LACTATION BIOLOGY I

1222 (M144) relationship between dry period length and production and reproduction in grazing Jersey and Holstein cows in Costa Rica. J. M. Sánchez*[1], A. Saborío-Montero[1], and A. Córdoba-Roldán[2], [1]*Centro de Investigaciones en Nutrición Animal y Escuela de Zootecnia, Universidad de Costa Rica, San José, Costa Rica,* [2]*Programa de Transferencia Tecnológica, Cooperativa de Productores de Leche Dos Pinos, San José, Costa Rica.*

The aim of this study was to evaluate relationships between dry period length (DPL) of Jersey and Holstein grazing cows and succeeding productive and reproductive performance. The study was conducted on 29 dairy herds in the highlands of Cartago, Costa Rica. The predominant pasture grass on the farms was kikuyu (*Kikuyuocloa clandestina*) and cows were supplemented with 1 kg of a grain mixture/2.5 to 3 kg of milk. A total of 4792 completed lactations (Jersey n = 3000, Holstein n = 1792) were registered from 2009 to 2012. DPL in Jersey (mean = 75.6 d, 95% CI: 74.1–77.0 d) differed (P < 0.001) from Holstein (mean = 80.7 d, 95% CI: 78.2 to 83.2 d). In Jersey cows the DPL was inversely correlated with actual milk yield (-0.23, P < 0.001) and 305-d milk yield (-0.27, P < 0.001). Open period length in Jersey cows (mean = 120 d, 95% CI: 117 to 123 d) differed (P < 0.001) from Holstein (mean = 150 d, 95% CI: 145 to 154 d). Jersey cows with DPL less than 58 d produced more 305-d milk (mean = 5583 kg, 95% CI: 5480 to 5687 kg, P < 0.001) than cows with a DPL greater than 73 d (mean = 5208 kg, 95% CI: 5086 to 5331 kg). Cows in both those dry groups produced less (P < 0.01) milk than cows with a DPL of 58 to 73 d DPL (mean = 5949 kg, 95% CI: 5885 to 6012). Jersey cows with DPL greater than 73 d had shorter (P < 0.001) lactation length (mean = 305 d, 95% CI: 299 to 310 d) than those below that threshold (mean = 324 d, 95% CI: 321 to 327 d). Calving interval for Jersey cows with DPL less than 58 d (mean = 403 d, 95% CI: 398 to 409 d) was greater (P < 0.05) than those with DPL between 58 and 63 d (mean = 392 d, 95% CI: 388 to 397 d). 305-d milk yield was greater (P < 0.01) in Holstein cows with DPL between 55 and 63 d (mean = 7620kg, 95% CI: 7434 to 7807 kg) than those with DPL less than 55 d (mean = 7162kg, 95% CI: 6964 to 7361kg) or higher than 86 d (mean = 7066kg, 95% CI: 6830 to 7301kg). Open period in Holstein cows was greater (P < 0.01) in those with DPL greater than 86 d (mean = 163d, 95% CI: 153 to 173d) compared to those cows with DPL between 55 and 86 d (mean = 144d, 95% CI: 138 to 150d). These results suggest that in grazing Jersey and Holstein cow herds milk yield and reproduction performance could be influenced by DPL.

Key Words: dry period length, grazing cows, dairy cows

1223 (M145) Effect of insulin on mRNA expression of genes related to milk synthesis in primary bovine mammary epithelial cells cultured in vitro. 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.*

The crucial role for mTOR in the regulation of milk protein synthesis in the bovine mammary gland has been reported, but the molecular events associated with regulation of milk fat synthesis remain unknown. This study was conducted to examine the potential role of insulin in the presence of two lactogenic hormones, hydrocortisone and prolactin, on milk protein and fat synthesis. Primary bovine mammary epithelial cells cultured in vitro were treated in three different ways (no hormones (NH, control), 50 ng/mL hydrocortisone and 200 ng/mL prolactin (FP), or 100 ng/mL insulin, 50 ng/mL hydrocortisone and 200 ng/mL prolactin (IFP)). Expression of 17 key genes involved in four pathways were detected by real-time PCR. Statistical significance was evaluated by unpaired t test analysis with SAS 9.0 software. Significance was declared at P < 0.05. Results showed that IFP group significantly increased the mRNA level of β-casein (CSN2), κ-casein (CSN3), Acetyl-CoA carboxylase (ACACA), fatty acid synthase (FASN) and Sterol Regulatory Element Binding Protein1 (SREBP1) compared to NH and FP groups (P < 0.05). Relative to the other groups, IFP group significantly up-regulated the expression of signal transducers and activators of transcription 5B (STAT5B) and E74-like factor 5 (ELF5) in JAK-STAT5 pathway, as well as Phosphatidylinositol 3-kinase (*P*I3K), Protein Kinase B (AKT1) and Eukaryotic initiation factor 4E (EIF4E) in PI3K/Akt/mTOR signaling pathway (P < 0.05). But the IFP hormone combinations had no effect on TSC1, TSC2 or RHEB transcription in AMPK signal pathway (P > 0.05). The results demonstrated that insulin may stimulate milk protein synthesis by JAK-STAT5 and PI3K/Akt/mTOR signaling pathways, and stimulate milk fat synthesis by PI3K/Akt/mTOR and SREBP signaling pathways in bovine mammary epithelial cells cultured in vitro. This research indicated insulin has an important role in milk protein and fat synthesis as the other two lactogenic hormones, hydrocortisone and prolactin.

