M142 Distribution and analysis of milk fat globule and crescent in Murrah buffalo and crossbred cow. Ratan K. Choudhary*, Harmanjot Kaur, Shanti Choudhary, and Ramneek Verma, *School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab, India.*

India is the largest buffalo milk producer in the world. Buffalo milk is rich in minerals like calcium and magnesium, contains 100% more fat and less cholesterol than cow milk. Physiochemical properties of dairy products depend upon the size and distribution of milk fat globules (MFG). Individual MFG from Murrah buffalo were analyzed as a function of size and distribution and comparisons were made with MFG of crossbred (Holstein Friesian × Sahiwal) cows. Main objectives of this study was to measure areas of buffalo MFG and analyze percentage of crescents associated with MFG using a simple microscopic technique. Crescents of MFG can provide an alternative source of RNA of mammary epithelial cells origin. Acridine orange staining of fresh milk was visualized by light microscopy under red and green channel filters. MFG and MFG with crescents were analyzed using ImageJ image analysis software. The percentage of large sized MFG (area >20 μm^2) was greater and the percentage of small sized MFG (area <10 μm^2) was lower in buffalo than cow milk. The percentage of crescents was < 1% of total MFG in both the species. These results indicate that MFG in buffalo milk is larger than that MFG of cow milk. RNA isolation from buffalo milk fat and its suitability for microarray analysis remains to be determined.

Key Words: buffalo, milk fat globule, crescent

M143 Fatty acid synthase (FASN) gene polymorphism and early lactation milk fat composition in Xinong Saanen goats. Abiel b Haile*, Zhang Wei, Wang Wei, Yang Dikun, Yi Yongqing, and Luo Jun, *Northwest A&F University, Yangling, Shaanxi, China.*

The fatty acid synthase (*FASN*) gene codes for a multifunctional protein, catalyzing the de novo fatty acid synthesis. Its high mRNA expression in GMEC (goat mammary epithelial cells), coinciding with medium to high heritability of milk fat, substantiated its role as a candidate gene for association analysis with milk fat profile. The main objectives of the study were to develop markers of *Capra hircus* FASN gene to improve healthfulness of goat milk FAs (fatty acids) and to investigate early lactation milk fat profile. A Linear mixed model association analysis of 46 dependent variables, analyzed from 300 milk samples 30 d postpartum, with 3 intragenic SNPs and 2 haplotype groups was conducted. The results had revealed associations of FASN SNPs and haplotype groups with myristic acid (C14:0), palmitic acid (C16:0) and linoleic acid (C18:2 n-6, cis). Herd was the predominant factor affecting fatty acid levels followed by herd-test-day (HTD) and parity, indicating the niche of nutrition and management in modulating early lactation milk fat profile. De novo FAs (C6:0 to C14:0) showed suppressed levels with strong negative correlations with C18 FAs. Contradicting to the presumed source of origin, butyric acid (C4:0) was impervious to the observed de novo FA depression, it had shown unique correlation patterns. Oleic acid (C18:1 *cis*-9) scored the highest mean value followed by palmitic acid. Palmitic acid had shown normal levels with weak negative correlations with C6:0 and C8:0. Here, its dual source of origin supposedly had played a role, in replenishing the diminished levels of de novo contribution, by the high C16 FAs bioavailability of body fat mobilization, which masked the inhibitory effect of long chain FAs. The correlation patterns of odd chain FAs with CLA and rumen derived biohydrogenation intermediates reinforce the supposition that odd chain fatty acids could serve as a diagnostic tool of rumen condition and biohydrogenation patterns. The SNP markers developed in the present study will assist in marker assisted selection, and the comprehensive analysis of early lactation milk fat profile will help to decipher main factors of variation, thereby aiding fatty acid profile modulation.

Key Words: dairy goat, SNP marker, milk fat

M144 Pattern of immunoglobulin A in milk from gorillas throughout lactation. M. Garcia*[1], M. L. Power[2], and K. M. Moyes[1], *[1]Department of Animal and Avian Sciences, University of Maryland, College Park, MD, [2]Smithsonian Conservation Biology Institute, Washington, DC.*

