M172  **Rapid and efficient method of total RNA isolation from milk fat for transcriptome analysis of mammary gland.** S. Choudhary and R. K. Choudhary*, School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab, India.

Isolation of good quality ribonucleic acid (RNA) from mammary glands of elite animals is often hindered by invasive method of mammary tissue sampling, ethical permission, time consuming, and repeated biopsies from the same animal at different time points. The aim of this study was to optimize a protocol for RNA isolation from milk fat globules (MFG) that is suitable for transcriptome analysis. We isolated good quality RNA from milk fat of goats and buffalo milk by combining 2 methods namely Trizol and GenElute mammalian RNA isolation kit. The concentration of RNA ranges from 385 to 3000 ng/µL (1267.5 ± 186.5 ng/µL) in 20 µL of elution volume. Our improved protocol resulted in optical density (OD) ratios of RNA close to 2.0 (OD260/280 = 2.05 ± 0.01 and OD260/230 = 1.99 ± 0.03) indicating its purity. RNA integrity number (RIN) value of representative sample was 8.1 indicating suitability of RNA samples for next generation sequencing like RNA-Seq. Functional validation of total RNA isolated from MFG, were tested for the expression of milk protein genes like α-lactalbumin (LALBA), β-lactoglobulin (BLG4), β-casein (CNS2) and ribosomal protein genes like RPS23 for quantitative PCR analysis. We concluded that our results could be used to obtain high quality and abundant quantity of RNA for transcriptome analysis of mammary glands and could be serve as non-invasive method of expression analysis in other species.

**Key Words:** milk fat globule, RNA isolation, transcriptome analysis


Lactation activity is extremely important for dairy cows, but the underlying metabolic mechanisms is not well understood. This study was conducted to investigate the lactation-related maintenance and initiation metabolic mechanisms in dairy cow using overall biofluid and partial tissue metabolomics. Six mid-lactation Holstein cows were used to analyze the relationships between 4 biofluids (rumen fluid, serum, milk and urine) and to compare mammary gland metabolome with 6 nonlactating cows using gas chromatography-time of flight/mass spectrometry and multivariate analysis. Totally, 33 mutual metabolites and 274 metabolites were identified in 4 biofluids and mammary gland tissues, respectively. The sub-clusters of heatmap analysis for rumen fluid and serum were grouped together and highly correlated with each other, but separated from milk. Creatine was identified as key metabolite to explain the biological variation among 4 biofluids. Pathways of gluconeogenesis, pyruvate metabolism, tricarboxylic acid (TCA) cycle, glycerolipid metabolism and aspartate metabolism, demonstrated most functional enrichment among 4 biofluids (false discovery rate <0.05, fold enrichment >2). Clear discriminations were observed between lactating and nonlactating cows, with 54 significantly higher (P < 0.05, VIP >1) metabolites in lactation group. Lactobionic acid, citric acid, orotic acid and oxamide were extracted by S-plot as putative biomarkers. The TCA cycle, glyoxylate and dicarboxylate metabolism, glutamate metabolism and glycine metabolism were determined as functional impact pathways (P < 0.01, impact value >0.1) in lactation group. Extremely upregulated function of the TCA cycle pathway (P < 0.0001) in lactating cows was identified along with 70% substrates increased in the mammary gland cell. These results provide the first integrated insight into better understanding of lactation-related overall and partial metabolic mechanisms and will be beneficial in developing regulated strategies for lactating dairy cows. More importantly, novel systematic investigation can be obtained from this study to address complex biological questions.

**Key Words:** dairy cow, lactation, metabolomics

M174  **Conjugated linoleic acid (CLA) reduces milk fat content in sows without altering litter performance.** E. C. Sandri, P. C. Carraro, and D. E. Oliveira*, Santa Catarina State University, Lages, Santa Catarina, Brazil.

