W148 Estradiol enhances apoptosis in bovine mammary epithelial cells in vitro. L. Yart*[1,2], L. Finot[1,2], V. Lollivier[2,1], P. G. Marnet[2,1], and F. Dessauge[1,2], *[1]INRA, UMR1348 Pegase, Saint-Gilles, France, [2]Agrocampus Ouest, UMR1348 Pegase, Rennes, France.*

Previous studies conducted on mid- or late-lactation dairy cows showed that the administration of exogenous estradiol induced a severe decrease in milk yield and accelerated mammary gland involution. However, the effects of estradiol on mammary epithelial cells remained unknown. The aims of this study were to investigate the in vitro effects of estradiol (i) on a bovine mammary epithelial cell line (Mac-T) and (ii) on apoptose-induced bovine mammary epithelial cells. In the first part of this study, the Mac-T cells were treated with increasing doses of estradiol (10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M), during increasing times (6h, 12h, 24h and 48h). It appeared that the higher treatment doses of estradiol (10^{-4} and 10^{-3}M) reduced the cell proliferation by 8 and 19% respectively at 24h and by 5 and 10% respectively at 48h, and induced apoptosis of the mammary epithelial cells. In the second part of this study, the Mac-T cells were either treated with estradiol (E_2, 10^{-4}M) or with camptothecin (Ct, 10 μM) to induce apoptosis or with camptothecin combine with estradiol (Ct+ E_2), during increasing times (2h, 6h, 24h and 48h). After 6h of treatment, we observed an increase in apoptotic markers (Caspase 3 activity and Annexin V-positive cell rate) in both Ct and Ct+ E_2 treatment batches. This increase was more important in the Ct+ E_2 treatment batch at 6h (+ 61.5%) and 24h (+ 9.8%) compared with the Ct treatment batch. Taken together, these results suggest that estradiol reduces mammary epithelial cells expansion and enhances apoptosis pathways in vitro.

Key Words: estradiol, mammary epithelial cell, apoptosis

W149 Evaluation of mitogenic properties of colostrum and colostrum replacer (CR) on growth of bovine mammary epithelial cells (BMEC) in vitro. K. E. Stemm,* C. M. Jones, J. L. Collier, and R. J. Collier, *University of Arizona, Tucson.*

Ingestion of colostrum is necessary for neonatal calves to develop a functioning immune system. A viable alternative source of immunoglobulins for management and safety reasons is colostrum replacer (CR). Colostrum has also been implicated in improved milk yield at maturity, possibly due to increased mammary growth. A comparison of mitogenic effects of colostrum versus CR has yet to be established. In this study, pooled whole colostrum (WC) from a commercial dairy was compared with Convert ImmPower Colostrum Replacer supplied by Agrarian Marketing (Middlebury, Indiana). A clonal BMEC cell line (MAC-T), was used to screen potential mitogenic agents in colostrum. Concentrations of DNA were determined by CyQuant DNA Assay (Life Technologies, Grand Island, NY) and by proxy mitogenic activity. Protein concentrations of WC and CR were 187.01 and 181.56 mg/mL, respectively, and therefore treatments were delineated as percentages by weight. Cells treated with 10% WC or CR exhibited a decline in cell numbers ($P < 0.05$). Therefore, only treatments of 1% and 5% WC or CR were utilized. The treatment of MAC-T cells with WC or CR at 1 or 5% produced higher DNA concentrations than cells that were untreated, ($P < 0.05$). The use of WC at 1% provided the best growth results, which was not different from the normal in vitro growth media, containing 10% FBS and insulin. When the comparison was made between the 2 treatments, WC promoted more growth than CR at 1%, ($P < 0.001$). The mitogenic agents epidermal growth factor (EGF) and insulin-like growth factor I (IGF-I) were then added to CR to determine if it improved mitogenesis. The addition of 25 ng/ml EGF and 100 ng/ml IGF-I alone and in combination with 1% CR promoted growth of cells greater than 1% CR alone, ($P < 0.001$), but not different from 1% WC ($P < 0.05$).