In cases when mother's milk is not available to a great ape infant (e.g., rejection), zoo nutritionists/veterinarians are responsible for formulating a milk replacer to maximize the chances of survival of the neonate. To accomplish this, they generally use milk from commercially available species (e.g., cow and goat) as well as nutritional supplements and other additives. As is the case in humans, placental transfer of immunoglobulin (Ig)A occurs throughout pregnancy in the gorilla. After birth, neonates rely solely on nutrients and Igs via the mother's milk until approximately 5 mo of age and are weaned at 3 to 4 years of age. Unfortunately, there is currently no useful information regarding the IgA profile of milk and how stage of lactation alters IgA concentration in gorilla milk. The objective of this study was to identify the pattern of IgA concentrations in milk from gorillas throughout lactation. Milk samples from 4 gorillas were collected between January 2009 and October 2014 and stored at −80°C until further analysis. Concentrations of IgA in milk were measured using a Human IgA ELISA kit. Data were grouped according to month in milk (MIM; 0 < MIM = 5, 5 < MIM = 10, 10 < MIM = 15, 15 < MIM = 36, and MIM >36). The data were analyzed as a complete randomized design with gorilla nested within MIM as a random term and MIM as a fixed effect. Month in milk had a significant ($P < 0.01$) affect IgA concentrations. Concentrations of IgA were highest for MIM >36 (81.3 µg/mL) followed by 0 < MIM = 5 (53.9 µg/mL). The increase in concentrations of IgA in later lactation may reflect reductions in milk production and this warrants further investigation. Results from this study provided a better understanding of the IgA profile of milk from gorillas. Additional knowledge regarding fluctuations of other immunoglobulins as well as nutritional profiles in gorilla milk will assist professionals charged with their care in captivity to develop improved hand-rearing protocols.

Key Words: gorilla, immunoglobulin A, milk

M145 Peroxisome proliferator-activated receptor gamma (PPARγ) agonist does not stimulate mammary lipogenic gene expression or overcome the effect of *trans*-10,*cis*-12 conjugated linoleic acid (CLA) in lactating ewes. Eveline C. Sandri[1], Elvis Ticiani[1], Monica Urio[1], Maurício Camera[1], Ana P. Povaluk[1], Kevin J. Harvatine[2], and Dimas E. Oliveira*[1], *[1]Santa Catarina State University/CAV, Lages, Santa Catarina, Brazil, [2]Penn State University, State College, PA.*

Milk fat synthesis involves biochemical processes, including fatty acid synthesis, uptake, transport and desaturation. *Trans*-10,*cis*-12 CLA inhibits milk fat synthesis by decreasing the expression of genes

and transcription factors. Peroxisome proliferator-activated receptor gamma (PPARγ) is a key regulator of lipid synthesis in many tissues and is affected by *trans*-10,*cis*-12 CLA but, the mechanisms by which *trans*-10,*cis*-12 CLA suppresses the expression or activity of PPARγ and expression of its targeted genes are not clear. This study used a chemical PPARγ agonist to evaluate the effect of PPARγ on mammary lipid synthesis and its interaction with *trans*-10,*cis*-12 CLA in lactating ewes. Twenty-four crossbred lactating ewes [70 ± 3 DIM; 60 ± 0.45 kg body weight (BW)] were randomly assigned to one of the 4 treatments (n = 6/treatment) for 7 d. Treatments were (1) Control (intravenous infusion of 100 mL/d of saline); (2) TZD (intravenous infusion of 4 mg/kg of BW per d in 100 mL of saline); (3) CLA (27 g/d orally dosed methyl ester containing 29.9% *trans*-10,*cis*-12 CLA); and (4) TZD+CLA. Mammary and adipose tissue biopsies were taken, RNA was extracted, cDNA synthesized and qRT-PCR analysis conducted for PPARγ, SREBP1 and SCD1. Compared with control, fat content was 22.3% lower in CLA ($P = 0.05$), tended to be 20.7% lower in TZD+CLA ($P = 0.06$). In the mammary gland, CLA decreased expression of PPARγ, SREBP1 and SCD1 by 64.4, 60 and 19% compared with control ($P = 0.02$, $P = 0.01$ and $P = 0.005$, respectively), confirming its negative effects on the expression of lipogenic genes. However, TZD did not stimulate the expression of these genes or overcome the effect of CLA in mammary tissue. In adipose tissue, expression of PPARγ were not affected by treatment, whereas SREBP1 expression was increased by TZD, CLA and TZD+CLA compared with control and SCD1 expression was higher in TZD+CLA compared with the other treatments. Overall, CLA negatively affected mammary expression of genes involved in lipid synthesis and TZD was unable to overcome those effects demonstrating that the mechanism of CLA is not dependent on inhibition of PPARγ

