M123 Use of ash and nitrogen concentrations in manure to estimate loss of ammonia over time. H. A. Paz* and W. P. Weiss, *The Ohio State University*, *Wooster*.

The aim of this study was to validate a mass balance method based on N and ash in manure to estimate NH_3 losses from manure. Six multiparous Holstein were used in a replicated 3 x 3 Latin square with three 14 d periods. Two diets were balanced to contain 15.5% CP but in one the forage (58% of diet DM) was mainly corn silage (CS) and alfalfa silage in the other (AS). A third diet (CSU) was the same as the CS diet except that 0.5% of urea was added (17.3% CP). During total collection digestion trials (last 4 d of each period), urine and feces samples were used to prepare a slurry mixture (1200 g) in the same proportion as excreted by each cow. Slurries were sampled immediately after mixing, incubated at ambient temperature (12 °C) for 3 d and sampled again. Initial and final N concentrations and slurry masses were used to determine N losses. Nitrogen loss was estimated using the mass balance method as: (N Intake - N Milk) - N/Ash*(Ash Intake - Ash Milk), where N and Ash Intake and N and Ash Milk are g/d and N and Ash concentrations in slurry samples at 3 d constitute the N/Ash ratio. Average daily DM intake and milk yield were 22.4 and 32 kg/d, respectively, and were not affected by treatment. Average manure output was 65.4 kg/d with cows fed AS and CSU excreting an average of 18.6 kg/d of urine followed by CS at 14.5 kg/d (P= 0.02). Manure N excretion was highest for cows fed CSU followed by AS and CS (422, 392 and 343 g/d, P<0.01). Urinary N as percent of excreted N was 34, 41, 53% for AS, CS, CSU, respectively (P<0.01). Ash excretion in manure was highest for cows fed AS (1134 g/d); CS and CSU had similar ash excretion (936 g/d) (P<0.01). Urine contributed 46, 42, and 40% of excreted ash for AS, CSU and CS. Measured NH_3-N loss was 30, 41, and 70 g/3 d for AS, CS, and CSU diet and calculated losses were 17, 31, and 75 g/3 d, respectively (AS = CS < CSU for both measured and calculated losses, P<0.01). Average estimated NH_3-N losses did not differ from measured losses (P= 0.15), but calculated values averaged 6 g less. The method underestimated NH_3-N losses in cows with negative N balance and overestimated it for cows in positive N balance.

Key Words: nitrogen, ash, NH_3

M124 The effects of metaphylactic therapy on health and growth of neonatal Holstein bull calves. K. S. Holloway*, G. A. Holub, J. E. Sawyer, and M. A. Tomaszewski, *Texas A&M University, College Station*.

A study evaluating effects of metaphylaxis and milk replacer additives on health and growth was conducted with newborn Holstein bull calves (n = 52; mean BW = 42.28 ± 3 kg) < 7 d of age. Calves were randomly assigned to receive tilmicosin phosphate (TIL), ceftiofur crystalline free acid (CEF), or saline (CON). All calves received a commercial milk replacer program (25% CP, 20% fat), and within metaphylaxis treatment, were randomly assigned to receive milk replacer with: 1) 4 g/d for 7 d and then 2 g/d for the next 14 d of an egg-based additive (PR); 2) 2 g/d of 96% betaine (BE); 3) both PR and BE (BP); or 4) without additives (NA). Calves were housed in individual fiberglass hutches with ad libitum access to a commercial calf starter and water. Body weight was recorded twice weekly and fecal scores (1=firm, 4=watery) were recorded daily for 54 d. Number of treatments per calf for scours, incidence of respiratory symptoms, and febrile events were recorded on a daily basis, and the cumulative incidence of each response was used as an index of morbidity. All data were analyzed as a completely randomized design with a 3 X 4 factorial treatment arrangement. Neither metaphylaxis, additives, nor their interaction affected ADG (P>0.60); overall, calves gained .45 kg/d. Fecal scores were reduced by 39% for CEF compared to CON (P<0.01), but were not affected by additives. Metaphylaxis influenced neither incidence of fever (P>0.3) or respiratory symptoms (P>0.2) nor were they reduced by additives. Overall, calves were treated an average of only 0.39 times for respiratory symptoms and 0.66 times for fever. Scours were not influenced by metaphylaxis (P>0.6), additives (P>0.5), nor their interaction (P>0.8). Other than fecal score, metaphylaxis did not enhance productivity or reduce morbidity in this study, but disease challenge may have been mild. Feed additives influenced neither measures of health and performance nor did the metaphylaxis and feed additive interaction.

