TH59 Microbial nitrogen synthesis in lambs fed corn silage inoculated with *Lactobacillus buchneri* associated with levels of concentrate. F. C. Basso*, C. H. S. Rabelo, E. C. Lara, L. G. O. Jorge, G. R. Siqueira, and R. A. Reis, *Department of Animal Science, UNESP/FCAV, Jaboticabal, SP, Brazil.*

This study aimed to evaluate microbial N synthesis in lambs fed corn silage inoculated with *L. buchneri* associated to levels of concentrate. Corn hybrid was harvested at 31% DM, chopped and treated without (control) or with 1×10^5cfu of *L. buchneri* 40788 (LB) per gram of forage. Two bunker silos were filled with 60 t of forage each, remaining closed for 70 d. Twenty-eight crossbreed lambs (BW = 25.7 ± 4.3 kg), males, were divided in 7 blocks into 4 treatments: control and LB silages associated with 40 and 60% of concentrate (corn meal, soybean meal, wheat meal and mineral supplement, DM basis). Lambs were housed in individual pens and fed ad libitum twice daily (0700 and 1700 h). Orts were weighed daily and DMI was determined. Silage, ingredients of concentrate and orts were collected twice per week and analyzed for DM, CP, NDF and NSC. On d 36 of trial, spot urine samples were collected from all animals (BW = 32 ± 4.1 kg; 4 h after feeding) for later analysis of purine derivatives (PD: allantoin, uric acid, xanthine and hypoxanthine). Microbial N supply and microbial N synthesis were estimated and analyzed as randomized block design in a 2 × 2 factorial arrangement by ANOVA using MIXED procedure of SAS. Inoculation of corn silage reduced concentrations of NDF (361 vs. 390 g/kg of DM) and increased the NSC content (514 vs. 491 g/kg of MS). Lambs fed with control silage and 40% of concentrate consumed less that to animals fed with control silage and 60% of concentrate ($P < 0.05$), observing similar nutrients intake between lambs fed LB silage. Lambs fed LB silage and 40% of concentrate had higher microbial N supply (7.6 vs. 6.5 g/d) than those fed LB silage and 60% of concentrate; however microbial N synthesis was similar between levels (13.6 vs. 12.4 g of N/ kg of DOMR). Otherwise, lambs fed with control silage and 40% of concentrate had a lower microbial N supply (5.8 vs. 8.3 g/d) and microbial N synthesis that to those fed with control silage and 60% of concentrate (12.1 vs. 16.1 g of N/ kg of DOMR) in the diet ($P < 0.05$). Inoculation of corn silage increased the efficiency of diet utilization. Financial support by Fundacao de Amparo a Pesquisa do Estado de Sao Paulo– FAPESP, Sao Paulo, SP, Brazil.

Key Words: fiber composition, purine derivative

TH60 Influence of endotoxin and thermolysin on claw explants in an ex vivo laminitis model. S. Schaumberger*, M. Penner, N. Reisinger, and G. Schatzmayr, *Biomin Research Center, Tulln, Austria.*

Laminitis in ruminants has a multifactorial etiology. As a key factor feeding of increased fermentable carbohydrate has been identified. This feed change may lead to rumen acidosis. Coincident with the change in the rumen pH is the release of endotoxin from gram-negative bacteria. An enhanced absorption of bacteria, endotoxins, exotoxins, lactic acid and histamine through the rumen, leads to a direct or indirect disruption in the micro-circulation of the corium and later to the lesions observed in laminitis. Objective of our ex vivo study was to test the influence of endotoxins and thermolysin on the lamellar separation, which is observed during laminitis. Claws were obtained from the slaughter house and dissected. Claw explants consisted of inner claw wall, epidermal lamellae and of connective tissue. Explants were cultured with 1 mL culture medium and with different concentrations (200 – 50 µg/mL) of lipopolysaccharides (LPS) from *Escherichia coli* O55:B5 and thermolysin (100 – 1 µg/mL) from *Bacillus thermoproteolyticus rokko* for 24 h at 37°C and 5% CO_2. Explants cultured only in medium were used as a negative control. After incubation explants were tested for their integrity with a force transducer. Explants cultured in medium remained intact and were viable. Thermolysin showed a concentration-dependent separation. Separation force was significantly decreased in explants treated with 50 and 100 µg/mL thermolysin ($P = 0.004$ and $P = 0.006$) compared with control. In explants treated with 50 µg/mL LPS a separation force of 30 ± 6 N was necessary. For an unknown reason, separation force increased (35 ± 3 N and 36 ± 3 N) at 100 and 200 µg/mL LPS. In all runs, average separation force in the control group was 35 ± 11 N. No significant differences in separation force were revealed for explants incubated in LPS. Further experiments will be carried out to elucidate the contribution of LPS on the lamellar separation.