Key Words: lipogenesis, milk fat depression, milk synthesis

M146 Effect of *trans*-10,cis-12 CLA on the expression of genes involved in milk fat synthesis in ovine mammary gland tissue cultured in vitro. Monica Urio, Ana P. Povaluk, Humberto T. Borges, June A. Favaretto, Maurício Camera, and Dimas E. Oliveira*, *Santa Catarina State University/CAV, Lages, Santa Catarina, Brazil.*

Culturing lactating mammary explants allows evaluating the effect of bioactive molecules in a controlled environment while maintaining the same characteristics found in vivo (Keys et al., 1997; In Vitro Cell. Dev. Biol. Animal, 33:206–21). However, studies using ovine mammary tissue are still scarce. The objective of this study was to evaluate the effect of *trans*-10,*cis*-12 CLA on the expression of key genes involved in milk fat synthesis in mammary explants cultured in vitro. Mammary samples were obtained through biopsies in lactating ewes (120 DIM) and were grown on plates with growth area of 1.9 cm^2 in mammary epithelial cell growth media supplemented with fetal bovine serum, antibiotics, insulin and growth factors at 37°C with 5% CO_2 and humidity saturated. Tissues were cultured for 3 and 24h in triplicates using the following treatments: 75 µmol/L of *trans*-10,*cis*-12 CLA or Control (no CLA). After cultures were stopped RNA was extracted, cDNA synthesized and qRTPCR performed. The genes studied were SREBP1 (sterol regulatory element-binding protein), PPARγ (peroxisome proliferator-activated receptor gamma) and SCD1 (stearoyl-CoA desaturase). Statistical analysis was performed using the MIXED procedure of SAS (2002) and ribosomal protein S18 housekeeping gene was used as a covariate in the model. CLA treatment had no effect on gene expression of SREBP1, PPARγ, and SCD1 at 3 h ($P = 0.19$, $P = 0.52$ and $P = 0.20$, respectively). At 24 h, there was a trend for CLA-treated explants to have reduced gene expression of SREBP1 and PPARγ ($P = 0.07$ and $P = 0.08$, respectively), and a 12-fold decrease in SCD1 gene expression ($P = 0.009$). Overall, our results suggest that *trans*-10,*cis*-12 CLA downregulates expression lipogenic genes in ovine mammary tissue, however explants may need to be cultured for at least 24 h

Key Words: dairy ewe, gene expression, milk fat depression

M147 Gene expression of transcription factors and genes involved in milk fat depression in lactating ewes of different body weights fed the same dose of CLA *trans*-10,*cis*-12. Monica Urio, Eveline C. Sandri, Ana P. Povaluk, Elvis Ticiani, Camila Renneberg, Maurício Camera, June A. Favaretto, and Dimas E. Oliveira*, *Santa Catarina State University/CAV, Lages, Santa Catarina, Brazil.*

During milk fat depression (MFD) induced by the feeding of *trans*-10,*cis*-12 CLA the magnitude in the reduction of milk fat content and/or yield varies considerably among animals. The objective this study was to evaluate the gene expression of transcription factors and genes downregulated during MFD in lactating ewes fed a uniform dose of *trans*-10,*cis*-12 CLA but differing in body weight. Fourteen lactating ewes were selected according to BW and separated in 2 groups that averaged 51.0 ± 1.0 and 69.0 ± 4.0 kg of BW. Ewes received for 10 d a daily oral dose of 27g/d of CLA (rumen-unprotected, 29.9% *trans*-10,*cis*-12 isomer). Mammary and adipose tissue biopsies were performed on d 10. RNA was extracted, cDNA synthesized and qt-RTPCR carried out. Data were analyzed using the MIXED procedure of SAS (2002) and ribosomal protein S18 housekeeping gene was used as a covariate. Gene expression of SREBP1 (sterol regulatory element binding protein), S14 (thyroid hormone responsive) and SCD1 (stearoyl-CoA desaturase) were evaluated in mammary tissue samples and leptin in adipose tissue. Gene expression of SREBP1, S14 and SCD1 did not differ between the BW groups ($P = 0.29$, $P = 0.17$ and $P = 0.57$, respectively). Leptin gene expression was higher in the heavier BW group ($P = 0.02$). Overall, our results suggest that animals of different body weights receiving a uniform dose of *trans*-10,*cis*-12 CLA showed change in gene expression of leptin.

