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829 Effects of short- and long-chain fatty acids on expression of lipogenic genes in bovine mammary epithelial cells. A. A. Jacobs^{*1}, J. S. Liesman², M. J. VandeHaar², J. Dijkstra¹, A. M. van Vuuren¹, and J. van Baal¹, ¹Wageningen University, Wageningen, the Netherlands, ²Michigan State University, East Lansing.

Several long-chain polyunsaturated fatty acids can exert an inhibitory effect on the expression of lipogenic genes, including acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and stearoyl-CoA desaturase 1 (SCD1), in the bovine mammary gland. Nevertheless, the effect of the short-chain fatty acids acetate (Ac) and β -hydroxybutyrate (BHBA), which are the main precursors for de novo fatty acid synthesis, on expression of these lipogenic genes remains unclear. To examine these effects, we used a bovine mammary epithelial cell line (MAC-T) incubated in fatty acid-free media (CON) and added either 5 mM Ac, 5 mM BHBA or a combination of 5 mM Ac + 5 mM BHBA. Furthermore, MAC-T cells were treated with either 100 μ M palmitic acid (PA), 100 μ M stearic acid (SA), 100 μ M oleic acid (OA), 100 μ M trans-vaccenic acid (TVA), 100 μ M linoleic acid (LA) or 100 μ M α -linolenic acid (ALA), which were complexed with BSA. Cells were grown to confluence in DMEM-F12 with 10% serum and all treatments were performed in triplicate. Fatty acids were added to the cells in serum-free DMEM-F12 including the hormones insulin, prolactin, apo-transferrin, progesterone and hydrocortisone. After 12 h of incubation, total RNA was extracted from the cells and mRNA expression of ACC, FAS and SCD1 was analyzed by real-time PCR. In comparison with CON, expression of ACC was increased by Ac (+44%) and was reduced by LA (-48%) and ALA (-49%). Expression of SCD1 was increased by Ac (+61%) and was reduced by OA (-61%), LA (-84%) and ALA (-88%). The treatments did not significantly affect expression of FAS compared with CON. Our results demonstrate that Ac upregulates the expression of ACC and SCD1 in MAC-T cells, which indicates that Ac may increase de novo synthesis and desaturation of fatty acids in the bovine mammary gland.

Key words: mammary epithelial cells, short-chain fatty acids, lipogenic gene expression

830 Effect of timing of feed intake on circadian pattern of milk synthesis. L. W. Rottman^{*}, Y. Ying, and K. J. Harvatiné, *The Pennsylvania State University, University Park.*

The rate of feed intake varies over the day and is expected to create a daily rhythm of nutrient absorption. The objective of this study was to characterize the effect of timing of feed intake on the pattern of milk synthesis. Twenty Holstein cows were used in a crossover design with 21 d periods. Cows were milked every 6 h on d 14 to 21 of each period. Milk samples were collected at each milking on d 18 through 21, and 6 blood samples were collected on d 20 to 21. Treatments were feeding a TMR once daily (1x fed) or in 4 equal meals every 6 h (4x fed). All cows were fed ad libitum at 110% of daily DMI. Data were analyzed as a repeated measures design, and the model included the random effect of period, sequence, and cow nested within sequence, and the fixed effect of treatment, time, and the interaction of treatment and time. Treatment did not affect daily milk yield, and there was no treatment by time interaction. Milk yield was different by time ($P < 0.001$) with peak yield at 0200 h and 2000 h and a nadir at 1400 h. There was a treatment by time interaction for milk fat percent, but 4x fed resulted in higher milk fat percent at all time points compared with 1x fed (0.22 to 0.45% higher; $P < 0.05$). Daily milk fat yield was increased 0.13 kg/d

by 4x feeding ($P < 0.001$). However, milk protein percent and daily yield were higher in 1x fed (0.1% and 0.05kg/d; $P < 0.001$). Plasma nonesterified fatty acids had a daily pattern with a predominant peak at 0700 h and a second, smaller peak at 1800 h (Time, $P < 0.0001$). Interactions were detected between treatment and time for plasma glucose, insulin, and blood urea nitrogen. Plasma glucose nadirs occurred at 0200 h, 1400 h, and 2200 h for 4x fed whereas 1x fed showed a single, higher nadir at 1000 h ($P < 0.01$). More peaks were observed, and the daily range of plasma insulin was greater in 1x fed (21.56 vs. 11.43 μ IU/mg). Blood urea nitrogen peaked at 1400 h in 4x fed compared with 1000 h in 1x fed, but had similar daily ranges and nadirs. In conclusion, dairy cows have a circadian pattern of milk synthesis that is responsive to the timing of feed intake.

