NDMI. The correlation between DMI and NDMI was 0.52 (P<0.001) and were most strongly associated during the first 10 weeks of lactation, after which point DMI was less than NDMI. The correlation between FCM and DMI across all herds was 0.23 (P<0.001). However, there was considerable variation among herds, with correlations between FCM and DMI within herd ranging from -0.14 (P=0.32) to 0.66 (P<0.001). Dry matter intake can be estimated in commercial tie-stalls with moderate accuracy, which could facilitate genetic research for feed intake in commercial herds.

Key Words: intake, commercial, dairy

Graduate Student Paper Competition-National ADSA Production PhD Poster: National ADSA Production Poster PhD Only (Graduate)

M133 Metabolism of ferulic acid in ram lambs. M. A. Soberón* and D. J. R. Cherney, *Cornell University*, *Ithaca, NY*.

Little is known about the metabolism of free ferulic acid (FA) in the ruminant once FA leaves the rumen. Due to its soluble nature, FA may be absorbed into the bloodstream and metabolized. This study was conducted to elucidate the metabolic pathway of free FA in sheep. Eight male lambs were randomly assigned to one of four treatment levels (0g, 3g, 6g or 9g of free FA) as part of a replicated 4x4 Latin square design. Lambs were housed individually and fed chopped alfalfa hay (22.8% CP, 39.3% NDF, 0.73 Mcal/kg NE_G) ad libitum and 0.35 kg corn grain (9.1% CP, 11.2% NDF, 1.52 Mcal/kg NE_G) once daily. Basal levels of FA in hay, grain, blood, feces and urine were established following a 14 d adjustment to diet and housing. Sampling was repeated at the conclusion of five continuous days where an oral dose of free FA was administered via bolus after each morning feeding. Feed, refusal and fecal samples were dried (60°C), ground to pass through a 2mm screen and analyzed for [FA] and NDF. Plasma and urine were frozen (-20°C) until analysis for [FA]. Body weights were taken weekly and DMI was measured daily. In addition to treatments, each lamb ingested FA in its bound form via the offered hay (2.67 mg/g FA) and corn (3.17 mg/g FA). Hay DMI was different among treatments (P=0.039) with DMI averages of 0.903 kg (0g), 1.06 kg (3g), 1.06 kg (6g), and 0.938 kg (9g). Refusals across treatments were not different in NDF level (P=0.347) or [FA] (P=0.848), indicating that sorting had no effect on free FA ingested. Free FA treatment level did not affect lamb body weight (P=0.281), fecal NDF levels (P=0.111), or fecal [FA] (P=0.294). No free FA was found in the plasma analyzed, indicating that during the five hours that passed between bolus dosage and blood collection, free FA in the blood was metabolized. Urine [FA] increased with increasing levels of free FA fed (P<0.001; averages were 1.66 μm/mL, 100 μm/mL, 240 μm/mL, and 592 μm/mL respectively for 0g, 3g, 6g, and 9g doses). These data indicate that free FA in lambs is fully metabolized from the blood by five hours post-dosage and primarily excreted in the urine.

Key Words: phenolic, ruminant

M134 Effects of acetate and essential amino acids on protein synthesis signaling in bovine mammary epithelial cells in-vitro. J. A. D. R. N. Appuhamy*, C. T. Bray, J. Escobar, and M. D. Hanigan, *Virginia Polytechnic Institute and State University*, *Blacksburg*.

Protein synthesis responds to various signals such as hormones, amino acids and energy supply. Acetate plays a major role as an energy source for milk synthesis in bovine mammary glands. The objective of this study was to investigate the effects of essential amino acids (EAA) and acetate on the phosphorylation status (PS) of mammalian target of rapamycine (mTOR), and ribosomal protein S6 (rpS6) which are involved in cellular signaling associated with protein synthesis and the cellular energy sensor AMP-activated protein kinase (AMPK). Bovine mammary epithelial (BME) cells from the MacT cell line were deprived of EAA and acetate overnight and then cultured in complete or EAA-deprived DMEM/F12 with and without acetate (5 mM sodium acetate) in a 2×2 factorial design repeated in 2 experiments. After 1 h of incubation, BME were lysed in the presences of protease and phosphatase inhibitors. Cell lysates were subjected to Western immunoblotting with antibodies against phosphorylated mTOR (Ser^{2448}), rpS6 ($Ser^{235/236}$), and AMPK (Thr^{172}). The PS of each signaling protein was determined as a ratio of the phosphorylated form and total forms. Acetate deprivation increased PS of AMPK (PS_{AMPK}) by 34% (P<0.05) and reduced PS of mTOR (PS_{mTOR}) by 42% (P<.05). There was a weak correlation between PS_{AMPK} and PS_{mTOR} (r=-0.32). Acetate had no effect(P>.10) on PS of rpS6 (PS_{rpS6}). In the absence of EAA, PS_{mTOR} and PS_{rpS6} were reduced (P<.01) by 55% and 75%, respectively. The correlation between PS_{mTOR} and PS_{rpS6} was 0.74 (P<.05). There was no interaction (P>.10) between EAA and acetate on PS_{mTOR} and PS_{rpS6} suggesting their regulatory effects on cell signaling were independent of each other. Extracellular EAA availability appeared to have a stronger effect on protein synthesis signaling in BME cells compared to the effect of extracellular acetate availability mediated by AMPK.