Key Words: fat synthesis, mammary biopsy, ovine

M148 Expression of acetyl-CoA carboxylase alpha (ACCα) transcripts from different promoters in mammary and adipose tissue from lactating ewes at different stages of lactation. Grégory J. Cardoso[1], Elvis Ticiani[1], Monica Urio[1], Ana P. Povaluk[1], Maurício Camera[1], Rogério Ferreira[2], Kevin J. Harvatine[3], and Dimas E. Oliveira*[1], *[1]Santa Catarina State University/CAV, Lages, Santa Catarina, Brazil, [2]Santa Catarina State University/CEO, Chapecó, Santa Catarina, Brazil, [3]Penn State University, State College, PA.*

Initiation and maintenance of lactation are complex phenomena involving cellular and enzymatic changes including those in mammary gland and adipose tissue. Fat is the main energy component of the milk, originating from the diet, mobilization from adipocytes, and from de novo synthesis in the mammary gland. Milk fat synthesis is controlled in various ways and one example is via regulation of acetyl-CoA carboxylase α (ACCα), a key enzyme in the de novo fatty acid synthesis pathway. ACCα is encoded by mRNAs transcribed from 3 promoters (PI, PII and PIII) characterized as tissue-specific in the ovine genome. PI transcripts are restricted to the adipose tissue and liver, whereas PII is found in all tissues and PIII has been isolated from mammary tissue from lactating ewes. This study evaluated the transcription of ACCα from PI, PII and PIII in mammary and adipose tissue from dairy ewes at different stages of lactation. Mammary gland and adipose tissue biopsies were taken from 6 lactating ewes at 3 periods (15, 70 and 120 d in milk) to represent early, mid and late lactation. Subsequently RNA

was extracted, cDNA synthesized and qRT-PCR analysis conducted. Data were analyzed by SAS using ribosomal protein S18 housekeeping gene as a covariate. As expected, no expression of PI was detected in the mammary gland. Transcripts from PII in mammary gland were higher in early lactation and decreased as lactation advanced (early vs. mid $P = 0.02$; early vs. late $P = 0.002$; mid vs. late $P = 0.23$). Similarly, the PIII transcripts decreased throughout lactation in mammary gland (early vs. mid $P = 0.001$; early vs. late $P = 0.001$; mid vs. late $P = 0.005$). On the contrary, in adipose tissue the PI gene expression was higher in mid and late lactation ($P = 0.001$), and transcripts from PII and PIII did not differ among periods. Results suggest that PII transcripts in mammary glands are upregulated in early and mid lactation according to a higher requirement for fat synthesis and PI transcripts have a greater expression level in adipose tissue in late lactation likely due to a positive energy balance replenishing body fat reserves.

Key Words: adipose tissue, gene expression, mammary gland

M149 Effects of sodium salicylate on early lactation milk production parameters. Caroline M. Ylioja*, Abigail J. Carpenter, Laman K. Mamedova, and Barry J. Bradford, *Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS.*

Treatment with non-steroidal anti-inflammatory drugs (NSAID) after calving can have a positive effect on whole-lactation milk production in older dairy cows. Ongoing research aims to determine whether this response is due to increased mammary epithelial cell function and whether this is also tied to changes to inflammation in early lactation. For this study, cows in 3rd or greater lactation (n = 8/treatment) were enrolled at parturition to receive a daily drench of sodium salicylate (SS; 125g/d) or water (CON) for 3 d beginning approximately 24 h postpartum. Mammary biopsies were conducted on d 1, 4, and 45 after calving. Daily milk weights were recorded and milk composition was determined 2×/week. Blood samples were taken 2×/wk and analyzed for metabolites. Results up to 45 DIM show no difference between treatments for plasma glucose, insulin, β-hydroxybutyrate, or nonesterified fatty acid levels. Weekly means of milk production parameters revealed no detectable differences for fat content or yield, milk urea nitrogen, somatic cell score, energy-corrected milk yield, or fat-corrected milk yield. Treatment interacted with week for average protein yield ($P = 0.02$), with SS cows increasing more (1.07 to 1.49 ± 0.09 kg/d) over the 7 weeks of the study than CON (1.19 to 1.37 ± 0.09 kg/d). Lactose content tended to be greater for SS (4.89%) compared with CON (4.74 ± 0.05%; $P = 0.06$). Milk yield tended to increase more for SS (from 26.6 to 55.8 ± 2.6 kg/d) than CON (from 30.7 to 52.3 ± 2.3 kg/d) over time (interaction $P = 0.10$). Results agree with previous studies where milk production increased for salicylate-treated cows, although the small sample size in the present study limited power to observe production responses, and production data included only the first 45 d of lactation. Ongoing analysis of morphological and transcriptional changes in mammary tissue may reveal mechanisms that contribute to a sustained increase in milk production following early-lactation NSAID administration.