Key Words: calf, metaphylaxis

M125 Effects of single nucleotide polymorphisms in stearoyl CoA desaturase on milk fatty acid profile in lactating Holstein cows fed diets varying in fat content. L. Clark*, S. Moore, and M. Oba, *University of Alberta*, *Edmonton, Alberta, Canada*.

The objective of this study was to determine the effect of a single nucleotide polymorphism (SNP) in the stearoyl CoA desaturase (SCD) gene on the ability of dairy cows to produce 9*c*, 11*t*-18:2 (CLA), and to determine whether the CLA to 11*t*-18:1 (VCA) ratio can be used as a phenotypic indicator for CLA production capability. A SNP in SCD found on the fifth exon of chromosome 26, which encodes an amino acid change from alanine to valine, was evaluated in this study. Twelve lactating dairy cows in mid-lactation (180 ± 44 days in milk) were blocked by SCD genotype (AA, AV, and VV; n=4 for each genotype) and fed either a high or low fat diet in a cross-over design with 21-d periods. The high fat diet contained 5% sunflower seed oil on a dry matter basis, and dietary fat content was 3.2 and 8.3% for the low and high fat diet, respectively. Milk yield and milk fat concentration were not affected by dietary treatment or genotype, averaging 29.0 kg/d and 3.61%, respectively. The CLA concentration in milk fat was greater for cows fed the high fat diet compared with those fed the low fat diet (4.4 vs. 0.6%; $P < 0.0001$), but was not affected by genotype. The CLA-to-VCA ratio was 0.62 when AV and VV cows were fed the low fat diet, but decreased to 0.50 when they were fed the high fat diet. However, the CLA-to-VCA ratio was not affected by dietary fat content for AA cows, averaging 0.57 ($P < 0.06$ for diet × genotype interaction). These results indicate that AA animals maintained consistent activity of SCD when they were fed the high fat diet. Because the CLA-to-VCA ratio is affected by dietary fat content, it may not be used as a phenotypic indicator to identify animals with greater capability for CLA production in the field, in which animals are fed diets varying in dietary fat content.

Key Words: CLA, SNP, stearoyl CoA desaturase (SCD)

M126 Influence of subclinical hypocalcemia on plasma biochemical parameters in dairy cows. W. G. Chamberlin*, J. R. Middleton, and J. N. Spain, *University of Missouri*, *Columbia*.

The purpose of this study was to evaluate the effects of calcium status at calving on plasma biochemical parameters and fat mobilization in

Holstein cows. It was hypothesized that cows with subclinical hypocalcemia at calving would have greater elevations in liver associated biochemical parameters and non-esterified fatty acid (NEFA) concentrations compared with normocalcemic cattle. Forty-four multiparous Holstein cows were assigned to one of two groups 1) hypocalcemic (n=28; ionized calcium [iCa] < 1.0 mmol/L) or 2) normocalcemic (n=16; iCa > 1.0 mmol/L) based on whole blood iCa concentrations on the day of calving. Blood samples were collected from all cattle for measurement of iCa concentrations, NEFA concentrations, and serum chemistry profiles at -14 d, 0 (calving), 3, 7, 14, 21, 28, and 35 d. Data are reported as LSMeans ± SE. On d 0, [iCa] differed between groups with hypocalcemic cows having a lower [iCa] (0.87 ± 0.01 mmol/L) compared with normocalcemic cows (1.10 ± 0.01 mmol/L; P < 0.001). Hypocalcemic cows also had lower total plasma [Ca] on d 0 (7.17 ± 0.09 mg/dL vs. 8.26 ± 0.12 mg/dL). Hypocalcemic cows had greater blood pH on d 0 (7.37 ± 0.02 vs. 7.27 ± 0.03; P = 0.01). Total plasma bilirubin concentration tended to be greater in hypocalcemic cows on d 21 (0.24 ± 0.02 mg/dL vs. 0.17 ± 0.03 mg/dL, respectively; P = 0.08). Gamma-glutamyl transferase activity was greater in hypocalcemic cows on d 0, 3, and 7 (18.8 ± 0.8 vs. 16.0 ± 1.0 U/L; 18.4 ± 0.8 vs. 15.2 ± 1.1 U/L; and 20.8 ± 0.9 vs. 17.1 ± 1.0 U/L, respectively; P ± 0.03). These results indicate a relationship between hypocalcemia at calving, blood pH, and altered liver associated biochemical parameters.