In lactating sows, a great proportion of the energy consumed is prioritized to milk production and synthesis of its components, resulting in an intense catabolism of body stores. As shown in dairy cows, ewes and goats, C18:2 trans-10,cis-12 conjugated linoleic acid (CLA) decreases milk fat synthesis and it may be an option to minimize the energy costs of lactation without compromising the piglet performance. This study evaluated the effect of CLA on sow milk yield and composition, and on piglet performance. Twenty multiparous sows from a commercial lineage, with a mean body weight (BW) of 200 ± 10 kg were randomly assigned to one of the 2 treatments (n = 10/treatment) for 18 d: (1) Control (no fat) and; (2) 1% of CLA (29,9% of trans-10,cis-12 and 29.8% of cis-9,trans-11) mixed in the ration. The diet was formulated to meet the nutritional requirements for the breed. Sows were kept in a controlled environment (temperature, humidity, and ventilation) and the CLA treatment was administered from d 7 through d 25 of lactation. Milk samples were collected from all sows from d 0 to d 25 to evaluate milk concentrations of fat, protein, lactose, and total solids. Data were analyzed as a complete randomized design using the Mixed Procedure of SAS. The model included the random effect of sow, and the fixed effects of treatment and d 0 measurements, the latter used as a covariate in sows without altering litter performance.

**Key Words:** milk fat synthesis, milk fat depression, piglet performance

M175  **The gene expression of fatty acid transporters and triglyceride coding genes changes according the stage of lactation in dairy ewes.** M. Camêra1, E. Ticiani1, K. J. Harvatine2, E. C. Sandri1, and D. E. Oliveira*,1, Santa Catarina State University, Lages, SC, Brazil, 2Penn State University, State College, PA.

During lactation the mammary gland produces a substantial amount of triglycerides using fatty acids synthesized in the mammary gland and from the plasma, prioritizing milk fat synthesis over adipose tissue,
especially at the beginning of lactation. Specific fatty acid transporter proteins and enzymes are involved in fatty acid uptake by mammary cells and triglyceride synthesis. The objective of this study was evaluate gene expression of long chain acyl-CoA synthetase (ACSL1), fatty acid binding proteins (FABP3 and FABP4), fatty acid translocator CD36 (FATCD36), lipo-protein lipase (LPL), acylglycerol phosphate acyltransferase (AGPAT6), lipin (LIPIN1), diacylglycerol acyltransferase (DGAT1), and peroxisome proliferator-activated receptor gamma (PPARγ) at different stages of lactation in dairy ewes. Mammary gland biopsies were taken from 6 lactating ewes at 15, 70, and 120 DIM, to represent early, mid, and late lactation. Total RNA was extracted, cDNA synthesized and quantitative real-time PCR analysis conducted. Data were analyzed by PROC MIXED (SAS Institute) procedure using stage of lactation as a fixed effect, animal as random, and the geometric mean of the housekeeping genes (ribosomal protein S18 and β-actin) as a covariate. Data points with Studentized residuals outside of ± 2.5 were considered outliers and excluded from analysis. There was no effect of stage of lactation for ACSL1, SLC27A6, and FABP4 transcripts (P > 0.05). The expression of FABP3 and FATCD36 was higher in early lactation and decreased as lactation progressed (P < 0.05). Similarly, the transcripts of AGPAT6 and DGAT1 were higher in early lactation (P < 0.05) and LIPIN1 tended to be increased in early lactation (P = 0.09). In addition, LPL and PPARγ were increased in early lactation compared with mid and late lactation (LPL P = 0.01 and P = 0.002 and PPARγ P = 0.03 and P = 0.03, respectively). Our results show a higher expression of fatty acid transporters and key enzymes in mammary tissue at early stages of lactation prioritizing milk fat synthesis.

Key Words: fatty acid synthesis, mammary gland, milk fat

M176  Milk yield differences between xanthosine treated and control glands are associated with changes in milk protein gene expression. R. K. Choudhary1, S. Choudhary1, D. Pathak2, R. Udehiya3, R. Verma1, S. Kaswan3, A. Sharma3, M. Honparke4, and A. Capuco*,5 1School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab, India, 2Department of Veterinary Anatomy, GADVASU, Ludhiana, Punjab, India, 3Department of Livestock Production & Management, GADVASU, Ludhiana, Punjab, India, 4Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana, Punjab, India, 5Department of Veterinary Gynaecology & Obstetrics, GADVASU, Ludhiana, Punjab, India, 6Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD.