Key Words: NSAID, inflammation, mammary function

M150 Effect of Leu and His on casein protein synthesis via mTOR signaling pathway in bovine mammary epithelial cells. Haina Gao[1,2], Han Hu[1,3], Nan Zheng[1,3], and Jiaqi Wang*[1,3], *[1]Ministry of Agriculture-Milk Risk Assessment Laboratory, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]College of Animal Science and Technology, Gansu Agricultural University, Lanzhou, Gansu, China, [3]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Assessing the regulatory effect of individual amino acids (AA) on milk protein synthesis is vital to AA requirement models for lactation. This study employed the immortalized bovine mammary epithelial cells (CMEC-H) as a model to investigate the effects of Leu or His on mTOR signaling and casein synthesis by Western blotting. Cells were cultured in the Earle's balanced salts (EBSS) with Leu (0.45, 1.35, 5.4, 10.8 mmol/L) or His (0.15, 1.2, 4.8, 9.6 mmol/L) addition for 6h, respectively, and the EBSS without AA was set as negative control, the EBSS supplemented with 10% FBS was used as positive control. The protein band values from the AA-supplemented cells were related to their AA-deprived controls. The experimental data were analyzed using the Duncan's test for post-hoc multiple comparisons of treatment means by SAS. Meaningful relationships among phosphorylation of the signaling proteins and casein expression were quantified with simple linear regression models using the REG procedure of SAS. Differences between experimental groups were considered significant at a $P < 0.05$. The results showed that, compared with the negative control, Leu or His significantly increased the phosphorylation of mammalian target of rapamycin (mTOR, Ser2481), a binding partner of target of rapamycin (raptor, Ser792), the ribosomal protein S6 kinase 1 (S6k1, Thr389), eukaryotic initiation factor 4E (eIF4E, Ser209), eukaryotic elongation factor 2 (eEF2, Thr56) and casein synthesis ($P < 0.05$). These results suggest that the supplement of Leu or His could activate the mTOR pathway and in turn catalyze the phosphorylation of signaling protein and increase milk protein synthesis. Our linear regression model assay declared that the expression of αs1-casein was positively correlated with P-mTOR ($R^2 = 0.7820$, $P < 0.01$), P-S6k1, ($R^2 = 0.7881$, $P < 0.01$) and eEF2 ($R^2 = 0.7835$, $P < 0.01$) with a dose-dependent effect of Leu. While the expression of β-casein ($R^2 = 0.9638$, $P < 0.01$) and κ-casein ($R^2 = 0.9048$, $P < 0.01$) were positively correlated with P-eEF2 with a dose-dependent effect of His. In conclusion, our results can provide certain basic information for the further study of the regulation mechanism of Leu or His on casein expression via mTOR pathway in CMEC-H.

Key Words: leucine, histidine, mammalian target of rapamycin (mTOR)

M151 MicroRNA miR-200b regulates lactation and cell proliferation by concurrently targeting *Dnmt3a* and *Dnmt3b* in bovine mammary epithelial cells. Yanjie Bian, Chunmei Wang, Na Zhang*, Yingjun Cui, and Qingzhang Li, *Key Laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, Harbin, P.R. China.*

With the rapid development of dairy industry and improvement of human living standards, the consumption market of dairy products constantly expanding. How to increase the milk yield and optimize milk quality has become the focus of dairy industry. Although numerous microRNA (miRNA) have been verified to play critical roles in the regulation of milk protein synthesis and mammary gland development in cows, but the mechanisms underlying their effects remain unknown. Here, 6 healthy, multiparous Chinese Holstein cows with similar genetic backgrounds were divided into 2 groups according to milk quality (n = 3 per group), statistical analyses were conducted using Student's test, and $P < 0.05$ was considered statistically significant. We found that the expression of miR-200b was significantly different between high-milk-quality cows and low-milk-quality ones by using small RNA sequencing and qRT-PCR techniques. Increasing evidences suggested that epigenetic mechanism was involved in the regulation of mammary function in the

