M142 Expression of the development gene CAMK2G in the virgin mammary gland of the dairy goat. L. N. Wang, C. Li, Q. Z. Li*, and C. Y. Yuan, *Key Laboratory of Dairy Science of Education Ministry, Northeast Agricultural University*, *Harbin, China*.

Identification of the key genes related to mammary gland development in the dairy goat is important for optimizing milk production. In this research, after comparing the expression of ESTs in 7 Long-SAGE libraries corresponding to different stages of development (early virgin, late virgin, early pregnancy, late pregnancy, middle lactation, early involution and late involution) and sequence alignment with BLAST in the sheep library, we found the gene CAMK2G to be an important gene that affected the development of the mammary gland in virgin dairy goats. mRNA was extracted from healthy virgin mammary glands of dairy goat, and then amplified by RACE (rapid amplification of cDNA ends). We synthesized the 3′- and 5′-RACE-fragments by using the CAMK2G's EST from the virgin mammary gland long-SAGE library as the gene-specific primer, then cloned and sequenced RACE fragments to obtain the full-length gene. The anticipated length of the 3′cDNA of gene CAMK2G was about 500 bp, and 5′cDNA was about 1500 bp. Comparing the homology with the sheep EST library provided by NCBI GenBank confirmed that the cloned gene was CAMK2G. We next performed an RNAi (RNA interference) experiment. The synthesized siRNA was based on the full-length of the gene CAMK2G and transfected into cells that were subcultured from primary epithelial cells of the virgin mammary gland. After the detection of cell viability analyzer and flow cytometry, both the proliferation and the activity of mammary epithelial cells were inhibited ($P < 0.05$). Real-time PCR showed that the expression of CAMK2G was lower after RNAi ($P < 0.01$). Addition, Western blotting showed that the expression of β-casein was decreased ($P < 0.05$). The experiments above were repeated 3 times. Thus, the gene CAMK2G may be important in mammary gland development of the virgin goat.

This work was supported by the National High Technology Research and Development Program of China (Grant No. 2006AA10Z1A4).

Key Words: dairy goat, mammary gland development, CAMK2G

M143 Effects of thyroxine, glucagon and insulin on mRNA levels of heat shock proteins in bovine mammary epithelial cells under heat stress in vitro. R. L. Cui[1], J. Q. Wang*[1], H. Y. Wei[1], D. P. Bu[1], H. Hu[1,2], and L. Y. Zhou[1], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*Faculty of Animal Science & Technology, Gansu Agriculture University*, *Lanzhou, China*.

The objective of this study was to establish the effects of thyroxine (T4), glucagon and insulin on the mRNA levels of heat shock proteins (HSPs) in bovine mammary epithelial cells under heat stress in vitro. The mammary epithelial cells were cultured in DMEM/F12 media containing 10% fetal bovine serum (FBS) at 38°C until they reached 80% confluence. T4 (0, 0.01, 0.1, 0.5, 1 and 2 μmol/L), glucagon (0, 0.01, 0.1, 0.5, 1 and 2 μmol/L) and insulin (0, 0.005, 0.01, 0.1, 0.5 and 1 μmol/L) were separately added into the media then cells were exposed at 42°C for 12 h as a heat stress model and 38°C for 12 h as the control. Levels of HSP mRNA were detected by RT-qPCR. Each treatment had 3 replicates in this experiment, and one-way ANOVA of SAS was used to analyze the experimental data. The results indicated that (1) At 38°C, HSP90 did not significantly change with insulin (0.005, 0.01, 0.1, 0.5 and 1 μmol/L) and T4 (0.01, 0.1, 0.5 and 1 μmol/L) ($P > 0.05$); HSP70 increased markedly with 0.005 and 1 μmol/L insulin ($P < 0.05$), and did not change markedly with 0.01, 0.1 and 0.5 μmol/L insulin ($P > 0.05$); However, other treatments all significantly decreased the mRNA levels of HSP27, 70, 90 and heat shock factor-1 (HSF-1) ($P < 0.05$). (2) The mRNA levels of HSP27, 70, 90 & HSF-1 were all significantly lower after cells were cultured with different concentrations of T4, glucagon and insulin separately at 42°C for 12 h ($P < 0.01$). In conclusion, T4, glucagon and insulin could inhibit mRNA levels of HSPs in mammary epithelial cells under heat stress.

Key Words: bovine mammary gland epithelial cells, heat shock proteins, FQ-PCR

M144 Immunodetection of the secreted forms of osteopontin in bovine milk. N. Bissonnette[1,3], C. Thibault[1], and G. Robitaille*[2], [1]*Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre*, *Sherbrooke, Qc, Canada*, [2]*Agriculture and Agri-Food Canada, Food Research and Development Centre*, *Saint-Hyacinthe, Qc, Canada*, [3]*Université de Sherbrooke*, *Sherbrooke, Qc, Canada*.