Key Words: laminitis, endotoxin, ruminant

TH61 Evaluation of a protocol to measure endotoxin activity in rumen fluid. S. Schaumberger*, C. Kalteis, N. Reisinger, and G. Schatzmayr, *Biomin Research Center, Tulln, Austria.*

Ruminants are fed high-grain diets to maximize their energy intake and productivity. These high fermentable diets often cause excess fermentation and lead to an acidotic state in the. During low rumen pH gram-negative bacteria lyse more rapidly and endotoxin concentration increases. The complexity of this imbalance leads to a reduced barrier function in the rumen. Endotoxin is translocated into blood and stimulates an inflammatory cell response. Clinical outcomes of a (sub-) acidotic state are, for example, rumenitis, metabolic acidosis, laminitis, reduced feed intake, abomasal displacement and others. Objective of our investigations was, to evaluate a reproducible method for detecting endotoxins in rumen fluid via kinetic chromogen Limulus-Amebocyte-Lysate assay (LAL). Following a literature search, 2 methods for sample preparation were compared: Thawed rumen fluid samples were heat inactivated and then either centrifuged or filtered (0.45 µm), before diluted and tested. Additionally tests were performed with and without Beta-Glucan buffer (Glucashield). LAL test was performed according to producers' guidelines. Best results (lowest standard deviations within repetitions) were recovered with the combination of filtering and the use of glucan buffer. Values recovered in rumen fluid were around 2.960 ± 110 EU/mL. Compared with this method, highest values were recovered with the method of combined centrifugation and Glucan buffer (5.680 ± 1.350 EU/mL). Tests methods with the use of glucan buffer revealed significant differences in their endotoxin activity ($P = 0.05$) in rumen fluid. Further tests will be performed to explain the differences in endotoxin activity in treatments with filters and centrifugation.

Key Words: acidosis, endotoxin, rumen fluid

TH62 Effect of corn silage with microbial inoculants on performance of feedlot lambs. F. C. Basso*, E. C. Lara, C. H. S. Rabelo, M. F. C. Miranda, G. S. Goncalves, L. G. O. Jorge, and R. A. Reis, *Department of Animal Science, UNESP/FCAV, Jaboticabal, SP, Brazil.*

This study aimed to evaluate the lambs performance fed corn silage inoculated with microbial inoculants. Corn hybrid was harvested at 40.4% DM, chopped, treated and ensiled in 3 stack silos with 40 t of forage each (closed for 85 d). The treatments were 1×10^5 cfu of

Lactobacillus plantarum MA18/5U combined with 1×10^5 cfu of *L. buchneri* CNCM I-4323 (LPLB) or combined with 1×10^5 cfu of *B. subtilis* AT553098 (LPBS) per gram of fresh forage, remaining a treatment uninoculated (control silage). Thirty crossbred lambs (BW = 29 ± 3.0 kg), males, were divided in 10 blocks into 3 treatments. Diet was composed of 60% of silage and 40% of concentrate (ingredients: corn meal, soybean meal, urea and mineral supplement, DM basis) and was formulated to daily gain of 200 g/d. Lambs were housed in individual pens and fed ad libitum twice daily (0700 and 1700 h). Orts were weighed daily and DMI was determined. Animals were weighed after fasting (16 h) at the beginning and end of the experimental period to obtain the ADG and feed conversion (FC). Animals were harvested with 40 kg (approximately 53 d of feedlot), then, carcasses were weighed to find the hot carcass weight (HCW), stored at −4°C for 24 h and weighed again to find the cold carcass weight (CCW). Thus, losses by cooling (LC), hot and cold carcass yield (HCY and CCY) were calculated. Subcutaneous fat thickness (FT) was measured over the loin eye between the 12th and 13th rib. Data were analyzed as randomized block by ANOVA using MIXED procedure of SAS (version 9.0). Lambs fed control silage showed higher DMI (1.5 kg/d) than those fed control silage (LPLB: 1.3 kg/d; LPBS: 1.4 kg/d; $P < 0.05$), whereas ADG was similar among animals (219 g/d). However, FC improved ($P < 0.05$) in lambs fed LPLB silage (6.0; Control: 6.5; LPBS: 6.8). The HCW (19.0 kg), CCW (18.3 kg), LC (2.8%), HCY (46.7%), CCY (45.0%) and FT (2.1 mm) were similar among animals fed different silages ($P > 0.05$). Inoculation in corn silage did not promote improvements on carcass characteristics, but feed conversion was enhanced in the lambs fed corn silage inoculated with *L. plantarum* combined *L. buchneri*. Financial support by Fundacao de Amparo a Pesquisa do Estado de Sao Paulo – FAPESP, $$Sao Paulo, SP, Brazil.