dairy cow. In recent studies, DNA methylation, as the major epigenetic regulators, has been shown to regulate the expressions of milk proteins in mammary epithelial cells. In vitro, subcultured bovine mammary epithelial cells (BMEC) were cultured in an incubator at 37°C in the presence of 5% CO_2 and were maintained in DMEM/DF-12 with 10% FBS. Our results showed that miR-200b regulated the DNA methylation level of BMECs by inversely targeting both DNMT3A and DNMT3B. We also found that the overexpression of miR-200b promoted BMECs proliferation and significantly decreased the expression of α_{S1}-casein and β-casein. The treatment of BMECs with 5-aza-2′-deoxycytidine (5-Aza-dC) increased the expression of α_{S1}-casein and β-casein. This study provided new insight into the molecular mechanisms of miR-200b in regulating lactation performance through DNA methylation, and provided a potential to optimize milk quality not only of the cows but also of their next generations.

Key Words: miR-200b, DNA methylation, bovine mammary epithelial cells (BMEC)

M152 MicroRNAs miR-181a and miR-194 can regulate the biosynthesis of milk fat and protein by targeting ACSL1 and STAT5a. S. Lian[1,2], X. M. Nan[2,3], D. P. Bu*[2,4], F. Wang[2], and J. R. Guo[1], *[1]College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, Heilongjiang, China, [2]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [3]Synergetic Innovation Center of Food Safety and Nutrition, Harbin, China, [4]CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, Beijing, China.*

MicroRNAs (miRNA) are a class of small noncoding RNAs and act as important post-transcriptional regulators of gene expression. *ACSL1* is an important gene in milk lipid synthesis and *STAT5a* is one of the key genes in milk protein synthesis. In a previous study dealing with incubation of stearic acid in bovine mammary epithelial cells, an opposite expression pattern was observed between *ACSL1* and miR-181a, *STAT5a* and miR-194. *ACSL1* and *STAT5a* were also predicted as potential target genes of miR-181a and miR-194 respectively by TargetScan and PicTar, which are the most popular target gene prediction softwares. The objective of this work was to determine the potential function of miR-181a and miR-194 on milk fat and protein synthesis by defining the regulatory relationship between miR-181a and *ACSL1*, miR-194 and *STAT5A*. In the study, primary bovine mammary epithelial cells harvested from mid-lactation cows were cultured in DMEM/F12 medium added with 10% FBS and lactational hormones. Cells were transfected with miR-181a mimic, miR-181a inhibitor, miR-194 mimic and miR-194 inhibitor, and non-transfected cells were set as control. After an 48 h treatment, expression of miR-181a, miR-194, *ACSL1*, *STAT5a* were determined by real-time reverse-transcription PCR. The results showed that the overexpression of miR-181a and miR-194 inhibited the expression of *ACSL1* and *STAT5a* respectively ($P < 0.05$), while the downregulation of the 2 miRNAs increased *ACSL1* and *STAT5a* expression ($P < 0.05$). Therefore suggesting that miR-181a and miR-194 may play important roles in milk fat and protein synthesis.

Key Words: miR-181a, miR-194, bovine mammary epithelial cells

M153 Establishment of a mammary epithelial cell line from a Chinese Holstein dairy cow and effects of different concentrations of insulin-like growth factor-I on expression of genes related to milk synthesis. T. Qin[1], H. Y. Wang[1], D. P. Bu[2], and H. B. Zhu*[1], *[1]Embryo Biotechnology and Reproduction Laboratory, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

This experiment was conducted to establish an efficient culture method for bovine mammary epithelial cells (bMEC) in vitro,a nd to research the effect of different concentrations (0, 0.1, 10 and 100ng/mL) insulin-like growth factor-I (IGF-1) on the mRNA expression of genes related to milk protein and fat synthesis in bMEC. Bovine mammary epithelial cells were cultured by tissue mass culture method, and cells were purified according to different sensitivity to trypsin of fibroblasts and bovine mammary epithelial cells. The growth curve of purified cells was detected by cell counting assay method. The expression of keratin 18 was detected by immunofluorescent histochemistry staining method. mRNA expression of 8 genes were measured by real time quantified PCR(RT-qPCR).The results showed that mRNA abundance of insulin-like growth factor-I receptor (*IGFIR*), insulin-like binding protein 3 (*IGFBP3*), α_{S1}-casein (*CSN1S1*) and k-casein (*CSN3*) did not differ ($P > 0.05$) within different concentrations of IGF-1. However, within the increasing concentrations of IGF-1, the mRNA abundance of β-casein (*CSN2*), acetyl-CoA carboxylase (*ACACA*), fatty acid synthase (*FASN*) and fatty acid binding protein 3 (*FABP3*) increased significantly ($P < 0.05$). These results indicated that IGF-1, as an important cytokine, might involve in regulating the mRNA expression of genes related to milk protein and fat synthesis in bovine mammary epithelial cells in vitro.