Key Words: cell signaling, acetate, amino acid

M135 Molecular cloning, distribution and ontogenetic expression of b0,+AT and the oligopeptide transporter PepT1 mRNA in Tibetan suckling piglets. W. Wang*[1], G. Wu[4], W. Gu[1], T. Li[1], M. Geng[1], W. Chu[2], R. Huang[1], M. Fan[3], D. Fu[1], Z. Feng[1], and Y. Yin[1], [1]*The Chinese Academy of Sciences*, *Changsha, Hunan, P. R. China*, [2]*Changsha University*, *Changsha, Hunan, P. R. China*, [3]*University of Guelph*, *Guelph, Ontario, Canada*, [4]*Texas A & M University*, *College Station*.

The AA transporter system b0,+ mediates apical uptake of basic amino acids, especially lysine and arginine. But some dietary protein digestion products are absorbed as oligopeptides rather than free AA. The aim of our study was to clone Tibetan porcine AA transporter b0,+AT and PepT1 for comparing the sequences of Tibetan pig with other species, and investigating tissue distribution and ontogenetic expression in the small intestine of Tibetan suckling piglets. Purebred Tibetan piglets (n=42) were obtained from multiparous sows and slaughtered randomly at 1, 4, 7, 14, 21, 28 and 35 d of age (6 piglets/age). The Tibetan porcine b0,+AT cDNA cloned from the small intestine was described first. The ORF of b0,+AT is 1464 bp and codified 487 AA residues, having a higher degree of sequence similarity with common pig (99.59%) and horse counterparts (91.2%) than with human (88.7%). The tibetan porcine PepT1 cDNA encodes 708 deduced AA residues that have high sequence similarity with its ovine and bovine counterparts. Both 2 putative proteins have 12 putative transmembrane domains. In this study, both b0,+AT and PepT1 mRNAs were detected in duodenum, jejunum, ileum, and liver by PCR. We investigated the expression of two genes in

duodenum, anterior jejunum, posterior jejunum and ileum from 1 to 35 d. The b0,+AT mRNA in duodenum and jejunum was greatest and least, respectively. It has a similar pattern in duodenum and anterior jejunum, which level decreased early in the suckling period and increased until d 35. Posterior jejunum expression uncreased steadily with age, except d 7. The ileum had the greatest expression at d 14 and became steady after d 28. The jejunum also had the greatest expression of PepT1 compared with the duodenum and ileum. PepT1 mRNA expression in the duodenum and proximal jejunum increased continuously from d 1 to 14; expression was greatest ($P<0.01$) at d 14 and then decreased from d 21 to 35. Our findings show that PepT1 expression in the distal jejunum increased gradually with age and the jejunum is the major absorption site for AA in Tibetan suckling piglets.