In vivo and in vitro treatment of mammary glands with xanthosine has been shown to increase mammary stem/progenitor cell population in heifers. Inosine, a ribonucleoside that is related to xanthosine, has been reported to increase milk production in transgenic goats. However, the underlying mechanisms of these effects are poorly understood. The goal of this study was to examine the effects of xanthosine on the mammary stem cell population and milk production in dairy goats. Primiparous Beetle goats (n = 7) were assigned to the study. Five d after kidding, one gland (either left or right) was infused xanthosine (TRT) twice daily (2×) for 3 d and the other gland served as control (CON). Mammary biopsies were collected at 10 d and RNA was isolated. Daily milk yield per gland was recorded 10.5 ±1.3 d after biopsies for 7 wk. Average milk yield in TRT glands was increased 2% (P = 0.04, paired t-test) relative to CON glands until 7 wk. After 7 wk, milk yield of TRT and CON glands did not differ. Analysis of milk composition revealed that protein, lactose, fat and solids-not-fat percentages remained the same in TRT and CON glands. Expression of transcripts for β-lactoglobulin (BLG4), β-casein (CSN2), estrogen receptor-α (ESR1) and aldehyde dehydrogenase 1 (ALDH1, a mammary stem cell marker) was significantly increased and α-lactalbumin (LALBA) and casein α-S2 (CSN1S2) tended to be increased in TRT glands. These results support the hypothesis that xanthosine increases milk production and the mammary stem cell population.

Key Words: goat lactation, xanthosine, mammary stem cell

M177  Peroxisome proliferator-activated receptor gamma (PPARγ) agonist and conjugated linoleic acid (CLA) have different effects on expression of milk protein genes in lactating ewes. M. Camerα1, E. C. Sandri*,1 K. J. Harvatine2, and D. E. Oliveira1, 1Santa Catarina State University, Lages, Santa Catarina, Brazil, 2Penn State University, State College, PA.

Milk protein is very important to the dairy industry. Milk protein synthesis is impacted by animal genetics, but is less responsive to nutrition. In a previous study using thiazolidinedione (TZD) trying to overcome the milk fat depression effect of trans-10,cis-12 CLA we observed an increase in milk protein content in lactating ewes treated with TZD. This study used a specific chemical PPARγ agonist and CLA to evaluate their effect on expression of milk protein genes (caseins and whey) and their interaction. Twenty-four crossbred lactating ewes (60 ± 45 kg body weight) 70 ± 3 DIM, producing 1.2 ± 0.34 kg of milk/d 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 (Rosiglitazone, 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% of trans-10,cis-12 CLA and 29.8% of cis-9,trans-11); and 4) TZD+CLA. Mammary biopsies were taken, RNA was extracted, cDNA synthesized and qRT-PCR analysis conducted for casein genes (CSN1S1, CSN1S2, CSN2, CSN3) and whey proteins genes [β-lactoglobulin (BLACTO) and α-lactalbumin (LALBA)]. Compared with control, TZD increased milk protein concentration 18.7% and expression of CSN1S1 (P = 0.05), CSN1S2 (P = 0.01), CSN2 (P = 0.01), CSN3 (P = 0.03) and BLACTO (P = 0.02) by 4, 8.6, 5, 4.9 and 4.7 fold, respectively. CLA increased the expression of CSN1S2 (P = 0.03), CSN2 (P = 0.001), CSN3 (P = 0.01) compared with control in 5.5, 5.3 and 3.9 fold, respectively. TZD+CLA tended (P = 0.06) to increase milk protein concentration 11.5% compared with control, but decreased expression (P = 0.05) of all genes studied. Overall, TZD positively affected mammary expression of genes encoding the major milk proteins, while CLA had a partial effect.