Key Words: *Bacillus subtilis*, *Lactobacillus buchneri*, *Lactobacillus plantarum*

TH63 Influence of yeast viability on rumen fermentation parameters and nutrient digestibility in beef heifers. D. Vyas*[1], A. Uwijeye[1], R. Mohammed[1], W. Z. Yang[1], K. A. Beauchemin[1], and N. Walker[2], *[1]Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, [2]AB Vista, Marlborough, Wiltshire, UK.*

The study was aimed at determining the importance of yeast viability for reducing the incidence of subacute ruminal acidosis (SARA) and improving total tract nutrient digestibility in cattle. Six ruminally cannulated beef heifers (680 ± 50 kg BW) were used in a replicated 3 × 3 Latin square design and were fed a diet consisting of 40% barley silage, 10% chopped grass hay, and 50% barley grain based concentrate. Treatments were (1) control (no yeast); (2) active dried yeast (ADY; 4 g providing 10^{10} cfu/g; AB Vista, UK); and (3) killed dried yeast (KDY; 4 g autoclaved ADY). The treatments were directly dosed via the rumen cannula daily at the time of feeding. The periods consisted of 2 wk of adaptation (d1–14) and 6 d of measurements (d15–20). Ruminal pH was continuously measured (d14–20) using an indwelling system. Rumen contents were sampled on d15 and d17 at 0, 3, 6, 9 and 12 h after feeding. Total-tract nutrient digestibility was measured using an external marker ($YbCl_3$) from d15–20. Dry matter intake tended to be higher with KDY ($P = 0.07$) while no treatment effects were observed for apparent total-tract nutrient digestibility. Both ADY and KDY elevated minimum and mean rumen pH ($P = 0.07$) while no effects were observed on maximum pH ($P = 0.17$). Both treatments were effective in reducing time that ruminal pH was below 5.8 ($P < 0.01$) and 5.6 ($P < 0.01$) compared with the control. No treatment differences were observed in the ruminal VFA profile and lactate concentration; however, NH_3-N was significantly higher with ADY compared with the other treatments. The relative population size of *S. bovis* was higher with both yeast treatments ($P < 0.01$) while *R. flavifaciens* tended to be higher with KDY ($P = 0.05$). However, *F. succinogenes*, *S. ruminantium*, and *M. elsdenii* remained similar for all the treatments. The study demonstrates the positive effects of yeast treatments in reducing the incidences of SARA irrespective of its viability. However, further studies are required to evaluate the importance of yeast viability for other dietary conditions, particularly when the risk of acidosis is high.

Key Words: active dry yeast, rumen acidosis, ruminal pH

TH64 Influence of tannins extract supplementation at low level on feedlot performance of Katahdin × Pelibuey hair-lambs. B. Ortiz*[1], A. Camacho[2], N. E. Villalba[3], L. R. Flores[2], M. A. Mariezcurrena[1], M. D. Mariezcurrena[1], and R. Barajas[2], *[1]Universidad Autonoma del Estado de Mexico, Toluca, Edo. de Mexico, Mexico, [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Sinaloa, Culiacan, Sinaloa, Mexico, [3]Agricola Ganadera Mojolo, Culiacan, Sinaloa, Mexico.*