Key Words: hypocalcemic, NEFA, transition cows

M127 Risk factors for multi-drug resistance of *Staphylococcus aureus* obtained from cases of mastitis. L. Oliveira*, H. Langoni, and P. Ruegg, *University of Wisconsin*, *Madison*.

The objective was to study risk factors for multi drug resistance (MDR) of *Staphylococcus aureus* isolated from milk samples obtained from cows with mastitis. Minimum inhibitory concentrations (MIC) of 12 antimicrobials were determined for S. aureus obtained from clinical (n = 58) and subclinical (n = 58) cases of mastitis that occurred on commercial dairy herds (n = 13). Of isolates, 76 (66%) did not exhibit resistance to any of the antimicrobials tested, 22 (19%) exhibited single drug resistance (SDR) and 18 (15%) exhibited multi drug resistance. Isolates with SDR were observed in 9 farms and MDR was exhibited in 18 isolates obtained from 4 farms. Farms could present isolates with SDR and MDR. Among resistant isolates, the distribution of MDR was 15 (66.7%) to two antimicrobials, and 3 (33.3%) to three antimicrobials. No isolate demonstrated resistance to more than three antimicrobials. MDR were observed in 7 (12%) and 11 (19%) of *S. aureus* obtained from subclinical and clinical cases, respectively. The combination of MDR to erythromycin and tetracycline (30.0%), and ceftiofur and enrofloxacin (27.8%) accounted for 57.8% of all MDR combinations. Enrofloxacin resistance was found in 7 isolates obtained from clinical cases that originated on one farm. Oxacillin resistance (indicating resistance to methicillin) was observed in a single isolate. Resistance type (SDR or MDR) was not associated with case type (P = 0.75), colony forming units (P = 0.12), somatic cell count (P = 0.98), days in milk (P = 0.30), or milk yield (P = 0.63). In this study, MDR was uncommon among the *S. aureus* isolated from bovine mastitis and there was no association between MDR and selected risk factors.

Key Words: *Staphylococcus aureus*, antibiotic resistance, multi-drug resistance

M128 Evaluating the impacts of a ruminally protected lysine product in dairy cows. N. Swanepoel*[1,2], P. H. Robinson[2], and L. J. Erasmus[1], [1]*University of Pretoria*, *Pretoria, South Africa*, [2]*University of California*, *Davis*.

The objective was to estimate the rumen escape potential of a prototype ruminally protected lysine (Lys) product (RPL; Ajinomoto Co., Tokyo, Japan) and to determine its effects on milk production and composition of high producing dairy cows. Rumen Lys escape of the RPL (42% L-Lys, 42% fatty acids) was estimated to be 18-23% using an in situ bag technique, increasing calculated intestinal absorption of Lys by 8-10 g/cow/d (the RPL was fed at 97 g/cow/d). The design was a double (early and mid lactation) 2x2 factorial with RPL crossover in two 28 day periods. Multiparity cows, grouped according to days in milk (DIM), allocated to one of two similar pens (~180 cows/pen), were fed the same total mixed ration (TMR), except for the RPL mixed into the treatment TMR. Both TMR were based on alfalfa hay, corn silage, steam flaked corn and dried corn distillers grains. Metabolic model evaluation suggested the TMR were limiting in Lys due to the high inclusion of corn products (47.6% of DM) causing a low model predicted Lys:Met ratio (i.e., ~3.1) in intestinally delivered protein of control cows. Dry matter intake was not impacted by RPL feeding in either early or mid DIM cows. Milk and true protein yields were reduced (41.8 vs. 40.9 kg/d and 1.27 vs. 1.25 kg/d; P = 0.05) by RPL in mid DIM cows but remained unchanged for early DIM cows. RPL decreased milk fat yield (1.86 vs. 1.77 kg/d and 1.56 vs. 1.43 kg/d; P<0.01), as well as fat concentration (3.50 vs. 3.29% and 3.74 vs. 3.54%; P<0.01) in early and mid DIM cows. Reduced concentrations of most essential amino acids (AA) in blood plasma with RPL feeding suggest that Lys was limiting. Since leucine (Leu) and isoleucine (Ile), as well as methionine (Met) and tryptophan (Trp) share intestinal transport systems, supplementary Lys from RPL may have reduced absorption of Ile and Trp through stimulation of Met and Leu absorption. Due to the involvement of these AA in metabolic processes (i.e., fatty acid synthesis, the citric acid cycle, muscle protein degradation), this could have changed energy metabolism in this study, reducing milk fat and/or protein synthesis when a small amount of Lys as RPL was supplemented.

