Lactation Biology 1

M193 Essential amino acids significantly contribute to the energy status in short-term MAC-T cell cultures. V. S. Lyman[1], M. L. Bell[1], W. A. D. Nayananjalie*[1], E. M. England[1], J. A. D. R. N. Appuhamy[2], and M. D. Hanigan[1], *[1]Virginia Polytechnic Institute and State University, Blacksburg, [2]University of Guelph, Guelph, ON, Canada.*

Acetate is a major energy source for ruminant mammary glands. Supplementation of AA increases milk protein synthesis, but the mammary glands have been found to take up some essential AA (EAA) beyond requirements for protein synthesis. Catabolized AA contribute to the energy supply of mammary cells. The in vivo contribution to ATP production is small, but that may not be the case in vitro. The present study was designed to investigate the effects of acetate and EAA on ATP levels and phosphorylation of AMP-activated protein kinase (AMPK). Reduced AMPK phosphorylation is indicative of improved energy status. A bovine mammary epithelial cell line, MAC-T was incubated in glucose free Dulbecco's Modified Eagle's Medium Ham's Nutrient Mixture F-12 (DMEM/F12) with 0 or 3.5 m*M* EAA and 0 or 5 m*M* acetate in a 2 × 2 factorial arrangement with 3 replicates. All media contained 10 ng/mL insulin. Cells were harvested after 1 h of incubation in treatment media and cell lysates were subjected to analysis for ATP (μmol/mg of protein) and total protein (mg/ml) content. The experiment was repeated on a different day and the cell lysates were subjected to Western immunoblotting analysis of AMPK phosphorylation state (phosphorylation at Thr172: total AMPK). Supplementation of EAA increased ($P = 0.07$) ATP content in MAC-T cells by 27% (60.4 ± 4.1 vs.78.2 ± 4.6 μmol/mg of protein) whereas added acetate was noneffective ($P = 0.46$). Consistent with the ATP results, EAA supplementation reduced ($P = 0.04$) phosphorylation of AMPK by 33% (0.95 ± 0.10 vs. 0.64 ± 0.10) whereas added acetate was associated with a numerical ($P = 0.20$) decline of 21% (0.89 ± 0.10 vs. 0.70 ± 0.10). There were no significant interactions between acetate and EAA on the ATP content or the phosphorylation of AMPK. ATP concentrations were highly correlated (r = - 0.90) with AMPK phosphorylation. In MAC-T cells, AMPK phosphorylation was responsive to ATP concentrations as observed in other cell types. Essential AA were much more potent in eliciting an ATP and AMPK response suggesting that these cells have limited ability to metabolize acetate.

Key words: amino acids, energy status, mammary cells

M194 Mammary uptake of fatty acids varying in chain length and unsaturation supplied by intravenous triglyceride infusion. J. A. Stamey*, J. K. Suagee, C. Caldari-Torres, and B. A. Corl, *Virginia Tech, Blacksburg.*

Supplementing dairy cows with feeds rich in omega-3 fatty acids does not readily increase excretion in milk fat of dairy cows. Previous results demonstrated that very long chain omega-3 fatty acids are primarily transported in the phospholipid fraction of blood, making them largely unavailable to the mammary gland for enrichment of milk fat. Our objective was to compare fatty acids of increasing chain length and unsaturation delivered intravenously as triglyceride emulsions to uncover any regulation of fatty acid uptake by the mammary gland. Late lactation dairy cows were assigned to a completely randomized block design to prevent carryover effects. Cows were fed a total mixed ration formulated to meet nutrient requirements and allowed an acclimation period of 5 d. Treatments were intravenous triglyceride emulsions enriched with oleic, linoleic, linolenic, or docosahexaenoic acid and were delivered continuously at 16 mL/h for 72 h. Each treatment supplied 30 g/d of the target fatty acid. Treatments did not affect feed intake, milk yield, or milk composition. Each target fatty acid demonstrated increased proportion in plasma triglyceride. Increases of target fatty acids, especially linolenic and docosahexaenoic, were evident in plasma phospholipid and cholesterol ester fractions, suggesting re-esterification in the liver. Transfer efficiencies were 31.6, 46.4, and 13.0%, and d 3 total milk fatty acyl yields were 37.5, 19.4, and 3.9 g (±1.36 pooled SEM) for linoleic, linolenic, and docosahexaenoic acid. Variation in oleic acyl yield prevented calculation of oleic acid transfer efficiency. Mammary uptake of fatty acids was reduced with increased chain length and unsaturation. Both liver and mammary mechanisms might regulate transfer of long chain polyunsaturates.