Key Words: gene expression, milk protein synthesis, thiazolidinedione


Heat stress (HT) of cows in the dry period (DP) decreases immune function and lowers milk yield in the next lactation compared with cooled dry cows. The objective of this study was to evaluate the effects of a dietary treatment (OmniGen-AF) fed to HT cows before, during and after the DP on immune function, hematology and immune related gene expression. Sixty days before dry-off, cows were cooled (i.e., shade, fans and coolers) and divided into 2 groups: control (fed 56 g/d of AB20; CON) and OmniGen-AF (fed 56 g/d of OmniGen-AF; OG). Cows were dried-off 45 d before parturition and further split into cooling (shade, fans and
soakers; CL) or HT (only shade) pens, which resulted in 4 treatments: HT (n = 17), CL (n = 16), HT + OG (HTOG, n = 19) and CL + OG (CLOG, n = 14). In the DP, rectal temperature (RT; °C), respiration rate (RR; breaths per min) and temperature humidity index (THI) were recorded to evaluate heat strain. Blood samples were collected before dry-off, during the DP and lactation from a subset of cows (HT, n = 12; CL, n = 12; HTOG, n = 11 and CLOG, n = 9) to evaluate L-selectin (CD62L, copies per ng of total mRNA) and CXCR2 mRNA (aka IL8-R) gene expression in immune cells. Other samples were used before dry-off and in the DP to evaluate neutrophil function and blood hematology (HT, n = 8; CL, n = 7; HTOG, n = 8 and CLOG, n = 6). HT increased RR (45.2 vs. 77.2 ± 1.6 bpm) and RT (38.9 vs. 39.3 ± 0.05 °C) versus CL (P < 0.01). OG increased L-selectin expression versus CON before dry-off (10229 vs. 5893 ± 2353; P = 0.09). L-selectin expression did not differ during the DP, but after calving there was an interaction of DP heat stress and dietary treatment (P = 0.05); CLOG cows had increased L-selectin expression versus CL cows (24,951 vs. 7,198 ± 5,061). Expression of CXCR2 and neutrophil function did not differ among groups. OG tended to increase neutrophil (10³/µL) count versus CON (3.6 vs. 3.3 ± 0.17; P = 0.13) and HT cows had lower hematocrit % versus CL (29.4 vs. 31.6 ± 0.6; P = 0.12). OG supplementation increased L-selectin expression before dry-off, and that may be related to improved immune status of cows during the DP and in the next lactation.

**Key Words:** immunity, heat stress, OmniGen-AF

**M179** Thiazolidinedione (TZD) does not modify the milk protein synthesis in lactating ewes. E. C. Sandri*, M. Camera, W. B. Junior, P. C. Carraro, E. D. Silva, and D. E. Oliveira, Santa Catarina State University, Lages, Santa Catarina, Brazil.

Thiazolidinedione (TZD) can stimulate insulin sensitivity by binding to transcription factors and indirectly acting on protein synthesis in mammary gland. In a previous study using ewes in early lactation (70 d in milk, DIM) we observed a positive effect of TZD on milk protein content. This study evaluated the effect of TZD on protein synthesis in late lactating ewes. Twenty-three lactating ewes with a mean body weight of 60 ± 0.45 kg, producing 0.98 ± 0.20 kg milk/day, 120 ± 3 d in milk and fed with a TMR of corn silage plus concentrate were randomly assigned to 1 of 2 treatments in a complete randomized block design: 1) Control (iv. infusion of 5 mL/d of saline solution); 2) TZD (iv. infusion of 4 mg/kg of BW/day in 5 mL of saline solution). The experimental period lasted 15d (5 of adaptation and 10 of measurements). Milk samples were collected from all ewes on d 1, 3, 4, 6, 7, 10 and pooled by ewe to evaluate the concentrations of milk fat, protein, lactose, casein and total solids. Data were analyzed using the PROC MIXED of SAS with ewe as random effect, and treatment, block and their interaction as fixed effects. There was no effect of treatment, block and their interaction for milk yield or for the concentrations and yields of protein, fat, lactose, and total solids. However, TZD reduced milk casein concentration (Control = 5.65 vs. TZD = 5.17%, P = 0.02). These results indicate that TZD does not stimulate the milk protein content in lactating ewes in late lactation.

**Key Words:** dairy ewe, milk composition, milk protein content