Osteopontin (OPN), a phosphoglycoprotein, presents in human a complex pattern of gene expression (splicing variants) which is tissue and physiological state-dependent. The concentration reaches 1 g/L and varies during lactation. Bovine OPN is secreted in lower but substantial amounts (10 mg/L) in bovine milk. The aim of this study was to monitor bovine OPN and to identify the different isoforms in milk. Four human and one mouse commercial anti-OPN targeting different portions of the human OPN were tested on bovine OPN. In addition, 2 antisera were raised against synthetic peptides designed from the NCBI refseq bovine sequence and one was raised against the protein that was purified from milk by HPLC; the purification method allows the isolation of a significant amount of pure protein. Purified and commercial bovine OPN were characterized by silver coloration and ECL Western blot analysis. The signature of both sources was identical as detected by silver coloration. One of the 3 human anti-OPN, which targeted the N-terminal part of the protein, profiled 2 bovine OPN isoforms at 65 and 40 kDa, whereas 3 human anti-OPN failed to detect purified OPN. The bovine anti-OPN (synthetic peptide) and the anti-mouse-OPN, which are highly specific to the C-terminal part of the protein, detected only the 65 kDa isoform. The bovine antisera (purified OPN from milk) recognized both forms and other forms in milk. Preliminary ELISA assays allowed a specific detection of bovine OPN. Using these antibody preparations it is possible to determine the abundance of each of these isoforms in milk. In conclusion, analysis of the bovine OPN reveals the presence of 2 isoforms. The immunodetection of the respective isoforms will allow to determine their relative abundance during lactation and to speculate on their local bioactivity.

Key Words: osteopontin, bovine, milk

M145 Differentiated immortalized porcine mammary epithelial cells grown on polysulfone hollow fiber provide a potential cell culture system for expression of recombinant proteins. T. C. Kuan*[1], Y. L. Sun[2], C. Y. Yen[1], and C. S. Lin[1], [1]*Department of Biological Science and Technology, National Chiao Tung University*, *Hsinchu, Taiwan*, [2]*Division of Biotechnology, Animal Technology Institute Taiwan*, *Miaoli, Taiwan*.

Spontaneously immortalized porcine mammary epithelial cells (PMEC) isolated from the mammary gland of lactating sows can express milk

proteins following differentiation into mammary gland-like structures (gland ducts, lateral buds, and alveoli). Hollow fiber bioreactors have been used for large-scale mammalian cell culture to produce monoclonal antibodies or recombinant proteins. The hollow fibers provide a culture system with a high surface to volume ratio. The system allows extremely efficient exchange of nutrients and waste products across the fiber wall. Therefore, we attempted to culture PMEC transformed with recombinant DNA in these hollow fiber bioreactors to produce recombinant proteins. In the present study, we investigated the optimum conditions for culturing the PMEC onto polysulfone hollow fibers (PHF). The results showed that the seeded PMEC could attach, grow and form monolayers on the surface of PHF. The PMEC could differentiate into mammary gland-like structures when the cells were grown on PHF coated with Matrigel. The regulatory region of the milk gene, α lactalbumin, was inserted upstream of luciferase cDNA to generate a recombinant DNA, pAL-luc. The pAL-luc was transformed into PMEC and used to test the potential for recombinant protein production by PMEC cultured on PHF. Luciferase activity expressed from the PMEC grown on the PHF coated with 2.5 mg/ml and 5 mg/ml Matrigel were increased by 2.7-fold and 7.0-fold compared with that of the cells grown on the PHF without Matrigel (n = 3). Moreover, prolactin supplementation enhanced the luciferase expression. In this established PHF-cell culture approach, the PMEC could be continuously cultured for one week and potentially express recombinant protein. In conclusion, we have provided a potential PHF-cell culture approach in which PMEC can differentiate and express recombinant protein.

Key Words: mammary epithelial cells, polysulfone hollow fiber, recombinant protein

M146 Effects of nutrient restriction on mammary cell activity and hormonal statement in lactating dairy cows. F. Dessauge*[1,2], V. Lollivier[1,2], E. Cutullic[1,2], J. Portanguen[1,2], C. Disenhaus[1,2], S. Barbey[3], B. Ponchon[1,2], and M. Boutinaud[1,2], [1]*INRA UMR 1080 Dairy Production*, *35590, Saint Gilles, France*, [2]*Agrocampus UMR 1080 Dairy Production*, *35000, Rennes, France*, [3]*INRA UE 326 Domaine Experimental du Pin au Haras*, *61310, Le Pin au Haras, France*.