Key words: fatty acid, lipid metabolism, transfer efficiency

M195 Conjugated linoleic acid-induced milk fat depression in lactating ewes is accompanied by reduced expression of genes involved in mammary lipid synthesis. M. Hussein*[1], K. H. Harvatine[2], W. M. P. B. Weerasinghe[3], L. A. Sinclair[3], and D. E. Bauman[1], *[1]Cornell University, Ithaca, NY, [2]Pennsylvania State University, University Park, [3]Harper Adams University College, Newport, Shropshire, UK.*

Conjugated linoleic acids (CLA) are produced during rumen biohydrogenation and exert a range of biological effects. The t10, c12 CLA isomer is a potent inhibitor of milk fat synthesis in lactating dairy cows and some aspects of its mechanism have been established. CLA-induced milk fat depression (MFD) has also been observed in small ruminants and our objective was to examine the molecular mechanism in lactating ewes. Multiparous lactating ewes (n = 16) were fed a basal ration (0.55:0.45 concentrates to forage; dry matter basis) and randomly allocated to 2 treatments. Treatments were zero CLA (Control) or 15 g/d of lipid-encapsulated CLA supplement containing c9, t11 and t10, c12 CLA isomers in equal proportions. Treatments were for 10 wk and CLA supplement provided 1.5 g/d of t10, c12. There were no effects on milk yield or milk composition for protein or lactose at wk 10 of the study ($P > 0.1$). In contrast, CLA treatment decreased both milk fat percent ($P < 0.01$) and milk fat yield (g/d) ($P = 0.07$) by almost 22%. The de novo synthesized fatty acids (FA) (<C16) decreased in proportion (15%) and daily yield (27%) due to CLA treatment ($P < 0.05$). In addition, the proportion of preformed FA (>C16) increased ($P < 0.05$) and there were numerical decreases in the yields of 16 carbon FA (15%) and >16 carbon FA (6%). Consistent with the FA pattern, mRNA abundance of FASN, ACACA and SCD1 decreased by 35 to 45% in the CLA-treated group ($P < 0.05$). Similarly, CLA treatment decreased mRNA abundance of GPAT ($P = 0.15$) and DGAT1 ($P = 0.09$), genes involved in fatty acid esterification, by almost 30%. The mRNA abundance for SREBP-1 and INSIG1, genes involved in regulation of transcription of lipogenic enzymes, was decreased by almost 60% with CLA treatment ($P < 0.05$). Furthermore, mRNA abundance of LPL decreased by almost 30% due to CLA treatment ($P = 0.06$). In conclusion, the mechanism for CLA-induced MFD involved the SREBP transcription factor family and a coordinated downregulation in transcript abundance for lipogenic enzymes involved in mammary lipid synthesis.

Key words: CLA, lactation, mammary

M196 Characterization of a novel bovine mammary epithelial cell line. P. Bernier-Dodier*[1,2], G. Tremblay[1], and P. Lacasse[2], *[1]Université de Sherbrooke, Sherbrooke, QC, Canada, [2]AAFC-Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada.*