Key Words: amino acids, milk protein, rumen bypass

M129 Effect of two CIDRs on progesterone concentrations and LH secretion in lactating dairy cows. C. Tritsch*, W. Silvia, S. Hayes, D. Ray, H. Hamilton, and A. Sanders, *University of Kentucky*, *Lexington*.

The concentrations of progesterone (P) maintained by 0, 1 or 2 EAZI-BREED CIDR® Cattle Inserts (Pfizer, New York, NY) were examined in lactating Holstein cows. Cows were presynchronized using prostaglandin (PG) F2a (Lutalyse® Sterile Solution, Pfizer, 25 mg each, i.m.) given at 50-120 days postpartum. Ten days later, ovaries were scanned ultrasonographically to confirm the presence of mature corpra lutea. CIDRs (0 CIDR controls n=5; 1 CIDR n=6; 2 CIDRs n=6) were inserted into each animal on the day of CL detection (experimental day 0). Animals received two injections of PGF2a as above, the first on the morning of day 1, the second 12 h later. Caudal blood samples were collected twice daily on days 0-2, then at 6 hr intervals beginning at 8 AM on day 3 for 24 h to quantify P. The concentration of P maintained by the CIDR (CIDR-P) was defined as the mean concentration in samples collected from 8 AM on day 3 through 8 AM on day 4. Jugular venous catheters were inserted on day 2. Blood samples were collected at 10 min intervals for 8 hours beginning at 8 AM on day 3, then an injection of GnRH (Cystorelin ®; Merial, Duluth, GA, USA; 100 ug, i.m.) was administered and blood sampling continued for 4 h at

30 min intervals. The effect of number of CIDRs (0, 1, 2) on CIDR-P, LH interpulse interval and the magnitude of the LH response to GnRH were determined by ANOVA. There was an effect of CIDR number on CIDR-P ($p < 0.0001$). There was no effect of CIDR number on the LH interpulse interval ($P = 0.4$). There was suppression in the amount of LH released in response to GnRH in the presence of 1 CIDR ($P < 0.01$). An additional CIDR had no further effect. These data suggest that 1) implanting 2 CIDRs leads to a significant increase in P compared to implanting a single CIDR, 2) increasing the concentration of P using two CIDRs failed to exert a negative feedback effect on pulsatile secretion of LH and 3) increasing the concentration of P with one CIDR exerted a significant suppression in the ability of GnRH to induce secretion of LH. *This research was supported by Kentucky Agricultural Experiment Station and KABA/Select Sires.*

Key Words: progesterone, dairy cow, LH

M130 Effects of increasing glycerin in the diet on ruminal fermentation during continuous culture. D. E. Rico*, Y. -H. Chung, C. M. Martinez, T. Cassidy, K. S. Heyler, and G. A. Varga, *The Pennsylvania State University*, *University Park*.

The objective of this experiment was to evaluate the impact of increasing levels of glycerin on ruminal fermentation during continuous culture. A 4 × 4 Latin square design was used to study four levels (0, 3, 5, and 8% of ration DM) of dry glycerin (min. 65% food grade glycerol) in the diet when replacing corn starch. The trial had 4 experimental periods of 9 d each, the first 6 d for adaptation and the last 3 d for sampling of effluent for DM content. Samples for VFA and ammonia were taken hourly for 5 h after feeding on day 9. Fermenters (1015 to 1040 ml in volume) were incubated with ruminal fluid (1 L) and ruminal digesta (25 g) from a cow receiving a diet with 16% CP, 32% NDF, and 25% starch. Fermenters were fed 25 g DM of the experimental diets three times per day and the solids retention time was set at 24 h. During the first 5 h after feeding, production of total VFA increased linearly ($P <0.05$) (76, 81, 86 and 87 mmol/L) while ratio of acetate to propionate decreased linearly ($P < 0.01$) (2.7, 2.3, 1.8 and 1.5) by increasing level of glycerin in the diet. Ammonia concentrations (11.7 15.1, 12.8, and 14.3 mg/dl) increased linearly ($P < 0.05$) with increasing glycerin level during the first 5 h after feeding. Dry matter digestibility increased linearly ($P < 0.01$) with glycerin level and was 37, 38, 41, and 40% for 0, 3, 5, 8% glycerin treatments, respectively. Higher ammonia concentration may indicate lower efficiency of nitrogen utilization by the ruminal microorganisms. Under the present experimental conditions, replacement of starch with glycerin seemed to have positive effects, as shown by increased total VFA production, decreased acetate to propionate ratio, and higher DM digestibility.