Feed restriction results in milk yield (MY) decrease. However the consequences on mammary activity and the involvement of prolactin (PRL) in the lower mammary synthesis are not known. The aim of the study was to investigate the effects of nutrient restriction on mammary epithelial cell activity in lactating dairy cows. We used 15 Holstein × Normande crossbred dairy cows, divided into 2 groups submitted to 2 feeding levels. From calving to wk 11 postpartum, the cows were fed a total mixed ration composed either of 55% maize silage, 15% dehydrated alfalfa and 30% concentrate (Basal diet-group as control, n = 7) or of 60% grass silage and 40% hay (Restricted diet-group, n = 8). Cows were milked twice daily. MY and composition were measured. Blood samples were harvested at wk 11 postpartum for the determination of PRL concentration. After 11 weeks of lactation, cows were slaughtered and mammary glands were removed and weighted. Expression of proteins involved in secretory activity was evaluated on mammary tissue by real-time qPCR and immunohistochemical staining was performed. Restricted diet-group cows had lower 11-week average daily MY (20.5 kg/d vs. 35.5, $P < 0.001$) and lower milk protein and lactose content from calving to slaughter than Basal diet-group cows. The size of the mammary acini were lower (−41%, $P < 0.01$) in the Restricted diet-group. Nutrient restriction decreased kappa-casein ($P < 0.01$) and α-lactalbumin ($P < 0.01$) mRNA levels in the mammary gland. The decrease in mean PRL concentration was not significant (−27%, $P = 0.15$) in Restricted diet-group compared with Basal diet-group. In conclusion, nutrient restriction resulted in a lower MY in lactating dairy cows. This was partly due to a modulation of mammary epithelial cells activity regulated at mRNA level.

Key Words: nutrient restriction, mammary epithelial cell, prolactin

M147 Effects of incremental sunflower seed supplementation on milk composition and mammary expression of genes regulating fatty acid uptake and lipogenesis. J. W. Møller, T. Bjørn, P. K. Theil, M. T. Sørensen, and K. Sejrsen*, *Faculty of Agricultural Science, Aarhus University*, *Tjele, Denmark*.

Dietary supplementation with unsaturated fatty acids is a well-established strategy for enhancing milk fat content of unsaturated fatty acids. The objective was to examine the effects of increased sunflower seed supplementation (SFS) on milk fat composition and mammary expression of genes regulating fatty acid uptake and lipogenesis. Twenty 4 lactating Holstein Friesian cows (186 ± 20 DIM; 25.3 ± 2.5 kg/d) were randomly assigned to 4 groups and fed a control diet or diets supplemented with 5%, 10%, or 15% sunflower seeds (% of DM) for a 5 week experimental period. DM intake and milk yield was reduced in cows fed 10% and 15% SFS when compared with control, whereas 5% SFS did not differ from control. All levels of SFS tended to increase milk fat content ($P = 0.08$). SFS decreased content of C4–14 ($P = 0,008$) and C16 fatty acids ($P = 0.014$). Content of ≥ C18 fatty acids was increased ($P = 0.015$). SFS increased the level of unsaturated fatty acids ($P < 0.001$) when compared with control and increased in a linear manner ($P < 0.001$) the content of rumenic acid (C18:2 c9t11) from 0.3% to 0,9%. Gene expression was analyzed by RT-PCR on RNA from mammary biopsies using the ΔΔCT method. SFS (5–15%) reduced mRNA abundance of SREBP-1 ($P = 0.034$), SCAP ($P = 0.0075$), FASN ($P = 0.035$), FADS1 ($P = 0.006$), and SCD ($P = 0.046$). mRNA abundance of ACC tended to be reduced ($P = 0.06$). SFS did not affect expression of the lipid uptake and transport genes LPL ($P = 0.189$), FABP3 ($P = 0.862$), and FAT ($P = 0.403$). In conclusion, our results show that dietary supplementation with high levels of unsaturated fatty acids in form of sunflower seeds leads to increased milk fat content in spite of decreased de novo milk fat synthesis as substantiated by a reduction in lipogenic genes. Furthermore, the expression of genes regulating fatty acid uptake and transport were unaffected although the amount of dietary fat present in milk was increased.

Key Words: dietary supplementation, mammary gene expression, conjugated linoleic acid

M148 Principal component analysis of milk fatty acid composition and the relationships between stearoyl CoA desaturase genotype and conjugated linoleic acid production in dairy cattle. J. Thomson*, L. Clark, M. Oba, and S. Moore, *University of Alberta*, *Edmonton, AB, Canada*.