Milk contains several cell types, including exfoliated mammary epithelial cells. Since the available bovine cell lines poorly differentiate at best (meagre milk proteins expressions and leaky tight junctions), we have isolated primary mammary epithelial cells from milk for in vitro experimentations. Although a high proportion of contaminant immune cells were initially present, they were rapidly loss since they have a relatively short survival time. Out of the 7 isolations accomplished, one cell population, isolated from the milk of a Holstein cow at approximately 200 DIM, was successfully maintained in culture for a prolonged period. Interestingly, those epithelial cells have survived and to proliferated for at least 50 passages without any sign of senescence, suggesting that they have spontaneously immortalized. Because only a small number of bovine mammary epithelial cell (BMEC) lines are currently available, we undertook the characterization of this novel cell line named HERA-2. Their epithelial origin was confirmed by an immunohistochemical detection of cytokeratins and by PCR detection of the cytokeratin-18 gene expression. The bovine origin of the cell line was confirmed by karyotype analysis of 12 cells and by sequencing the gene *cytochrome C oxidase I*. At all the tested passages, the HERA-2 cells formed tight inter-cellular junctions as evaluated by transepithelial electrical resistance measurement that generally reached more than 1500 ohm*cm^2. When the HERA-2 cells are cultured on matrigel in presence of lactogenic hormones (prolactin, dexamethasone and insulin), they form mammospheres and duct-like structures. Up to now, none of the culture conditions tested was able to induce significant expression of lactogenic differentiation markers (αs1-casein, β-casein and α-lactalbumin encoding genes). In conclusion, the HERA-2 is a novel BMEC line that could constitute a good alternative to the MAC-T cells since they were not immortalized with the SV-40 large T-antigen. Their use as a model could facilitates the study on the regulation bovine mammary cells functions.

Key words: mammary gland, tight junction

M197 Further study on the role of SREBP-1 in lipogenesis in bovine mammary epithelial cells. L. Ma* and B. A. Corl, *Virginia Tech, Blacksburg.*

Sterol regulatory element binding proteins (SREBPs) are transcription factors that regulate lipid metabolism. Among the 3 isoforms, SREBP-1a, −1c and −2, SREBP-1a and −1c regulate fatty acid synthesis. The objective of this study was to further determine the role of SREBP-1 in regulating lipogenesis in bovine mammary epithelial cells (MAC-T cells). Cells were seeded to plates at a density of 2×10^4 cells/cm^2 and incubated in basal medium (DMEM+10% FBS) overnight. Then cells were transfected with small interfering RNAs (siRNA) against SREBP-1 (SSI), a random sequence as negative control (NEG), and no siRNA as untreated control (UNT), according to protocol (Dharmacon Inc.). After 48 h, cells were harvested for real-time quantitative PCR, immunoblotting, and radiolabeled-oleate incorporation assays. Our previous study showed that SREBP-1 mRNA and SREBP-1 proteins were reduced by SREBP-1 siRNA significantly ($P < 0.0001$). Acetate incorporation and mRNA expression of de novo lipogenic enzymes (acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS)) also decreased with SSI ($P < 0.001$). In the current study, we observed a significant reduction in mRNA expression of acyl-CoA synthetase short-chain family member 2 (ACSS2), isocitrate dehydrogenase 1 (IDH1), and fatty acid binding protein 3 (FABP3) ($P < 0.0001$). The protein expression of stearoyl-CoA desaturase 1 (SCD1) and ACC was decreased as well. Although we did not observe any reduction in triglyceride synthetic enzymes in previous study, phosphatidic acid phosphatase-1(Lipin1) mRNA level dropped with SSI treatment. However, adipophilin (PLIN2) had a 3.5-fold increase. De novo fatty acid synthesis starts with acetate which is converted to acetyl-CoA by ACSS2. Acetyl-CoA is used to synthesize malonyl-CoA by ACC, and FAS is responsible for fatty acid chain elongation. IDH1 generates NADPH as a reducing equivalent for de novo lipogenesis. In conclusion, SREBP-1 regulates lipogenesis in MAC-T mainly through de novo lipogenic enzymes. This project was supported by National Research Initiative Competitive Grant no. 2009–35204–05358 from the USDA National Institute of Food and Agriculture.