Key words: circadian, dairy cow

831 Long term effect of feeding rumen protected fish oil or microalgae on mammary gene expression in Holstein cows managed under pasture or confinement systems. P. Vahmani^{*1}, K. Glover², L. A. MacLaren², J. Green-Johnson³, and A. Fredeen², ¹Dalhousie University, Halifax, NS, Canada, ²Nova Scotia Agricultural College, Truro, NS, Canada, ³University of Ontario Institute of Technology, Oshawa, ON, Canada.

The objective of this study was to examine how milk fat percent and yield, and the expression of mammary lipogenic genes were affected by the source of dietary marine oil (fish oil vs. microalgae) in grazing or confined dairy cows. Forty 8 Holstein cows were deployed across 2 feeding systems: either grazing pasture (n = 23) from Apr08 to Sep08, or receiving a TMR in confinement (n = 25) from Oct 08 to Feb09. Cows within each feeding system were blocked by calving date and assigned randomly within block to a control (no supplement) or one of 2 isolipidic supplements: rumen protected fish oil (RPF) or rumen protected microalgae (RPM) for 125 \pm 5 d beginning 30 d pre-calving. The RPM supplement provided ~50 g/d of DHA, whereas the RPF supplement provided ~25 g/d each of EPA and DHA. Milk samples were taken at 90 \pm 5 DIM for compositional analysis. Four cows from each treatment within each feeding system were slaughtered at 95 \pm 5 DIM and mammary parenchymal tissue was sampled. Expressions of mammary genes involved in de novo fatty acid synthesis (ACACA, FASN), desaturation (delta5-, delta6- and delta-9 desaturases), fatty acid uptake (LPL), transcriptional regulation (SREBP1, ChREBP, INSIG1, SCAP, THRSP) and nuclear receptor signaling (PPAR α , PPAR γ) were determined using qPCR. Milk fat percent was higher ($P = 0.02$) for control (3.52 \pm 0.18) compared with RPM (3.02 \pm 0.19), but was not different ($P = 0.23$) from RPF (3.28 \pm 0.19). However, milk fat yield was not affected ($P = 0.25$) by the lipid supplements (1.34 \pm 0.07, 1.25 \pm 0.08 and 1.22 \pm 0.09 kg/d for control, RPF and RPM, respectively). Expression of SREBP1, the main transcriptional regulator of milk fat synthesis, was reduced ($P < 0.05$) by 26% with RPM or RPF compared with the control, but the rest of measured genes were not altered by the treatments. The lipid supplements did not affect the regulation of the mammary lipogenic complex except for a reduced expression of SREBP1. This effect was apparently insufficient to alter the expression of lipogenic genes and milk fat yield.

Key words: fish oil, microalgae, mammary gene expression

832 Reduced milking frequency increases the concentration of host-defense proteins in milk. K. Stelwagen^{*1}, M. K. Broadhurst², K. Kim², A. J. Molenaar², D. P. Harris², and T. T. Wheeler², ¹*Agri-Search Ltd., Hamilton, New Zealand*, ²*AgResearch Ltd., Hamilton, New Zealand*.