Table 1. b0,+ and PepT1 expression from d 1 to 35

tissue/ages	duo(b0,+/PepT1)	proximal jeju	distal jeju	ileum
1	$1.028^{a}/1.009^{bc}$	$1.025^{b}/1.244^{b}$	$1.019^{c}/0.785^{b}$	$1.061^{b}/0.573^{c}$
4	$0.346^{bc}/1.035^{c}$	$0.468^{b}/0.727^{b}$	$1.332^{c}/1.113^{b}$	$2.072^{b}/0.975^{ab}$
7	$0.113^{c}/1.294^{b}$	$0.437^{b}/0.730^{b}$	$3.127^{b}/1.729^{ab}$	$2.106^{b}/1.093^{ab}$
14	$0.459^{b}/3.575^{a}$	$0.809^{b}/2.257^{a}$	$1.178^{c}/2.588^{a}$	$6.483^{a}/0.177^{d}$
21	$0.475^{b}/1.709^{b}$	$0.759^{b}/1.090^{b}$	$2.367^{bc}/3.072^{a}$	$4.035^{ab}/0.819^{bc}$
28	$0.498^{b}/0.531^{c}$	$0.528^{b}/0.885^{b}$	$3.682^{ab}/2.784^{a}$	$2.969^{b}/0.527^{c}$
35	$0.857^{a}/0.424^{c}$	$4.511^{a}/1.023^{b}$	$5.212^{a}/2.850^{a}$	$3.704^{ab}/1.411^{a}$

Key Words: amino acid transporter, PepT1,b0,+, Tibetan pig

M136 Milk fatty acid composition of whole fluid milk in the United States. A. M. O'Donnell*, D. M. Barbano, and D. E. Bauman, *Cornell University*, *Ithaca, NY*.

Consumers are increasingly aware that food components have the potential to influence human health maintenance and disease prevention, and dietary fatty acids (FA) have been of special interest. It has been 25 years since the last survey of US milk fatty acid composition, and during this interval there have been substantial changes in dairy rations, including increased use of total mixed rations and by-product feeds as well as the routine use of lipid and FA supplements. Furthermore, analytical procedures have improved allowing greater detail in the routine analysis of FA, especially *trans* fatty acids. Our objective was to survey US milk fat and determine its fatty acid composition. We obtained samples of fluid milk from 56 milk processing plants across the US every 3 months for one year to capture seasonal and geographical variations. Processing plants were selected based on the criteria that they represented the major volume of milk produced in that area. An overall summary of the milk fat analysis indicated that saturated fatty acids (SFA) comprised 63.7% of total milk FA with palmitic and stearic acids representing the majority (44.1% and 18.3% of total SFA, respectively). Unsaturated fatty acids (UFA) were 33.2% of total milk FA with oleic acid predominating (71.0% total UFA). *Trans* FA (TFA) represented 3.2% of total FA, with vaccenic acid being the major *trans* isomer (46.6% total TFA). *Cis*-9, *trans*-11 18:2 conjugated linoleic acid represented 0.56% total milk FA, and the major omega-3 FA (linolenic acid, 18:3) composed 0.39%. Analyses for seasonal and regional effects indicated statistical differences for some FA, but these were minor from an overall human nutrition perspective as the FA profile for all samples were numerically similar. Overall, the present study provides a valuable database for current fatty acid composition of US fluid milk, and results demonstrate that the milk fatty acid profile is remarkably consistent across seasons and geographic regions from the perspective of human dietary intake of milk fat.

Key Words: fatty acids, milk fat composition, survey

M137 Polymorphisms in lipogenic genes and variations in milk fatty acid composition in Holstein dairy cows. R. A. Nafikov*[1], J. P. Schoonmaker[1], J. M. Reecy[1], D. Moody-Spurlock[1], J. Minick-Bormann[2], K. J. Koehler[1], and D. C. Beitz[1], [1]*Iowa State University*, *Ames*, [2]*Kansas State University*, *Manhattan*.

The objective of this study is to determine variations in single nucleotide polymorphisms (SNPs) in lipogenic genes that are associated with differences in milk fatty acid composition in Holstein dairy cows. Three genes, sterol regulatory element binding protein 1 (SREBP1), SREBP cleavage-activating protein (SCAP), and insulin induced protein 1 (Insig1) that are involved in the transcriptional regulation of stearoyl-CoA desaturase (SCD) and other lipogenic genes, were sequenced in exonic and intronic regions to detect SNPs. Five hundred cows were genotyped for discovered SNPs using Sequenom MassARRAY system. Phenotypic data were comprised of milk samples that were collected once per month throughout a ten month lactation period for all the cows and analyzed for fatty acid composition using gas chromatography. The PROC MIXED procedure of SAS with a month of lactation as a repeated statement was used to analyze the data. The mixed model included genotype as a fixed effect, sire line as a random effect, and milk production, percentage of milk fat, and lactation number as covariates. Multiple numbers of SNPs from SREBP1, SCAP, and Insig1 were significantly associated with differences in unsaturated fatty acid composition in milk. In particular, depending on the SNP, the percentages of monounsaturated fatty acids, polyunsaturated fatty acids, total unsaturated fatty acids, saturated fatty acids, and/or the ratio of unsaturated to saturated fatty acids were affected. The effects of SNPs from the earlier mentioned genes might be explained by differential regulation of the SCD transcription in cows with different genotypes for those genes. The results of this study indicate the potential of using associated SNPs as DNA markers to select breeding stocks that have a healthier milk fatty acid composition.