Key words: SREBP-1, small interfering RNA, bovine

M198 Capturing circadian mammary gene expression of cows using RNA from milk fat globule. J. Crodian*, T. Casey, and K. Plaut, *Purdue University, West Lafayette, IN.*

The overall objective of this study was to measure mammary gene expression over a circadian cycle in the lactating cow. Rather than isolating RNA from repeated mammary biopsies, total RNA was isolated from the cytoplasmic crescent of the milk fat globule. Three lactating Holstein cows were housed in tie stalls with water and feed provided ad libitum. Cows at 125 d into lactation with an average SCC of 105,000 were milked every 4hrs over a 48hr period. Milk samples were analyzed by DHIA for %fat, %protein, %lactose and MUN. Milk fat was separated by centrifugation and total RNA was isolated using QIAZOL. qPCR analysis was performed and expression of ACACA (acetyl-CoA carboxylase α), CSN2 (β-casein), PER2 and ARNTL were measured. Expression of core clock genes, PER2 and ARNTL showed diurnal variation (Table 1), with peak (16–20h; dark phase) and trough (32–36h; light phase) of expression significantly different ($P < 0.05$). Although ACACA and CSN2 showed diurnal variation, peak and trough of gene expression were not different. Diurnal patterns of MUN and %fat were also evident. Mean peak of %fat (4.18+/−0.11; 32–36h; light phase) and trough (3.52+/−0.17; 16–20h; dark phase) were different ($P < 0.01$), and opposite in phase of PER2 and ARNTL. These results suggest that mammary clock genes and milk components exhibit circadian rhythms.

Table 1. Fold change in gene expression relative to average expression across 48 hrs (log base 2*).

Time (h)	PER2*	SEM	ARNTL*	SEM
0	−0.48	0.56	−1.18	0.69
4	0.51	0.06	−0.19	0.37
8	1.29	0.12	0.30	0.52
12	1.44	0.12	0.21	0.45
16	1.30	0.20	0.41	0.27
20	1.30	0.35	0.55	0.30
24	−0.74	0.38	−0.15	0.32
28	−0.41	0.70	−0.06	0.10
32	−2.75	0.64	−1.22	0.74
36	−2.31	0.74	−0.56	0.35
40	−0.16	0.29	0.47	0.21
44	0.49	0.35	−0.02	0.43
48	0.50	0.56	1.44	0.09

Key words: circadian, mammary, gene expression

M199 Expression of PEPCK isoforms in the mammary gland of dairy goats is regulated by insulin status. S. J. Mabjeesh*[1], A. Sahmay[2], N. Argov-Agrman[1], C. Sabastian[1], and B. J. Bequette[3], *[1]The Robert H. Smith Faculty of Agriculture, Food and environment, The Hebrew University of Jerusalem, Rehovot, Israel, [2]Institute of Animal Science, The Volcani Center, Bet Dagan, Israel, [3]University of Maryland.*

Phosphoenolpyruvate carboxykinase (PEPCK) isoforms (c, cytosolic; m, mitochondria) are expressed in the liver and mammary gland. PEPCK-c is a rate-controlling enzyme for gluconeogenesis and glyceroneogenesis whose activity is decreased by insulin, whereas PEPCK-m expression is constitutive and functions to channel lactate towards gluconeogenesis. We hypothesized that the increase in milk protein but decrease in milk lactose and fat when the hyperinsulinemic-euglycemic clamp (HIEC) is applied to dairy goats is due to a decrease in expression of mammary PEPCK-c mRNA. Late lactation goats (n=4;150 ± 30 DIM) were subjected to saline infusion and HIEC (104 µg insulin/h) for 4-d periods in a 2×2 cross-over design. Goats were milked two times per day and milk yields and components were determined. On day 4 of each period, a mammary biopsy (~1g) was taken from an udder half for expression of PEPCK-m and -c mRNA by rtPCR. Plasma insulin increased (P< 0.002) 3.5-fold due to the HIEC and euglycemia was maintained. The HIEC decreased (P < 0.005) DMI (40%) and milk yield (26%). Whereas milk fat content was not affected, HIEC increased (P < 0.001) milk protein content (2.82% vs. 3.09%) but decreased (P < 0.001) milk lactose content (4.22% vs. 4.03%). Expression of PEPCK-m mRNA was 8-fold higher (P < 0.031) than PEPCK-c. The HIEC decreased PEPCK-c mRNA 7-fold but increased PEPCK-m mRNA by 30%. These results demonstrate that insulin regulates mRNA expression of mammary PEPCK isoforms, and this may underlie the changes in milk component synthesis observed when the HIEC is applied.

Key words: insulin, casein, goat