Twenty-four hair lambs (3/4 Katahdin and 1/4 Pelibuey breed) with a mean weight of 24.07 ± 2.09 kg were involved in an experiment with the objective of determine the influence of tannins extract supplementation at low level on feedlot performance of Katahdin × Pelibuey hair-lambs. Lambs were weighed and in groups of 3, were placed in 8 elevated (0.6 m) plastic floor pens (0.9 × 1.9 m). In a completely randomized block design lambs were assigned to next treatments: (1) Feeding with a 95% concentrate corn-canola meal based diet (14.2% CP; 2.0 Mcal NEm/kg DM) without additional tannins extract (CTRL), or (2) Control diet plus 0.3% (DM basis) of tannins extract supplementation (TE). Tannins extract were obtained from a Condensed and hydrolysable tannins blend (BYPRO; Inudor, S.A., Argentina), that contains 72% of tannins. Lambs were weighed at starting experiment and when each block accomplishes the target final weight (35 to 40 kg), the block of heavy lambs required 42 d to reach the market weight, and the block of light lambs required 56 d to arrive at the target weight. Experiment was analyzed by ANOVA as a randomized complete block design, with 4 replicates for treatment, pen was considered as the experimental unit. Final weight was not influenced by treatments ($P = 0.32$). The average daily gain was improved 18% ($P < 0.05$) by TE supplementation, with means of 245 and 290 g/day for lambs in the CTRL and TE treatments, respectively. Dry matter intake and feed efficiency were not modified by treatments ($P > 0.90$). It is concluded that low tannins extract supplementation at level close to 0.3% of dietary DM, improves weight gain of Katahdin × Pelibuey hair-lambs.

Key Words: lamb, feedlot performance, tannin

TH65 Effects of dietary phytogenic feed additives on in vivo rumen fermentation, enzyme profile, and microbial ecology of crossbred cattles. S. L. Ingale*[1], A. K. Pattanaik[2], D. N. Kamra[2], and K. Sharma[2], *[1]: College of Animal Life Sciences, Kangwon National University, Chuncheon, Republic of Korea, [2]Clinical & Pet Nutrition Laboratory, Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar, India.*

An experiment was conducted to investigate the effects of dietary phytogenic feed additives on in vivo rumen fermentation, enzyme profile, and microbial ecology of crossbred cattle. Three rumen-fistulated Holstein-Frisian males (260 ± 14.8 kg) were arranged in a 3 × 3 switchover design involving 3 periods of 21 d duration each. Dietary treatments were

basal diet (CON) and basal diet supplemented with either 2.5% herbal combination comprising of *Withania somnifera*, *Boerhavia diffusa*, and *Holarrhena antidysentericum* (1:1:1; WiBH) or *Woodfordia fruticosa*, *Solanum nigrum*, and *Trigonelia-foenum-graceum* (1:1:1; WoST). The animals were fed on wheat straw:concentrate (60:40). In each of the 3 phases, at the end of 21d of feeding rumen liquor was sampled on 3 consecutive days, at 0, 2, 4, 6, and 8 h post-feeding and analyzed for pH, short chain fatty acids, nitrogen fractions, and protozoa population. For analysis of rumen enzymes activity and microbial quantification, whole rumen content was collected before feeding for 3 consecutive days. Dietary treatments had no effects ($P > 0.05$) on postprandial pH, short chain fatty acids, nitrogen fractions, and protozoa count of rumen liquor at any time of collection. The activity of rumen avicelase and xylanase were greater ($P < 0.05$) in animal fed diet supplemented with WoST compared with animal fed control and WiBH supplemented diets. Animals fed diets supplemented with WiBH or WoST had greater ($P < 0.05$) population of rumen *R. flavefaciens* and *F. succinogenes* and fungi compared with animals fed control diet. Moreover, *F. succinogenes* and fungi population were greater ($P < 0.05$) in WiBH supplemented groups than WoST supplemented group, whereas *R. flavefaciens* population was greater in WoST supplemented group than WiBH supplemented group. Results obtained in present study indicate that dietary supplementations of combination of phytogenic feed additives have potential to improve the fiber degrading bacteria, fungi populations and hydrolytic enzyme activity.