Key Words: insulin, bovine mammary epithelial cells, real-time PCR

1224 (M146) Conjugated linoleic acid (CLA) trans-10, cis-12 decreases ACC-α gene expression in lactating mammary gland by decreasing specific transcripts from different promoters. D. E. Oliveira[*1], D. E. Bauman[2], and K. J. Harvatine[3], *[1]Santa Catarina State University, Lages, Brazil, [2]Cornell University, Ithaca, NY, [3]Penn State University, State College.*

Transcription of acetyl-CoA carboxylase α (ACC-α) is initiated from multiple promoters in a tissue-specific fashion and mammary expression of ACC-α is decreased during CLA-induced and diet-induced MFD. The effect of *trans*-10, *cis*-12 CLA and a low forage and high oil diet on regulation of the different ACC-α isoforms in mammary tissue was investigated. Nine mid-lactation cows were arranged in a 3 × 3 latin square design with 14-d experimental periods. Treatments were Control, 3 d i.v. infusion of *trans*-10, *cis*-12 CLA (CLA-MFD), and feeding a low forage, high oil diet (Diet-induced MFD). Milk fat yield was decreased 38% percent by the low forage and high oil diet and by 24% percent by CLA. Mammary biopsies at the end of each treatment were taken. Quantitative real-time reverse transcriptase PCR assays were developed for ACC-α specific promoters of interest. Data were analyzed using the PROC MIXED of SAS to test expression of individual ACC-α promoters I, II, III (exon 5A) and a well described splice variant (-24NT). The geometric mean of 3 housekeeping genes was calculated and used as a covariant in the model. Differences in expression of individual ACC-α promoters was declared significant at $P < 0.05$ and trends at $P < 0.1$. There was no treatment effect on expression of ACC-α transcript from promoter I. Compared to Control, ACC-α promoter II transcript were reduced 32.9% with diet-induced MFD treatment ($P = 0.005$) and tended to be decreased 18.5% by CLA treatment ($P = 0.06$). Transcript from promoter III (exon 5A) was reduced 26.4 and 35% for CLA-induced and diet-induced MFD treatments ($P = 0.0001$), respectively, compared to Control. Transcript for the -24NT splice variant was reduced by 25.8% during det-induced MFD in comparison to control ($P = 0.03$), but CLA treatment was not different from control. Promoters II, III, and -24NT splice variant transcript of ACC-α are decreased during diet induced milk fat depression demonstrating a promoter specific role of bioactive fatty acids in ACC-α gene expression.

Key Words: acetyl-CoA carboxylase promoters, gene expression, conjugated linoleic acid

1225 (M147) Conjugated linoleic acid (CLA) affects in different ways acetyl-CoA carboxylase α (ACC-α) transcripts from different promoters in mammary and adipose tissue from lactating ewes. E. Ticiani[1], M. Urio[1], A. P. Povaluk[1], M. V. Camera[1], R. Ferreira[2], L. C. Miletti[1], K. J. Harvatine[3], and D. E. Oliveira[*1], *[1]Santa Catarina State University, Lages, Brazil, [2]Santa Catarina State University, Chapecó, Brazil, [3]Penn State University, State College.*