Key Words: insulin-like growth factor-I, bovine mammary epithelial cells, gene expression.

M154 Influence of heat stress and amino acid supplementation on microRNA expression in bovine mammary epithelial cells. M. Duque*[1], A. A. K. Salama[2], and J. J. Loor[3], *[1]Grupo de Investigación Biogénesis and GRICA, Facultad de Ciencias Agrarias, Universidad de Antioquia, Medellín, Colombia, [2]Grup de Recerca en Remugants (G2R), Departament de Ciència Animal i dels Aliments, Universitat Autónoma de Barcelona, Bellaterra, Spain, [3]Department of Animal Sciences, University of Illinois, Urbana, IL.*

Heat stress and essential amino acid profiles are important factors that can affect milk fat and protein synthesis in the mammary gland of dairy cows. MicroRNAs (miRNA) are non-coding RNAs that play a critical role in post-transcriptional regulation of bovine mammary gene expression. Regulatory microRNAs affect a wide variety of cellular processes such as cellular differentiation, proliferation, apoptosis, genome stability and milk protein and fat synthesis pathways. Although miRNA expression profiling in various bovine tissues or cells have been performed, studies to elucidate the potential role of miRNAs under stress conditions are limited or nonexistent. The objective of this study was to determine using MACT cells the effects of heat stress and amino acid (AA) supplementation on microRNA expression. A thermo neutral (TN, 37°C) and heat stress (HS, 42°C) environment with one of 3 AA supplementation groups were studied: control (Con), control + methionine (Met) and control + arginine (Arg). A total of 6 treatments were evaluated: TN-Con, TN-Met, TN-Arg, HS-Con, HS-Met and HS-Arg. After 6 h of incubation cells were harvested, and miRNAs were extracted for quantitative RT-PCR analysis. Targets included miR-141, miR-221, miR-34a, miR-200a, miR-27a/b, miR-92a, miR-99a, miR-23a, miR-26a, miR-103, and miR-184. Both HS and AA increased ($P < 0.05$) the expression of miR-141 and miR-221, which were reported to decrease STAT5 protein expression and induce angiogenesis. An upregulation ($P < 0.05$) of miR-34a, miR-200a, miR-27a/b, miR-92a, miR-99a, and miR-184 was observed in the HS group, but not with AA supplementation. These miRNAs are reported to be involved in the regulation of

heat-shock proteins, milk fat synthesis, prolactin receptor expression, cell cycle regulation, and mTOR and insulin signaling. Both miR-23a (MAPK pathway) and miR-26a (cell proliferation) were not affected ($P > 0.05$) by temperature, but Met and/or Arg decreased ($P < 0.05$) their expression. The sole miRNA unaffected by temperature or AA was miR-103. The results suggest that AA supplementation and HS can alter miRNA expression and, as such, could serve as another regulatory factor of genes that play important functions in the mammary gland.

Key Words: bovine mammary cells, miRNA, amino acid

M155 Characterization of prolactin and adrenergic and serotonergic receptors in bovine apocrine sweat glands. Courtney A. Burger, Jayne L. Collier, and Robert J. Collier*, *University of Arizona, Tucson, AZ.*