Key Words: colostrum replacer, mitogen, mammary cells

W150 Effects of intra-mammary infusions of casein hydrolysate, EGTA, and lactose at drying-off on mammary gland involution. B. Ponchon*[1], P. Lacasse[2], N. Silanikove[3], S. Ollier[2], and X. Zhao[1], *[1]Department of Animal Science, McGill University, Sainte-Anne-de-Bellevue, QC, Canada, [2]AAFC-Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada, [3]Volcani Center, Bet Dagan, Israel.*

The transition from lactation to the dry period in dairy cows is a period of high risk for acquiring new intra-mammary infections. This risk is reduced when the involution of the mammary gland is completed. Consequently, strategies that accelerate the involution process after drying-off could reduce the incidence of mastitis. The objective of this study was to assess the effect of 3 different treatments on mammary gland involution markers. Each quarter of 8 Holstein cows in late lactation was randomly assigned at drying-off to an intra-mammary infusion of iso-osmotic solutions of a casein hydrolysate (CNH; 70mg), EGTA (5.7g), lactose (5.1g) or saline (control). Milk samples were collected on the last 2 d before and 1, 3, 5, 7, 10 and 14 d after the last milking. Milk somatic cell count (SCC), lactoferrin and BSA concentrations gradually increased ($P < 0.001$) during the first 2 weeks following the last milking whereas milk citrate concentration decreased ($P < 0.001$). The increases in SCC, lactoferrin and BSA concentrations after the last milking were similar in quarters infused with saline and lactose. Intra-mammary infusion of CNH hastened the increase in SCC and BSA and their concentrations were greater ($P < 0.05$) than in milk from control quarters on d 1, 3, 5 and 7. Similarly, the CNH treatment induced a faster rise of lactoferrin concentration, which was greater ($P < 0.05$) than in milk from control quarters on d 1, 3 and 5 after the drying-off. Milk citrate concentration was unaffected by CNH but the citrate:lactoferrin ratio was lower ($P < 0.05$) in CNH-treated quarters at d 3 and 5 than in control quarters. Infusion of EGTA increased SCC on d 1 and 3 but it had no effect on the other parameters. This study suggests that an intra-mammary infusion of CNH at drying-off hasten the involution of the mammary gland.

Table 1. Milk SCC, BSA and lactoferrin concentrations and citrate:lactoferrin ratio 3 days after drying-off

	Control	CNH	EGTA	Lactose
SCC (x 1000)	182	3 162*	436*	323
BSA (g/L)	1.14	2.63*	1.37	1.48
Lactoferrin (G/L)	0.73	1.26*	0.76	0.78
Citrate:Lactoferrin	3.64	1.58*	2.94	2.25

*Different from control ($P < 0.05$).

W151 Expression of amino acid transporter LAT1 and the regulation by prolactin in mammary gland of dairy cow. L. Feng, Y. Lin, Q. Li,* X. Gao, and N. Zhang, *Key laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, Harbin, Heilongjiang, China.*