Feeding *trans*-10, *cis*-12 CLA to lactating ewes reduces milk fat by down-regulating gene expression of enzymes involved in lipid synthesis in mammary gland. An example is acetyl-CoA carboxylase α (ACC-α), a key enzyme in the de novo fatty acid synthesis pathway. ACC-α is encoded by mRNAs transcribed from three promoters, PI, PII and PIII, characterized as tissue-specific in the ovine genome. This study evaluated the effects of a rumen-unprotected *trans*-10, *cis*-12 CLA supplement fed to crossbred Lacaune/Texel lactating ewes, on gene expression of different mRNA transcripts of ACC-α. Twelve ewes arranged in a completely randomized design received for 15 d one of the following treatments: Control (forage + 1 kg of concentrate) and CLA [forage + 1 kg of concentrate + 28 g/d of CLA (29.9% *trans*-10, *cis*-12)]. The CLA supplement (an oil mixture) was orally dosed. Mammary gland and adipose tissue biopsies were taken on Day 15. Subsequently RNA was extracted, cDNA synthesized and quantitative real-time reverse transcriptase PCR analysis conducted. Data were analyzed by PROC MIXED using ribosomal protein S18 housekeeping gene as a covariate in the model. Compared to Control, in the mammary gland CLA reduced by 29.1% ($P = 0.037$) transcript from PIII with no changes on transcripts from PI and PII ($P > 0.05$). In the adipose tissue, transcript from PI was increased by 379.8% ($P = 0.028$) in the CLA treated group when compared to Control. There was no treatment effect on transcripts from promoters II and III in the adipose tissue. Overall, our results suggest that *trans*-10, *cis*-12 CLA downregulates ACC-α gene expression by decreasing expression from promoter III in mammary tissue and increases ACC-α gene expression by increasing expression promoter I in adipose tissue.

Key Words: adipose tissue, gene expression, mammary gland

1226 (M148) Effect of different hormones on â-casein and lactoferrin expression in mammary epithelial cells. *W. Q. Li[1,2], J. Q. Wang[*1], D. P. Bu[1], and X. M. Nan[1], [1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]College of Life Science, Henan Agricultural University, ZhengZhou, China.*

An inverse relationship in expression patterns for lactoferrin (LTF) and caseins occurs throughout the lactation cycle: when LTF expression is high, casein expression is low and vice versa. The objective of this study was to investigate the effect of different hormones on β-casein (CSN2) and LTF expression in mammary epithelial cells. Primary bovine mammary epithelial cells were treated with eight kinds of induction mediums to induce expression of CSN2 and LTF, including group A dexamethasone&prolactin&insulin (1 μg/mL, 5 μg/mL, 5 μg/mL), group B dexamethasone&prolactin&insulin (1 μg/mL, 10 μg/mL, 5 μg/mL), group C β estradiol (5 μg/mL), group D 10 ng IGF-I, group E 100 ng IGF-I, group F 200 ng IGF-I, group G 400 ng IGF-I, group H 1000 ng IGF-I. Materials administered in groups C-H did not include dexamethasone&prolactin&insulin. Relative mRNA expression of CSN2 and LTF was detected at 24h after induction by qPCR. Data was analyzed by One-way ANOVA and pearson correlation procedure of SAS 9.0. The results showed that 100ng IGF-I was the most suitable inducing material for β-casein ($P < 0.05$) and no significant difference of LTF expression among groups was observed ($P > 0.05$). Correlation coefficient of CSN2 and LTF expression was -0.277. It was concluded that CSN2 expression in bovine mammary epithelial cells depended on hormone and IGF-I, but not LTF. More research is needed to explore the relationship between β-casein and lactoferrin.

Key Words: β-casein, lactoferrin, mammary epithelial cell

1227 (M149) Effects of methionyl-methionine on milk protein synthesis in bovine mammary gland. J. X. Yang[1], H. Y. Liu[1], C. H. Wang[1], Q. B. Xu[*1], and J. X. Liu[2], [1]*Institute of Dairy Science, Zhejiang University, Hangzhou, China,* [2]*Zhejiang University, Hangzhou, China.*

This study was conducted to evaluate effect of methionyl-methionine (Met-Met) on milk protein synthesis in cultured lactating bovine mammary tissues. Mammary tissues were obtained from three lactating Holstein dairy cows and incubated in DMEM/F12 for 72 h. Triplicate isolated cultured mammary tissues were treated with conditional medium containing certain essential amino acid (AA) for 3 h before treatment exposure. The concentration of Met-Met was adjusted through replacing the medium Met with Met-Met at ratios of 0, 5, 10, 15, 20, and 25%, respectively. After incubation with experimental medium, tissues were collected for determination of α_{s1} casein expression by RT-qPCR and western blot. The AA absorption was determined by the change of free AA concentration in the medium. The mRNA abundance of AA transporters, mTOR and JAK2-STAT5 signaling pathway were measured for the control and optimal ratio of Met-Met treatment. Replacement of Met-Met for single Met significantly increased α_{s1} casein expression, with the highest expression at 15% of Met-Met substitution. Compared with the control, mRNA abundance of AA transporters including ATB (0, +) and CAT1 was increased, and AA absorption were significantly improved in 15% Met-Met group ($P < 0.05$), in which the mRNA abundance of mTOR, S6K1, 4E-BP1, JAK2 and STAT5 ($P < 0.05$) was also increased. From these results, it is indicated that Met-Met promoted milk protein synthesis and AA absorption in cultured bovine mammary tissues, and mTOR and JAK2-STAT5 signaling pathways may be involved in the Met-Met-stimulated milk protein synthesis.