Key Words: crossbred cattle, enzyme profile, microbial ecology

TH66 Effect of tannin extract supplementation on apparent digestibility of crude protein and plasma urea nitrogen of implanted and non-implanted finishing hair-lambs. L. R. Flores*[1], J. J. Lomeli[1], J. I. Macias[1], E. A. Velazquez[1], N. E. Villalba[2], A. Camacho[1], E. Vazquez[1], I. Quintero[1], and R. Barajas[1], *[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Sinaloa, Culiacan, Sinaloa, Mexico, [2]Agricola y Ganadera Mojolo, S.A. de C.V., Culiacan, Sinaloa, Mexico.*

Twenty-four Katahdin lambs (25.05 ± 3.08 kg) were involved in a total feces collection experiment to evaluate the effect of tannin extract supplementation on apparent digestibility of crude protein and plasma urea nitrogen of implanted and non-implanted finishing hair-lambs. In groups of 3, lambs were placed in 8 plastic floor elevated pens (1.5 × 1.6 m). In agreement with a replicated cross over design were assigned to receive follows treatments: (1) Feeding a 95% concentrate diet based in whole corn grain and canola meal (CTRL); (2) Control and ear anabolic-implant (IM); (3) Control added with 0.3% (DM basis) of tannin extract (TE); and (4) Control added with 0.3% (DM basis) of tannin extract and ear anabolic-implant (IMTE). Experiment was divided in two 14-d periods; within a period, the first 10 d for adaptation and last 4 d for sample collection. Last day of each period blood samples were taken from jugular vein. Each cross over was integrated by 4 pens; lambs from 2 pens were ear implanted with 2 pellets of commercial implant Component (Elanco) equivalent to a dose of 40 mg of trenbolone and 8 mg of estradiol. One pen with implanted lambs and another with no implanted animals receiving TE supplementation, remainder pen were assigned to basal control diet. Once completed 14 d of first period, diets were switched. Experiment was analyzed by ANOVA for a replicated crossover design. Treatments had not effect ($P > 0.15$) on intake, fecal excretion and apparent digestibility of dry matter, organic matter or crude protein. Mean values of plasma urea nitrogen (PUN) concentration were 8.29, 5.91, 6.45, and 6.24 mg/dL for CTRL, IM, TE, and IMTE treatments, respectively. Both, IM and TE decreased ($P = 0.02$) PUN values compared with CTRL. PUN values were similar between IM and TE treatments. Results suggest than supplementation of tannin extract had no effect on apparent digestibility of crude protein and decreases PUN values in nonimplanted lambs, whereas in implanted lambs tannin supplementation did not affect PUN values.

Key Words: lamb, PUN, tannin

TH67 Effects of bismuth subsalicylate and dietary sulfur level on in vitro rumen fermentation in continuous culture. S. W. Fessenden*, A. J. Carpenter, M. Ruiz Moreno, and M. D. Stern, *Department of Animal Science, University of Minnesota, St. Paul, MN, USA.*

In ruminants, excess dietary sulfur is associated with reduced DM intake, poor feedlot performance, and sulfur-associated polioencephalomalacia. Bismuth subsalicylate (BSS) has been shown to decrease hydrogen sulfide in vitro; however, negative effects on fermentation were reported when BSS was included at 1% of diet DM. The objective of this experiment was to evaluate effects of BSS inclusion at 0.5% of diet DM on microbial fermentation in continuous culture when provided with differing levels of dietary sulfur. Eight dual-flow continuous culture fermenters were used during 2 consecutive 10-d periods consisting of 7 d for stabilization followed by 3 d of sampling. Treatments were arranged in a 2 × 2 factorial design, with 2 levels of BSS (0 and 0.5% of DM) and 2 levels of dietary sulfur (0.21% and 0.42% of DM). A pelleted feedlot diet containing 39% dry rolled corn, 32% earlage, 21% wet distillers grains, 3.2% corn silage, 1.5% soybean meal, 0.6% urea, and 2.7% mineral premix was provided to the fermenters at a rate of 75 g of DM/fermenter/d. Effluents from sampling days were composited by fermenter within period, resulting in 4 reps/treatment. BSS inclusion decreased ($P < 0.01$) true OM digestion, while no effects were observed for NDF and ADF digestion. Total VFA concentrations, molar proportions of acetic, propionic, and branched-chained VFAs decreased ($P < 0.01$) with BSS addition. The ratio of acetic to propionic acid and the molar proportion of butyric acid increased ($P < 0.01$) with BSS addition. In regard to nitrogen metabolism, BSS increased NH_3-N concentration, NH_3-N, and dietary-N flows ($P < 0.01$), and decreased non-NH_3-N flow, microbial-N flow and CP degradation ($P < 0.01$). Inclusion of BSS increased mean, minimum, and maximum fermentation pH ($P < 0.01$). Dietary sulfur level and the interaction of BSS and dietary sulfur had no effects ($P > 0.05$) on any measured parameter of fermentation. Results from this experiment indicate that BSS included at 0.5% of diet DM has detrimental effects on in vitro rumen fermentation in continuous culture. These effects are not dependent on level of dietary sulfur.