Milk is a very complex fluid and while its main components, fat, lactose, caseins and major whey proteins, have been reasonably well studied, increasingly its minor components are being elucidated. Many of these minor components have known bioactive properties, including those related to innate immune function, and may have potential to add economic value to the dairy industry. We have previously shown that once-daily milking (ODM), compared with twice daily (TDM), can be used as an on-farm tool to increase the content and yield of the host-defense protein lactoferrin in milk. In the present study we show the effect of ODM on the level of several additional host-defense proteins in milk. Lactating (128 ± 15 DIM) New Zealand Friesian dairy cows (n = 10), grazed on pasture, were continued on normal TDM for the first 3 d of the experiment and then switched to ODM for a further 9 d. At each milking samples were collected for analysis of gross milk composition, SCC, and the abundance of the host-defense proteins lactoferrin, serum amyloid A3 (SAA3), angiogenin/RNase5 and RNase4. The level (µg/mL) of all 4 proteins was increased ($P < 0.05$) during ODM (TDM vs. ODM: lactoferrin, 81%, 110.1 vs. 199.8 ± 38.7; SAA3, 82%, 0.09 vs. 0.16 ± 0.02; angiogenin/RNase5, 12%, 8.63 vs. 9.63 ± 0.27; RNase4, 38%, 1.77 vs. 2.39 ± 0.11). The moderate increase of angiogenin/RNase5 is likely to be a consequence of the corresponding decrease in milk yield of 8% due to ODM, as the total yield of angiogenin/RNase5 did not differ significantly between TDM and ODM. Based on indirect indicators of mammary tight junction permeability and a small but significant increase in SCC (TDM vs. ODM: 30 vs. 42 ± × 1000/mL; $P < 0.05$), the increase in milk of host-defense proteins is likely to be due to a mild sterile intramammary inflammatory response accompanying the transition from TDM to ODM. This study shows that ODM may be used as a management tool to increase the level of valuable immune-related minor proteins in milk.

Key words: milk bioactive, milking frequency, host-defense

833 Effect of milking frequency early post-partum on energy metabolism in grazing dairy cows. C. V. C. Phyn¹, T. M. Grala², J. K. Kay¹, A. G. Rius¹, S. R. Morgan¹, and J. R. Roche^{*1}, ¹*DairyNZ Ltd., Hamilton, New Zealand*, ²*DairyNZ Ltd., Cl- ViaLactia Biosciences (NZ) Ltd., Auckland, New Zealand*.

In grazing dairy cows, short-term once-daily (1X) milking immediately post-calving results in long-term decreases in milk, fat and protein yields, and improves BCS, whereas thrice-daily (3X) milking does not increase energy-corrected milk yields. This study investigates the immediate and long-term effects of post-calving milking frequency (MF) on energy metabolism. Multiparous Holstein-Friesians (n = 150) were randomly assigned to one of 5 groups following calving: milked 1X for 3 or 6 wks and twice daily (2X) thereafter; milked 2X for the entire lactation (control); or milked 3X for 3 or 6 wks and 2X thereafter. Weekly blood samples (wks 1 to 12 post-calving) were analyzed for plasma NEFA, glucose, and aspartate aminotransferase (AST). Liver and adipose tissue was collected at 3, 6 and 9 wks post-calving (n = 12 cows/trt), and gene expression measured using RT-qPCR. Data were analyzed using mixed models fitted with REML in GenStat including: treatment and contrasts to test MF, duration and their interaction as fixed effects, and cow as a random effect. Gene expression of growth hormone receptor (GHR)-total and GHR1A was reduced

($P < 0.05$) and insulin receptor-B increased ($P < 0.01$) at 3 wks post-calving in the liver of cows milked 3X. Cows milked 1X had greater insulin-like growth factor (IGF)-I expression at 3 wks compared with cows milked 2X or 3X. Expression of lipogenic genes (SCD, LPL, THRSP) in adipose tissue was greater ($P < 0.05$) in cows milked 1X at 3 and 6 wks. In contrast, at 9 wks post-calving, cows milked 3X for 6 wks had decreased lipogenic (SCD, THRSP), lipolytic (hormone sensitive lipase) and GHR-total expression. Plasma glucose was greater ($P < 0.001$) and NEFA was lower ($P < 0.001$) during wks 1 to 6 in cows milked 1X. Cows milked 1X for 6 wks also had greater glucose post-treatment (wks 7 to 12). During wks 1 to 3, cows milked 3X had lower glucose and greater NEFA, while AST was greater ($P < 0.05$) throughout (wks 1 to 12). Results indicate that grazing cows milked 1X post-calving maintained a better energy balance and greater lipogenesis than cows milked 2X, whereas 3X milking induced a more severe negative energy balance.

Key words: milking frequency, dairy cow, energy metabolism

834 Regulation of STAT and IGF signaling during reversible and irreversible involution of the bovine mammary gland. K. Singh^{*1}, J. Dobson¹, K. Oden¹, A. Molenaar¹, R. Murney¹, K. Swanson¹, and K. Stelwagen², ¹*AgResearch Ltd., Ruakura Research Centre, Hamilton, New Zealand*, ²*Agri-Search Ltd., Hamilton, New Zealand*.