Key Words: SNP, fatty acids, candidate genes

M138 Regulation of bovine pyruvate carboxylase promoters by fatty acids. H. M. White*, S. L. Koser, and S. S. Donkin, *Purdue University*, *West Lafayette, IN*.

Pyruvate carboxylase (PC) is a critical enzyme in gluconeogenesis and TCA cycle carbon flux. Bovine PC (EC 6.4.1.1) gene contains three promoter sequences (P3, P2, and P1 from 5′ to 3′) which regulate its expression. Physiological stressors that evoke changes such as elevated NEFA or elevated glucocorticoid levels, including the onset of calving and feed restriction, also activate PC. The objective of this experiment was to determine the direct effects of stearic, oleic and linoleic acids (1 mM), dexamethasone (1 μM) and Wy14643 (10 μM; a PPAR aganonist) on promoter activity. Rat hepatoma (H4IIE) cells were transiently transfected with bovine PC promoter-luciferase constructs containing either P1, P2 or P3 and exposed to treatments for 23 h. Activity of P1 was suppressed ($P \leq 0.05$) with exposure to stearic acid (1.75 vs. 5.85 arbitrary units for stearic vs. control, respectively), and enhanced ($P \leq 0.05$) with exposure to Wy14643 (9.31 vs. 5.85 arbitrary units for Wy14643 vs. control, respectively). Conversely, stearic acid enhanced

($P \leq 0.1$) P3 activity (1.87 vs. 0.34 arbitrary units for stearic vs. control, respectively). Activity of P2 was not altered ($P \geq 0.05$) by any treatments and there was no response to glucocorticoids or unsaturated fatty acids for any of the promoters tested. Stearic acid suppresses the activity of bovine PC promoter 1 but enhances the activity of promoter 3 creating a net increase in promoter activity. These data demonstrate the role of fatty acids in regulating PC expression. The specificity of response for PC promoter function to stearic acid in the current data suggests a physiological role for elevated stearic acid concentrations previously observed in transition cows at calving.

Key Words: pyruvate carboxylase, promoter, fatty acids

Lactation Biology

M139 Effects of restricted feeding of prepubertal ewe lamb on growth performance, mammary gland development and first lactation. L. Villeneuve*[1], D. Cinq-Mars[2], and P. Lacasse[3], [1]*Centre d'expertise en production ovine du Québec*, *LaPocatière, QC, Canada*, [2]*Laval University*, *Québec, QC, Canada*, [3]*AAFC, Dairy and Swine Research and Development Center*, *Sherbrooke, QC, Canada*.

The aim of this study was to determine the effects of restricted feeding before puberty on growth performances, mammary gland development and milk production in replacement ewe lambs. At weaning, 72 Dorset ewe lambs were assigned to either an ad libitum diet (A), a restricted diet with good quality forage (14.8% CP, 2.15 Mcal ME/kg DM, 34.7% ADF) (F), or a restricted diet with medium quality forage (13.3% CP, 1.81 Mcal ME/kg DM, 42.8% ADF) (R). The quantity of feeds offered to ewe lambs of group R and F was adjusted in order to get an ADG representing 70% of that of ewe lambs of A group. These diets were offered during 75 d following weaning to cover the allometric phase of mammary gland development. During this period, ADG were respectively 223 and 229 g/d for R and F group compared to 305 g/d for A group ($P<0.0001$). At the end of this period, 28 ewe lambs were slaughtered and their mammary gland was collected. Parenchymal tissue weight tended to be higher for R and F groups with 27,86g and 24,37g respectively compared to 19,34 g for A ($P<0.10$). Stroma weight was greater ($P<0.05$) for A lambs (91.2, 61.9, and 64.4g for A, R and F, respectively). Total DNA and total protein in parenchymal tissue tend to be greater ($P=0.09$ and $P=0.07$ respectively) for R and F. Dry fat free tissue were greater for R and F averaging 1.81mg and 1.50mg respectively against 1.19mg for A ($P<0.05$). The remaining ewe lambs were fed a diet composed of silage and barley until their first lambing. During this period, compensatory growth occurred and ADG were greater ($P<0.01$) for R and F (179 and 175 g/d) than for A ewe lambs (147 g/d). Feed conversion was better ($P<0.01$) for R and F while the DMI was similar for all three groups. Milk fat and protein content were comparable for all animals but standardize milk yield tend to be better for R and F group ($P=0.07$). The results of this study suggest that restricted feeding before puberty improve mammary gland development and milk production without compromising growth performances in ewe lambs.