System L-amino acid transport mediates the uptake of aromatic neutral amino acids and nutritionally essential amino acids. This study was to reveal the relationship between the expression of system L-amino acid transporter LAT1 and the development of dairy cow mammary gland, and illuminate the regulation mechanism of prolactin (PRL) to LAT1 expression. We used Western blotting and immunofluorescence triple staining technology to detect the expression of LAT1 and its auxiliary protein 4F2hc at 4 development stages of dairy cow mammary gland tissue, including virgin, pregnant, lactation, involution. The effect of prolactin on the expression of LAT1 was determined by qRT-PCR and Western blotting in dairy cow mammary epithelial cells during mid-lactation. Subsequently, we silenced the prolactin receptor (PRLR) gene by siRNA interference technology in dairy cow mammary epithelial cells, and detected the PRLR gene silencing effect silencing and the expression of LAT1 by qRT-PCR and Western blotting. We also tested Î²-casein in mammary epithelial cells by high performance liquid chromatography (HPLC). Immunofluorescence results showed that LAT1 and 4F2hc co-expressed in the apical and basolateral membrane of duct and acinus epithelium at different stages of mammary gland. The expression of LAT1 and 4F2hc were significantly upregulated during lactation ($P < 0.05$). LAT1 mRNA expression were greatly increased prolactin dose range (5, 10, 20, 40 and 60 Î1/4g/ml), and LAT1 had the highest expression level at 20 Î1/4g/ml prolactin stimulation group. This result conformed to the result of Western blotting. In siRNA-PRLR transfection group, the expression of LAT1 exhibited a differentially reduction ($P < 0.05$). In addition, both secretion of Î²-casein and cell activities were also obviously decreased in transfected cells. These results indicated that PRL-PRLR signaling pathways were most likely to play a major role in the regulation of LAT1 expression during lactation, and LAT1 may play a role during the development and lactation of dairy cow mammary gland.

Key Words: dairy cow, mammary epithelial cells, LAT1

W152 Bzw2 promotes proliferation and lactation of mammary epithelial cell in dairy goat. R. Sun, Q. Li,* H. Yan, J. Zhao, X. Gao, and N. Zhang, *The Key Laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, Harbin, Heilongjiang, China.*

Mitosis of mammary epithelial cell is the foundation of mammal lactation. We developed a strategy of combined application of serial analysis of gene expression (SAGE) tags for gene identification (GLGI) to screen and identify genes influenced lactating ability of mammary epithelial cell in the dairy goat. The Bzw2 gene was found as a candidate gene related to lactation by screening Long-SAGE library of mammary gland in the dairy goat. This study was to identify the Bzw2 gene function during lactation in the dairy goat mammary gland. The Bzw2 gene was cloned by SMART RACE from Long-SAGE tag. The mRNA level of Bzw2 was relatively higher in early lactation than in other development stages of the mammary gland. The proliferation of mammary epithelial cell was inhibited by transfecting specific shRNA of Bzw2, The mRNA level of Stat5, Csn2 and Prlr were also downregulated, which would suggest that the productivity of the mammary epithelial cell was attenuated after Bzw2 RNAi. The reduction of mammary cell growth and lactation by Bzw2 RNAi are rescued through overexpression of Bzw2. These results revealed that Bzw2 might play an important role in lactation though the molecular mechanism which was still to be discovered by studies.

Key Words: Bzw2, mammary epithelial cells;, RNAi

W153 CLA and diet induced milk fat depression reduces milk fat across the entire day. K. Cook[1], K. J. Harvatine*[1], and D. E. Bauman[2], *[1]Penn State University, University Park, [2]Cornell University, Ithaca, NY.*

Recently a circadian rhythm of milk and milk component synthesis has been characterized that is partially dependent on the timing of feed intake. Our objective was to determine if inhibition of milk fat synthesis during diet-induced milk fat depression occurred to a higher degree during certain phases of the day. A retrospective analysis was conducted of 2 experiments that induced milk fat depression while milking cows at equal intervals, 3 times per day. The response at each milking was analyzed using the Proc Mixed procedure of SAS with a repeated statement. The model included the fixed effect of treatment, milking time, and the interaction of milking time and treatment and the random effect the cow and period. The subject was cow by period with the ARH1 covariance structure. In Experiment 1, 9 multiparous cows were arranged in a 3 × 3 Latin square design. Treatments were control TMR, control TMR plus 3 d intravenous infusion of 7.5 g/d of trans-10, cis-12 conjugated linoleic acid (CLA), and a low forage and high fat diet for 10 d. In Experiment 2, 10 multiparous ruminally cannulated cows were arranged in a replicated design and milk samples were collected during a control period or after 5 d of abomasal infusion of 10 g/d of CLA. In Experiment 1 there was a significant effect of treatment and milking for milk fat concentration and yield ($P < 0.001$ and $P < 0.05$, respectively), but no interaction of milking time and treatment. In Experiment 2, there also was an effect of treatment and milking time on milk fat concentration ($P < 0.05$) and no treatment by milking time interaction. There was a treatment, but no milking time or treatment by milking time interaction on milk fat yield. Milk fat percent was 0.48 and 0.28 percentage units lower at the morning milking than the afternoon milking in Exp. 1 and Exp. 2, respectively. A daily rhythm of milk fat concentration and yield can be observed in cows milked 3 times a day. However, diet-induced milk fat depression decreases milk fat yield equally over the day.