STAT and IGF signaling play a major role at the onset of rodent mammary involution. The aim of this study was to investigate the reversibility of mammary STAT5/3 and IGF signaling following involution induced by not milking, and subsequent re-milking in dairy cows. Mammary alveolar tissue was obtained from non-pregnant cows (n = 5/group) slaughtered at mid-lactation 6h post-milking (control), cows not milked for either 7 or 28d, and cows that were re-milked for 7d following both non-milked periods. Western blot analyses showed that pSTAT5 (activated) protein levels following 7 and 28d involution were lower ($P < 0.001$; 8.5- and 9.5-fold, respectively), compared with lactating controls and cows returned to lactating levels when re-milked following both non-milked periods. In contrast, apoptosis marker pSTAT3 levels were much greater ($P < 0.001$; 40- and 16-fold, respectively) following both 7 and 28d of involution, compared with lactating controls and remained greater ($P < 0.01$; 5-fold) following re-milking after a 28d involution. Similar results were observed for other indicators of apoptosis, lactoferrin and α Bax. In contrast to rodent involution, IGF-I was increased in 7 ($P < 0.1$; 2-fold) and 28d ($P < 0.01$; 3.5-fold) non-milked cows and only returned to lactation levels when cows were re-milked after the 7d non-milked period. IGF-II and IGF-BP5 mRNA levels were similar between all groups ($P < 0.1$). Average expression of the major milk proteins α S1-, β -, and κ -CN, α -LA and β -LG declined (RT-PCR; $P < 0.05$) following 7 and 16d of involution (3.5 to over 200-fold) compared with lactating controls. Levels returned to those of lactating controls re-milked after 7d of involution. When re-milked after 28d non-milking, all but κ -CN returned to lactating levels. The variability in milk protein gene expression in response to involution and re-milking between animals was supported by histological analyses demonstrating differences in response rates between cows. These data suggest that STAT3 and IGF-I play a role in the irreversible phase of involution.

Key words: STAT5/3, IGF, bovine mammary involution

835 Variations in the mammary uptake of nutrients throughout an extended milking interval in dairy cows. J. Guinard-Fla-

ment*, C. Hurtaud, and S. Lemosquet, *UMR Production du Lait, INRA/Agrocampus Ouest, Saint-Gilles, France.*

The rate of milk secretion remains steady until 16 h after milking. However, little is known about milk synthesis and the way the udder adapts its nutrient uptake in relation to variations in arterial nutrient flow to sustain its metabolic activity. A trial was carried out to describe variations in mammary blood flow, and in arterial concentrations, mammary arteriovenous differences (AVD), extraction rates (ER), and uptake of nutrients in the course of milk accumulation in the udder over a 36-h period. The trial was performed twice on 2 consecutive wk with 3 dairy cows (34 kg/d of milk). Cows were fed and milked twice daily at the same time, except over the 2 36-h intervals. During milk accumulation, 20 blood samples were collected using 1- to 3-h intervals between samplings. Uptakes of glucose, acetate, BHBA, glycerol, O₂, and release of CO₂ were reduced by 27–38% on the last 12 h compared with the first 12 h of milk accumulation ($P < 0.05$). But patterns of variations in the course of time between feed distributions were preserved. The mammary uptake of glucose and glycerol did not vary or only slightly between feed distributions (1.4-fold variation; $P < 0.05$). In contrast, acetate and BHBA showed larger variations to a maximum of 50 to 100% ($P < 0.05$ except for BHBA on the last 12 h of milk accumulation). Uptakes of acetate and BHBA were maximal 3 h after feed distribution and were correlated to their arterial concentrations and AVD ($r = 0.73$ and 0.88 for acetate and $r = 0.63$ and 0.83 for BHBA, respectively) whereas those of glucose and glycerol were correlated to ER ($r = 0.80$ and 0.90 , respectively). Maximal uptakes of acetate and BHBA were also associated with greater uptakes of glucose, glycerol, O₂ and maximal releases of NEFA and CO₂ ($P < 0.05$). These results suggest that the de novo synthesis of milk fatty acids could vary throughout the day according to the arterial concentration of acetate or BHBA or both. In contrast, the uptake of glucose and glycerol would depend on the metabolic activity of the mammary gland, the udder modifying its efficiency of extraction from blood according to its metabolic needs.