Apocrine sweat glands are involved in thermoregulation via evaporative heat loss in the bovine and are most active at temperatures above thermoneutral. We have previously shown that serotonin (5-HT) receptors 5-HT 1b, 2b, 4, and 7 are found in whole skin, apocrine sweat glands, and epithelial cells, while 5-HT 2a is present in whole skin and epithelial cells. It has been shown that prolactin (PRL) plays a role in lactational homeostasis, because mammary glands are modified apocrine sweat glands, we hypothesized that PRL receptors would be present in sweat glands. Additionally, we looked at adrenergic (ADR) receptors, as epinephrine is known to influence sweating response. The present study was conducted to determine if the 5-HT, PRL, and ADR systems are involved in thermoregulation by examining the cDNA expression of receptors through conventional PCR in bovine whole skin, apocrine sweat glands, and epithelial cells. For the 5-HT system, brain was used as control and additional isoforms classified were 5-HT 1a, 1d, 1f, 5a, and 6. We identified isoforms 5-HT 1d, 1f, and 5a in whole skin, apocrine sweat glands, and epithelial cells, whereas 5-HT 1a is present in whole skin and epithelial cells, and 5-HT6 is present in whole skin and apocrine sweat glands. For the PRL system, bovine mammary epithelial cells (BMEC) were the control and 2 isoforms were identified: PRL receptor long (PRLr-L) and PRL receptor short (PRLr-S) which differ in their intracellular domain length and sequence. We found PRLr-S in whole skin, apocrine sweat glands, and epithelial cells, while PRLr-L is in whole skin only. For ADR receptors, BMEC and liver were used as control, and we found α1 ADR 1a, α2 ADR, β1 ADR, and β2 ADR receptors in whole skin, apocrine sweat glands, and epithelial cells. α1 ADR 1b was only present in BMEC and liver, while α1 ADR 1d was only in BMECs. The presence of these receptors in the apocrine glands and epithelial cells indicates that the 5-HT, PRL, and ADR systems are involved in regulation of apocrine sweat gland function.

Key Words: apocrine, bovine, serotonin

M156 RNA-sequencing analysis of milk somatic cells in heat-stressed dairy goats. A. A. K. Salama*[1], B. Badaoui[2], S. Hamzaoui[1], and G. Caja[1], *[1]Grup de Recerca en Remugants (G2R), Departament de Ciència Animal i dels Aliments, Universitat Autónoma de Barcelona, Bellaterra, Spain, [2]Integrative Biology Group, Parco Tecnologico Padano - CERSA, Lodi, Italy.*

Milk transcriptome characterization could help in understanding how milk synthesis and mammary cell turnover are affected by heat stress. Eight multiparous Murciano-Granadina dairy goats (44.1 ± 1.7 kg BW; 2 ± 0.04 L/d; 90 ± 3 DIM) were kept in metabolic cages and randomly assigned to 2 climatic treatments according to a crossover design (2 28-d periods). Treatments were:1) thermal neutral (TN; 15 to 20°C, 40 to 45% humidity, THI = 59 to 65), and 2) heat stress (HS, 12 h/d at 37°C and 40%, and 12 h/d at 30°C and 40%, THI = 86 and 77, respectively). Milk samples were collected at d 28 of each period, somatic cells were separated, and RNA was extracted. The RNA of 4 goats for each treatment at each period was mixed, resulting in final 4 RNA samples (2 TN and 2 HS) that were analyzed by Illumina RNA-sequencing (RNA-Seq). The RNA –Seq reads were mapped to the bovine genome by "tophat" and the expression level was quantified by "cufflinks." The RNA-Seq produced a total of ~170 million reads with an average of 85 million reads for each treatment (range of reads for the 4 samples was 38 to 46 million). We detected 140,39 transcripts expressed in milk cells, 700 of them were differentially ($P < 0.01$) expressed between HS and TN. The HS reduced ($P < 0.01$) the expression of genes related to de novo fat synthesis (*ACACA* and *FASN*), fatty acid desaturation (*SCD*), milk fat globule formation (*BTN1A1*, *XDH*, and *GLYCAM1*), and protein and lactose synthesis (*CSN1S1*, *CSN1S2*, *CSN3*, and *LALBA*). The HS upregulated ($P < 0.01$) cathepsin genes (*CTSB*, *CTSD*, *CTSZ*, *CTSS*, *CTSC*, and *CTSL1*) as well as genes related to plasminogen pathway (*PLAU*, *PLAUR*) and ubiquitin-proteasome pathway (*FBXW2*, *UBAP1*, *RPS27A*). Furthermore, HS increased ($P < 0.05$) the expression of the pro-apoptotic tumor necrosis factor receptors superfamily (TNFRSF1A and TNFRSF1B). Seven miRNA (miR-23a, miR-24–2, miR-27a, miR-29c, miR-29d, miR-142, and miR-221) had a greater ($P < 0.01$) expression with HS. In conclusion, heat stress reduces the synthetic capacity of mammary cells and increases the expression of proteases in milk. This increase in proteases expression could negatively affect milk coagulation properties and cheese making using milk produced from heat-stressed goats.

Key Words: RNA-sequencing, heat stress, dairy goat