Key Words: milk fat, CLA, circadian

W154 Dairy cows having various levels of *cis*-9, *trans*-11 CLA de novo synthesis differently express proteins in milk epithelial cells. H. G. Lee*[1], T. Wang[1], J. N. Lim[1], J. D. Bok[2], J. H. Kim[3], S. B. Lee[1], S. K. Kang[2], J. H. Hwang[1], K. H. Lee[1], H. S. Kang[1], and Y. J. Choi[2], *[1]Department of Animal Science, Pusan National University, Miryang, Gyeongnam, Korea, [2]Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea, [3]Research and Technology Center, Cargill Agri Purina, Seongnam, Gyeonggi, Korea.*

The objective of this study was to associate cows having various levels of *cis*-9, *trans*-11 CLA to different protein expression profiles of milk epithelial cells. Morning milks were collected individually from 26 multiparous Holstein cows in their third or fourth lactation fed the same diet. Total milk lipids of each sample were extracted following the Folch method and the lipid methyl esters were quantified by GC with a SP-2560 fused silica capillary column. Milk epithelial cells were extracted from the fresh milk by the Boutinaud method. All data were evaluated by GLM of SPSS using ANOVA. On the basis of *cis*-9, *trans*-11 CLA contents, the animals were grouped into group I (0.59% of total lipids;

n = 7), II (0.85% of total lipids; n = 8) and III (1.02% of total lipids; n = 11). Protein aliquots extracted from milk epithelial cells were pooled in each group. Differences in protein expression among these groups were compared with 2-DE. The differently expressed spots (III/I; ≥ 2 or ≤ 0.5) were identified using ESI-Q-TOF and a protein search engine. Although animals were offered the same diet, the content of *cis*-9, *trans*-11 CLA in group III (1.02 ± 0.10%) was twice as high as that in group I (0.59 ± 0.14%) ($P < 0.05$). The groups I, II and III showed significant differences in the protein expression profiles of milk epithelial cells ($P < 0.05$). One upregulated (CATHL5) and 3 downregulated proteins (ANXA1, ZWINT, CSN3) were found and they varied similarly as the pattern of *cis*-9, *trans*-11 CLA contents. In conclusion, the different levels of *cis*-9, *trans*-11 CLA de novo synthesis of dairy cows may associate with these identified proteins expressed in milk epithelial cells.

Key Words: *cis*-9, *trans*-11 CLA de novo synthesis, protein expression profiles, purified milk epithelial cells

W155 Modification of protein synthesis of bovine mammary epithelial cells induced by heat shock. H. Hu, J. Q. Wang,* D. P. Bu, L. Y. Zhou, and P. Sun, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing.*