Key words: milk synthesis, udder uptake, dairy cow

836 Effect of heat stress during the dry period on insulin sensitivity of multiparous dairy cows. S. Tao*, I. M. Thompson, A. P. Monteiro, M. J. Hayen, and G. E. Dahl, *University of Florida, Gainesville.*

Heat stress during the dry period affects hepatic gene expression and adipose tissue mobilization during the transition period. One of the possible outcomes may be altered insulin action on peripheral tissues. Our objective was to evaluate the effect of heat stress during the dry period on insulin sensitivity in the transition period. Cows were dried off 46 d before expected calving and assigned to 1 of 2 treatments: heat stress (HT, $n = 16$) or cooling (CL, $n = 16$). During the dry period, the average THI was 78, but CL cows were cooled with sprinklers and fans and HT cows were not. After calving, all the cows were housed together in the same barn and cooled. Rectal temperatures (RT) were measured twice daily (0730 and 1430h) and respiration rate (RR) recorded thrice weekly during the dry period. DMI was recorded daily from dry-off to 42 d relative to calving (RTC). BW and BCS were measured weekly

from dry-off to 42 DIM. Milk yield and composition were recorded daily to 126 DIM. Glucose and insulin tolerance tests were performed at dry-off, -14, 7 and 28 d RTC from a subset of cows (HT, $n = 8$; CL, $n = 8$). Relative to HT, CL cows had lower RT in the afternoon (39.3 vs. 39.0 °C; $P < 0.01$) and lower RR (69 vs. 48 breaths/min; $P < 0.01$). CL cows consumed more feed than HT cows prepartum (11.4 vs. 10.2 kg/d; $P = 0.05$), but not postpartum ($P = 0.25$). Compared with HT, CL cows gained more weight before calving ($P = 0.01$) but lost more weight in the early lactation ($P = 0.02$). Treatment did not affect BCS. CL cows produced more milk than HT cows (40.4 vs. 32.7 kg/d; $P < 0.01$), but prepartum cooling did not affect milk composition. Preliminary data from the glucose tolerance test indicate that CL cows had similar glucose disposal rates ($P = 0.3$) relative to HT cows 2 weeks before calving. Regardless of treatment, cows had increased glucose disposal rate at -14 d RTC compared with dry-off. We conclude that heat stress during the dry period compromises lactation performance but does not affect insulin sensitivity late in the dry period.

Key words: heat stress, insulin sensitivity, dairy cow

837 Dry period seasonal effects on the subsequent lactation. I. M. Thompson*, A. P. Monteiro, and G. E. Dahl, *University of Florida, Gainesville.*

Photoperiod and heat stress during the dry period influence subsequent lactational performance, and health. To determine the effects of heat stress abatement during the dry period on subsequent lactation under commercial conditions in north central Florida, cows were dried off approximately 45d before expected calving and randomly assigned to 2 treatments, heat stress (HT; $n = 77$) and cooling (CL; $n = 56$). Cool cows were kept outside and provided with sprinklers, fans and shade, whereas HT cows were outside under shade. Relative to HT, CL cows had greater milk production (45.62 vs. 43.21; $P = 0.02$) through 120 DIM. Having confirmed significant production effects of heat stress during the dry period, we analyzed records of 2,614 multiparous cows under commercial conditions in Florida over 3 years to determine seasonal effects during the dry period on subsequent lactation performance, reproduction and occurrence of health disorders during the first 60 DIM. Seasons were Cool (Dec, Jan and Feb) and HOT (June, July and Aug). Traits analyzed were lactation number, 305d milk production, calving interval, number of breedings, days open and occurrence of postpartum health disorders such as digestive problems, mastitis, metritis, retained fetal membranes, and respiratory problems. Cows dried during HOT months had lower milk yield in the subsequent lactation relative to COOL cows (10,351 vs. 10,902 kg; $P < 0.01$). Additionally, HOT cows had a higher incidence of mastitis ($P < 0.01$) and respiratory problems ($P < 0.01$) compared with COOL cows. Moreover, cows exposed to heat tended to have a higher incidence of retained fetal membrane ($P < 0.07$) compared with cows cooled while dry. Of interest, COOL cows had a higher incidence of postpartum digestive problems ($P < 0.01$). Therefore, environmental management strategies during the dry period may be needed to attain optimal lactation performance.

Key words: dry period, season, milk production