Key Words: ewe lamb, mammary development, restricted feeding

M140 Effects of intravenous infusion of *trans*-10, *cis*-12 18:2 on mammary lipid metabolism in lactating dairy cows. R. Gervais*[1], J. W. McFadden[2], A. J. Lengi[2], B. A. Corl[2], and P. Y. Chouinard[1], [1]*Université Laval*, *Québec, QC, Canada*, [2]*Virginia Tech*, *Blacksburg*.

It has been previously established that supplementation of *t*10*c*12 18:2 reduces milk fat content and fat deposition in a number of species. The objectives of the study were 1) to examine whether potential mechanisms by which *t*10*c*12 18:2 is reported to affect lipid metabolism in adipose tissue of different species could be partly responsible for the inhibition in milk fat synthesis observed in dairy cows; 2) to investigate the effects of *t*10*c*12 18:2 on the expression of a newly identified isoform of stearoyl-CoA desaturase in bovine mammary tissue. Four primiparous lactating Holstein cows fitted with a jugular catheter were used in a 2×2 crossover design. For the first 5 d of each period, cows were infused intravenously with a 15% lipid emulsion providing 10 g/d of either *c*9*c*12 18:2 (CTL) or *t*10*c*12 18:2 (CLA). On d 5 of infusion, mammary gland biopsies were performed and tissues were analyzed for mRNA expression of acetyl-CoA carboxylase (ACC), fatty acid synthetase (FAS), lipoprotein lipase (LPL), stearoyl CoA desaturase-1 (SCD1) and -5 (SCD5), sterol regulatory element-binding protein-1 (SREBP1), interleukin-6 (IL6) and -8 (IL8), and tumor necrosis factor-α (TNFα) by real-time RT-PCR. Compared to CTL, CLA reduced milk fat content and yield by 46 and 38%, respectively ($P<0.05$), and increased the *t*10*c*12 18:2 content in milk fat from 0.05 to 3.53 mg/g ($P<0.05$). Milk yield, milk protein, and DMI were unaffected by treatments ($P>0.15$). Infusion of the CLA treatment reduced the mRNA expression of ACC and FAS by 46 and 57%, respectively and tended to reduce the expression of SCD1 ($P=0.06$) and LPL ($P=0.14$). Abundance of SREBP1 mRNA was reduced by 59% in the CLA treatment ($P<0.05$). However, infusing *t*10*c*12 18:2 did not affect the expression of transcripts for SCD5, TNFα, IL6, and IL8 ($P>0.15$). Results from the current study corroborate the idea that effects of *t*10*c*12 18:2 observed on adipose tissue in animal models and humans are not part of the response in the inhibition of milk fat synthesis in dairy cows. They also support the hypothesis that SCD1 and SCD5 present important differences in their regulation and physiological roles.

Key Words: CLA, lipid metabolism, desaturase

M141 Selection of reference genes for quantitative real-time PCR in mouse mammary gland during different lactation days. X. L. Dong[1,2], J. Q. Wang*[1], D. P. Bu[1], K. L. Liu[1], H. Y. Wei[1], and L. Y. Zhou[1], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*Yangzhou University*, *Yangzhou, China*.

Real-time quantitative PCR (qPCR) is a method for rapid and reliable quantification of mRNA transcription. Selection of high quality reference genes is crucial to interpret the data generated by this method. The aim of this study was to investigate the expression stability of reference genes in the mammary gland of mouse during lactation circle. Six reference genes (*B2M*, *ACTB*, *GAPDH*, *SDHA*, *HPRT1* and *ARBP*) were chosen. The mammary gland samples were obtained from thirty lactating mice that were at 0, 1, 4, 8 and 12 days relative to parturition. Gene expression levels were measured by qPCR. During the different lactation days, the expression of the gene was analyzed by the ANOVA procedure of SAS (Version 6.12, SAS). The expression of *ACTB*,*GAPDH*, *SDHA*,