Key Words: αs1 casein, bovine mammary gland, methionyl-methionine

1228 (M150) Effect of bta-miR-145 overexpression and down-expression on the other microRNA expression in primary bovine mammary epithelial cells. W. Q. Li[1], D. P. Bu[*1], J. Q. Wang[2], and X. M. Nan[1], [1]*State Key Laboratory of Animal Nutrition, 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, Beijng, China.*

MicroRNA research has made great progress. MicroRNA overexpression by mimic or down-expression by inhibitor is a common method to study the function of microRNA. However, it is unknown that whether overexpression or inhibition of a certain microRNA affects other microRNAs expression or not. This study was performed to evaluate the effect of bta-miR-145 overexpression or down-expression on other microRNAs expression. Bta-miR-145 mimic (150pmol/well) or inhibitor (300pmol/well) was transfected in primary bovine mammary epithelial cells in 6-well plates. After 24h transfection, expressions of bta-miR-145, bta-miR-214, bta-miR-181a, bta-miR-21 were detected by RT-qPCR method. Relative quantification (ΔΔCt) method was used to analyze the data. The geometric mean of 5SrRNA and U6 was used to normalize the expression of microRNAs. All data were tested by one-way ANOVA program of SAS 9.1. The results showed that the overexpression effect of bta-miR-145 was achieved in cells by its mimic, and inhibiting effect of bta-miR-145 was achieved by its inhibitor. In addition, bta-miR-181a and bta-miR-145 had the same expression trend, while bta-miR-214 and bta-miR-145 had the opposite expression trend. And bta-miR-21 expression was almost not affected by bta-miR-145

overexpression and down-expression. It was concluded that one kind of microRNA overexpression or inhibition would cause expression change of other microRNAs. It is suggested that system biology views should be take to explain the related biological problems.

Key Words: bta-miR-145, overexpression, down-expression

1229 (M151) Stearic acid alters microRNA profiles in bovine mammary gland epithelial cells. Y. G. Chai[1], X. M. Nan[1], D. P. Bu[*2], J. J. Loor[3], and J. Q. Wang[2], *[1]State Key Laboratory of Animal Nutrition, 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, [3]University of Illinois, Urbana.*

MicroRNAs (miRNA) are important regulators of cellular processes. The objective was to study whether the addition of stearic acid would alter the microRNA profile of bovine mammary epithelial cells. Cells were cultured and passaged in DMEM/F12 basic medium with 10% fetal bovine serum. For experimental assays, cells at 80% confluence were cultured in lactation medium (containing insulin, epidermal growth factor, transferring, hydrocortisone, progesterone and fatty acid free bovine serum albumin) with or without stearic acid (SA) for 24 h. A customized microarray containing 672 bovine miRNA was used to investigate their functional roles in bovine mammary gland epithelial cells in response to supplemental stearic acid (SA). Total miRNA expressed in the control and SA incubations was 157 and 165. Seventeen of 165 miRNA were differentially expressed with SA, and 12 (bta-miR-452, bta-miR-30c, bta-miR-362–5p, bta-miR-181a, bta-miR-194, bta-miR-2368, bta-miR-2893, bta-miR-2888, bta-miR-2374, bta-miR-29c, bta-miR-19a, and bta-miR-2411) were verified by real-time PCR. Using TargetScan, PicTar, GO and KEGG for functional analyses revealed that gene targets of miRNAs affected by SA are associated with regulation of transcription and mTOR signaling. This study provides novel data on miRNAs responsive to fatty acid in vitro.

