 6 per treatment per time point) and dam mammary tissue and pup stomach milk clots were collected. Data were analyzed using ANOVA with treatment, time, and the interaction of treatment and time as fixed effects and second by fitting to a cosine function with a 24 h period for rhythm analysis. In control mice, no overt rhythm was observed for feed intake, milk fat synthesis or mammary gene expression, which is expected as FVB mice commonly do not express a robust circadian rhythm. Dams from DR treatment consumed more than 70% of daily intake during the dark phase, while NR mice consumed more than 76% of daily intake during the light phase (treatment by time, $P < 0.01$). Litters from DR and NR gained the most weight during the light (65%) and dark (72%) phase, respectively (treatment by time $P < 0.01$). Day restriction entrained a rhythm in de novo milk FA synthesis and the peak (amplitude 1.57 and 4.52% FA for DR and NR, respectively) was advanced about 10 h for NR ($P < 0.01$). Inverted rhythms (shifted by 11.3 h) were also observed for preformed FA between DR and NR treatments ($P < 0.01$). The mammary expression of core clock genes (BMAL1, CRY1 & 2, and PER1 & 2), lipogenic regulators (SREBP1c and Spot 14), and milk fat synthetic enzymes (FASN and SCD1) showed a rhythm in the mice with restricted feeding and the phase was shifted by 5.5 to 12.3 h ($P < 0.01$). In conclusion, timing of feed intake entrains the circadian rhythm in the mammary gland and milk fat synthesis by entraining the expression of mammary clock genes as well as regulators and enzymes involved in milk fat synthesis.

Key Words: FVB mice, feed restriction, circadian rhythm

87 Proteomic profiling of bovine mammary gland response to milk removal or increased milking frequency indicates roles for prolactin and leptin signaling. M. G. H. Stevens*[1], E. H. Wall[2], P. A. Bentley[3], A. Ruiz-Sanchez[3], and T. B. McFadden[1,3], *[1]University of Missouri, Columbia, [2]University of Vermont, Burlington, [3]University of Alberta, Edmonton, AB, Canada.*

Milking dairy cattle 4-times (4X) instead of twice-daily (2X) during early lactation increases milk yield and persistency. Our objective was to identify physiological processes involved in the mammary response to milk removal or increased milking frequency. Six cows were assigned at parturition to unilateral frequent milking (2X milking of left udder half, 4X milking of right udder half) and biopsies were taken from both udder halves at 5 DIM. In the first study (n = 2) the effect of milk removal was investigated by taking biopsies immediately after milking only the 4X udder half. In the second study (n = 4) biopsies were obtained at 2.5h after milking both udder halves to quantify the sustained effect of 4X. Proteins were quantified by iTRAQ and proteomes of paired udder halves were compared. Proteins differentially expressed by ≥1.5 fold were used for Ingenuity Pathway Analysis (IPA). The response to milk removal was characterized by differential expression of 479 peptides, mapped to 38 proteins; 35 were eligible for IPA. The top ranked network comprised 17 proteins (Score = 42) and associated with post-translational modification, carbohydrate and lipid metabolism. A weak activation of prolactin signaling is predicted as an upstream regulator and associated with 6 of the differentially expressed proteins (activation score: 0.686; $P < 0.001$). The sustained response to 4X-milking was associated with differential expression of 1302 peptides, mapping to 97 proteins; 67 were used for IPA. The top network was associated with cellular assembly and organization, molecular transport and protein trafficking (Score = 46). Activation of leptin signaling is predicted as an upstream regulator and associated with 19 differentially expressed proteins (activation score: 2.200; $P = 0.003$). Data suggest that the response to milk removal involves proteins associated with mammary metabolism, and may be driven by prolactin signaling. The sustained response to 4X appears to modulate proteins involved in cellular organization and function, and may be regulated by leptin.

Key Words: proteomics, bovine mammary gland, lactation biology