The objectives of this study were to assess the relationships between individual milk fatty acids and conjugated linoleic acid (CLA) concentration in bovine milk fat and to assess the relationship between a single nucleotide polymorphism in the stearoyl CoA desaturase gene and CLA production using principal component analysis (PCA). 215 cows from an Alberta commercial dairy farm were genotyped and milk samples were collected for milk fatty acid analysis. Forty-three variables including milk production parameters, individual fatty acid concentrations, and indices of desaturation were analyzed. The first 3 principal components explained 47.61% of the total variance (PC 1, 24.13%; PC 2, 13.95%; and PC 3, 9.53%). The first PC had high loadings for most of the short chain fatty acids, the second PC had high loadings for the yield

measurements, and the third PC had high loadings for long chain fatty acids. Thus, the majority of the variables were described by 3 principal components. There was a positive correlation among the short chain or de novo synthesized fatty acids (C4-C16) ($P < 0.05$), and a negative correlation between the de novo synthesized fatty acids and the long chain fatty acids which primarily come from peripheral circulation (C18 and C20 isomers) ($P < 0.05$). CLA concentration had a positive correlation with C18:1 t6–8, C18:1 t9, C18:1 t10, C18:1 t12, C18:1 t13–14 and 18:1 t11 ($P < 0.05$). CLA was negatively correlated with C18:1 c9 ($P < 0.05$). A high positive correlation between the concentration of C12:0 with CLA yield (coefficient of 0.85, $P < 0.0001$) was observed. The CLA desaturation index (CLA /18:1t11 + CLA) was positively correlated to C8:0, C10:0, C12:0, C14:0, C14:1, and C16. This relationship may warrant further research. Genotype did not explain a significant amount of the variation in these data. This suggests that fatty acid origin (de novo synthesized fatty acids vs. fatty acids from circulation) had a much bigger impact than genotype on fatty acid variation in milk.

Key Words: stearoyl CoA desaturase, milk fatty acids

M149 Improved lactation persistence and altered milk composition in growth hormone-treated mice is not linked to dramatic changes in mammary mitochondrial biogenesis or the degree of mTOR or AMP kinase phosphorylation. W. Olea*[1], A. Parlow[2], R. Collier[3], and D. Hadsell[1], [1]*Baylor College of Medicine*, *Houston, TX*, [2]*Harbor-UCLA Medical Center*, *Torrance, CA*, [3]*University of Arizona*, *Tucson*.

Previous work in our lab has shown that mice treated with growth hormone (GH) under both ad-libitum (AL) and reduced (4X) nursing frequency can support greater litter gain during prolonged lactation. However, the mechanism of this response is not well understood. Our hypothesis was that GH treatment would increase milk production or alter milk composition through effects on signaling pathways regulating protein synthesis or mitochondrial biogenesis. To test this hypothesis we compared milk concentrations of fat, protein, and lactose, mammary mitochondrial (mt) DNA copy number, and the phosphorylation of mammalian target of rapamycin (mTOR) and AMP kinase (AMPK) in mammary tissue from lactating mice treated (n = 6/treatment) with either saline (SAL) or recombinant murine GH (18 mg/kg/day) under conditions of either AL or 4X nursing frequency during prolonged lactation. GH treatment increased (main effect, $P < 0.05$) milk triglyceride (172.47 ± 25.42, 321.50 ± 33.33, 185.27 ± 51.75, and 271.45 ± 52.07 mM for AL-SAL, AL-GH, 4X-SAL, and 4X-GH, respectively), and protein concentrations (93.68 ± 7.02, 109.63 ± 9.84, 81.68 ± 6.32, 106.64 ± 9.78 mg/mL for AL-SAL, AL-GH, 4X-SAL,4X-GH, respectively). Mitochondrial DNA copy number, measured as the ratio of cytochrome-B to β-Actin by real time qPCR, was not significantly impacted by nursing frequency or GH treatment. Immunofluorescent staining for Phospho-mTOR and phospho-AMPK were also not significantly affected by nursing frequency or GH treatment. Staining for total mTOR, however, was higher in GH treated animals than SAL (main effect, $P < 0.01$). These results support the conclusion that although GH treatment increases milk production and alters milk composition during prolonged lactation in mice, this response is not mediated through dramatic alterations in the phosphorylation of mTOR or AMPK or through affects on mitochondrial biogenesis.

This project supported by National Research Initiative Competitive Grant no.2007–35206–17831 from the USDA CSREES.

Key Words: growth hormone, persistence, mitochondria