Key Words: dry glycerin, ruminal fermentation, continuous culture

M131 Evaluation of the economic impact of Optigen® use in commercial dairy herd diets with varying feed and milk prices. J. F. Inostroza*[1], V. E. Cabrera[1], R. D. Shaver[1], and J. M. Tricárico[2], [1]*University of Wisconsin*, *Madison*, [2]*Alltech Inc.*, *Brookings, SD*.

The objective of this study was to evaluate the impact of Optigen® (blended, controlled-release urea) use in commercial dairy herd diets on feed cost and milk income minus feed cost. Results from a field trial with 16 Wisconsin herds randomly assigned to treatment sequences of either Optigen® (OPT; 114 g/cow/d replacing an equivalent amount of supplemental CP to provide iso-nitrogenous TMR; TMR formulation space created by the use of OPT was filled with either corn grain or corn silage DM) to control (CON) or CON to OPT in a cross-over design with two 30-d feeding periods, showed that milk yield was 0.5 kg/d/cow greater ($P < 0.01$) for OPT than for CON; data were analyzed using the mixed model procedure of SAS with period, sequence and treatment as fixed effects and herd as a random effect. An economic simulation analysis was performed using the OPT feeding rate and milk yield response from the field trial and monthly soybean meal-48 ($0.373±0.054/kg), dry corn ($0.188±0.020/kg), corn silage ($0.059±0.005/kg), and high-moisture corn ($0.149±0.016/kg) prices (as-fed basis) and milk prices ($0.38±0.03/kg) for January through December, 2008. The cost of OPT was set at $1.63/kg. A total of 32 combinations of varying feed and milk prices were simulated. Results are provided in Table 1. Under the conditions of the simulations performed in this study, OPT reduced feed cost only when corn silage was used to fill formulation space while milk income minus feed cost was increased by OPT for all scenarios. A decision tool spreadsheet was developed to allow for further economic simulation analyses with the ability to vary the milk yield response to OPT, the cost of OPT, and the CP and energy supplements evaluated.

Table 1. Economic impact of Optigen® use in dairy herd diets.

CP Supplement Replaced by OPT	Ingredient Used to Fill Formulation Space	Feed Cost OPT - CON ($/cow/day)	Milk Income OPT - CON ($/cow/day)	Milk Income - Feed Cost ($/cow/day)
SBM-48	Dry Corn	0.047 (± 0.027)	0.192 (± 0.016)	0.145 (± 0.039)
SBM-48	Corn Silage	-0.020 (± 0.039)	0.192 (± 0.016)	0.212 (± 0.051)
SBM-48	HM Corn	0.042 (± 0.028)	0.192 (± 0.016)	0.150 (± 0.040)

Key Words: controlled-release urea, feed cost, dairy cows

M132 Dry matter intake measurements in commercial tie-stall dairy herds. M. W. Dekleva*[1], C. D. Dechow[1], J. M. Daubert[1], J. W. Blum[2], and G. A. Varga[1], [1]*The Pennsylvania State University*, *University Park*, [2]*University of Bern*, *Bern, Switzerland*.

The objective of this study was to determine the relationships among fat corrected milk (FCM), measured dry matter intake (DMI), and DMI predicted from National Research Council equations (NDMI) in order to determine if DMI could be measured with sufficient accuracy in commercial tie-stall dairy herds to facilitate genetic research. There were 1091 intake measurements made for 543 cows from 11 herds in Central Pennsylvania. All herds participated in monthly Dairy Herd Improvement Association testing, and intakes were measured once monthly over a 24-hour period within 7 days of a test date. Herd managers were instructed to follow their normal feeding routine and to distribute feed evenly to all cows. Intakes were then calculated by recording the total feed distributed to a group of cows, adjusting for observed variation, recording the amount of feed that was moved to or from individual cows, and subtracting weights of individual refusals the following morning. Feed samples were collected on each visit and analyzed for dry matter percentage. Body weights were estimated from hearth girth measurements at four points during lactation. Least-squares-means (LSM) for FCM, DMI and BW were estimated for week of lactation 1 to 40 using a four-trait model in ASREML. Fixed effects included herd-test day, herd-lactation and week of lactation. Random effects were cow and error. Least squares-means for BW and FCM were used to derive weekly

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