Key Words: stearic acid, microRNA, lactational response

1230 (M152) The peroxisome proliferator-activated receptor γ (PPARγ) agonist thiazolidinedione (TZD) does not overcome *trans*-10, *cis*-12 conjugated linoleic acid (CLA) inhibition of milk fat synthesis in lactating dairy ewes. E. C. Sandri[1], E. M. Sandri[2], M. V. Camera[1], A. P. Povaluk[1], M. Urio[1], E. Ticiani[1], K. J. Harvatine[3], and D. E. Oliveira[*1], *[1]Santa Catarina State University, Lages, Brazil, [2]Santa Catarina State University, Chapecó, Brazil, [3]Penn State University, State College.*

Peroxisome proliferator-activated receptor γ (PPARγ) is regulated by ligand activation and regulates lipid synthesis pathways, but a functional role for PPARγ in diet-induced MFD in vivo has not been established. This study evaluated the effect of PPARγ agonist thiazolidinedione (TZD) on milk fat synthesis and its interaction with *trans*-10, *cis*-12 CLA. Twenty-four lactating ewes (60 ± 0.45 kg of BW) were randomly assigned one of the four treatments (n = 6/treatment) for 7 d: Control (100 mL/d of saline); TZD (IV 4 mg of TZD/kg of BW per d in 100 mL of saline); CLA (oral dose of 27 g/d of rumen-unprotected CLA (oil mixture: 29.9% *trans*-10, *cis*-12), and TZD+CLA (TZD infusion initiated 1 d before CLA dosing). Milk was sampled and analyzed for fat, protein and lactose concentration. Compared to control, fat content was 22.3% lower in CLA (6.14 vs. 4.77%, P = 0.05), tended to be 20.7% lower in TZD+CLA (6.14 vs. 4.87, P = 0.06), and did not differ between control and TZD (6.14 vs. 6.70%, P = 0.39). Compared to TZD, fat content was 28.8% lower in CLA (6.70 vs. 4.77%, P = 0.008) and 27.3% in TZD+CLA (6.70 vs. 4.87%, P = 0.01). Fat content did not differ between TZD+CLA and CLA (4.87 vs. 4.77%, P = 0.87). Treatments did not affect the concentration of milk protein and lactose (P > 0.05). In conclusion, TZD did not stimulate milk fat synthesis and was not able to overcome CLA inhibition of milk fat synthesis, indicating that PPARγ does not mediate CLA-induced MFD

Key Words: milk fat depression, lipid synthesis pathway, peroxisome proliferator-activated receptor γ

1231 (M153) Fatty acid synthase is essential for milk fat formation in goat mammary gland. J. Zhu[1], J. Luo[*2], Y. Sun[1], and H. Shi[1], *[1]Northwest A&F University, Yangling, China, [2]Northwest A & F University, Yangling, China.*

The inevitable role of fatty acid synthase (FASN) on fatty acid metabolism has been a validated concept for a quite long period of time. However, the details of its effect on milk fat formation remain to be unclear. This study explored the potential role of FASN in regulating milk fat accumulation and secretion in lactating goat mammary gland epithelial cells (GMECs). Using quantitative real-time PCR, we detected that FASN was predominantly expressed in fat, small intestine and mammary

gland tissues among 10 different tissues (subcutaneous fat, small intestine, mammary gland, lungs, rumen, muscle, spleen, liver, kidney and heart) collected from three Xinong Saanen dairy goats at mid-lactation period. In addition, we also found a much higher expression level of FASN gene at mid-lactation mammary gland compared with dry-off period (17.9-fold) from three Xinong Saanen dairy goats. These results indicated the potential role of FASN in lactation. Using designed shRNA targeting FASN sequence to construct adenovirus vector with BLOCK-IT system, the effect of shRNA on mRNA expression of GMECs were determined after 48-h incubation of infected GMECs. Inhibition of FASN by C75 (10 ng/uL) and shRNA mediated interference, with the adenovirus as infection vector, markedly reduced cellular triglyceride (TAG) content by decreasing the expression of genes related to TAG synthesis (glycerol-3-phosphate acyltransferase, GPAT; 1-acylglycerol-3-phosphate O-acyltransferase, AGPAT6; diacylglycerol acyltransferase 2, DGAT2) and enhancing the expression of lipolysis related genes (adipose triglyceride lipase ATGL; hormone-sensitive lipase, HSL) (except ATGL response to shRNA treatment) in GMECs. Consistent with the markedly decreased expression of the genes related to lipid droplets formation and secretion (tail-interacting protein 47 gene, TIP47; adipose differentiation-related protein, ADRP; butyrophilin 1a1, BTN1A1; xanthine oxidoreductase, XOR), cellular lipid droplets were also reduced sharply after incubation with C75 or Ad-shRNA investigated by Oil red O staining method. The results provided evidence of FASN's essential role in TAG synthesis and secretion in GMECs. Hence, FASN may be future essential for goat milk fat formation.

Key Words: fatty acid synthase, milk fat, mammary gland epithelial cells, dairy goat