The objective of this study was to determine the effect of heat stress on protein synthesis by Chinese bovine mammary epithelial cells (CMEC). The CMEC has been identified to be a normal and available epithelial cell line. The CMEC cultured in vitro were exposed to 42°C for 0.5, 3, 5, 8, 12 h, and controls were exposed to 38°C. Heat shock protein (Hsp) and casein expression of gene and protein were detected by qRT-PCR and ELISA, separately. The mean, SD, and *P* values were calculated from triplicated experiments using SARS (9.0). The Student′s *t*-test was used to calculate *P*-values for comparison, and the significant statistics was set at a *P*-value < 0.05. The transcription of Hsp genes (*hsp27*, *70* and *90*) was enhanced ($P < 0.05$) as of 0.5 h of heat stress. The peak of transcription was observed at 3 h after the onset of thermal treatment. Although the transcription rates returned subsequently to baseline values, they were still upregulated ($P < 0.05$) compared with controls at 12 h. The expression of Hsp27, 70 and 90 increased at different rates. The expression of Hsp27 and Hsp70 increased gradually during the first 8 h, and then decreased, yet all values were higher than those of the control over the entire thermal stress period ($P < 0.05$). On the other hand, the Hsp90 expression only increased at 8 h. Among the Hsp, Hsp70 was acutely synthesized, and the transcription and protein expression of Hsp70 were 10 times and 3 times greater than the control group at 0.5 h and 8 h, respectively. The major milk protein genes β-casein (*CSN2*) and butyrophilin (*BTN1A1*), which are markers for the secretion capacity of bovine mammary epithelial cells, were both downregulated with heat stress from 0.5 h to 12 h ($P < 0.05$). Total casein synthesis decreased from 3 to 8 h ($P < 0.05$), corroborating the decline in milk protein secretion. Results suggest that under heat stress, normal biological activities of CMEC are disturbed and the decrease in milk protein synthesis of epithelial cells contributed to synthesize heat shock proteins thereby protecting cells from heat damage. This result might potentially explain the reduction of milk formation induced by heat stress.

Key Words: mammary epithelial cells, heat stress, heat shock protein

W156 Choline and methionine affect oxidative stress in a bovine mammary epithelial cell line. L. Pinotti*[1], E. Skrivanova[2], R. Rebucci[1], E. Fusi[1], F. Cheli[1], and A. Baldi[1], *[1]Department of Veterinary Sciences and Technology for Food Safety, Università degli Studi di Milano, Milan, Italy, [2]Institute of Animal Science, Prague, Czech Republic.*

The aim of the present study was to investigate the role of choline and methionine in modulating the oxidative stress induced by hydrogen peroxide in bovine mammary epithelial cells. The BME-UV1 cell line has been used as an in vitro model of the bovine mammary epithelium. Cells were incubated with choline and methionine at 2 different concentrations: LowCM,500 μM and 715 μM for choline and methionine, respectively, or HighCM, 1000 μM and 1430 μM, for choline and methionine, respectively. The ratio between choline and methionine has been established on a molar basis. In both treatments, the cells were cultured in presence of insulin (1 μg/ml). In dose response experiments, cells were exposed to increasing concentrations of hydrogen peroxide (0 to 500 μM) to establish the half lethal concentration (LC50) of hydrogen peroxide for the BME-UV1 cell line. Cell proliferation (MTT test) was measured at different incubation times (24, 48 and 72h), whereas apoptosis (TUNEL) was measured at 48h. Incubation time affected the response to hydrogen peroxide. At the lowest range of hydrogen peroxide concentration tested (15.62 to 62.5 μM) choline and methionine significantly ($P \leq 0.05$) enhanced cell viability on average by 19%, 21% and 25.8% after 24, 48 and 72h, respectively. Supplemental choline and methionine in the medium exerted a dose dependent effect on apoptosis of BME-UV1 cells: percentages of apoptotic cells were 0.42%, 1.35%, in HighCM and LowCM treated cells, respectively, whereas apoptosis in hydrogen peroxide treated cells was 6.81% for the same range of hydrogen peroxide concentration tested. Our results indicate that choline and methionine could play a role in counteracting oxidative damage induced by hydrogen peroxide in bovine mammary epithelial cells, even though the exact mechanism merits further investigations.

Key Words: choline, methionine, bovine mammary epithelial cell