Key Words: bismuth subsalicylate, rumen

TH68 Effect of an exogenous phytase on digestibility, performance and phosphorus balance of Holstein steers. G. Buendía-Rodríguez[1], S. S. González-Muñoz*[2], M. D. Montoya-Flores[1], N. I. Ortega-Álvarez[1], and C. Aceves-Hacebe[1], *[1]CENIDFyMA, INIFAP, Ajuchitlán, Querétaro, México, [2]Colegio de Postgraduados, Montecillo, Estado de México, México.*

Phytase added to diets for ruminants may increase bioavailability of inorganic phosphorus (P). Therefore, the objective of this experiment was to evaluate the effect of an exogenous phytase on digestibility, performance and P balance of 24 Holstein steers (215.45 ± 12.26 kg initial body weight) individually fed a concentrate diet (14% CP and 3.1 Mcal ME). The experimental design was completely randomized with 3 treatments: 0, 12 and 24 g/t phytase (FINASE, AB Enzymes, Darmstadt, Germany; from *Trichoderma reesei*; 40,000 FTU/g) in the

diet. The experiment lasted 60 d and the variables recorded were: average daily gain (ADG), dry matter intake (DMI), feed conversion (FC), dry matter digestibility (DMD), P excretion and P retention (phosphorus balance). Data were analyzed using MIXED procedure of SAS and treatment means were compared with the Tukey test ($P \leq 0.05$). Treatments affected ($P \leq 0.05$) ADG (1119b, 1292a, 1130b; g) and DMI (5997^{ab}, 6307^{a}, 5933^{b}; g), but did not change FC (5.39, 4.92, 5.29) and DMD (65.38, 66.52, 64.65%). Besides, daily fecal P excretion was decreased (13.45^{a}, 13.08^{a}, 10.89^{b}; g) and as a consequence average P retention was increased (6.49^{c}, 7.89^{b}, 8.80^{a}; g/animal) ($P \leq 0.05$). Thus, it may be concluded that an exogenous phytase changed average daily gain and DMI of Holstein steers, whereas fecal P excretion was reduced, which may decrease soil contamination.

Key Words: phytase, steer performance, fecal P excretion.

TH69 Effects of supplementing *Propionibacterium freudenreichii* on lipid biohydrogenation of beef finishing diets containing flax oils using a semi-continuous fermentation system (RUSITEC). S. Ding*[1,2], M. L. He[2], G. O. Ribeiro Junior[3,2], A. Y. Alazzeh[2], H. Holo[4,5], O. M. Harstad[6], T. A. McAllister[2], S. J. Meale[1,2], and A. V. Chaves[1], *[1]Faculty of Veterinary Science, University of Sydney, Sydney, NSW, Australia, [2]Lethbridge Research Center, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, [3]Veterinary School, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, [4]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway, [5]TINE SA, Oslo, Norway, [6]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway.*

Propionibacteria has been shown to play a role in the ruminal biohydrogenation (BH) of polyunsaturated fatty acids (PUFA). However, to-date, no studies have been undertaken regarding the ability of propionibacteria to alter lipid BH patterns in diets supplemented with flax oil using the artificial rumen simulation technique (RUSITEC). Thus, a 23 d RUSITEC was conducted to examine the effects of supplementing *Propionibacterium freudenreichii* (strain T54) or T54 and flax oil in a beef finishing diet (TMR), on BH. The experiment consisted of an 8 d adaptation period and 15 d of inoculation with T54. Treatments were: 1) CON [10 g TMR + 23 mL autoclaved mixture of sodium lactate broth (SLB) with T54 (10^9 cfu/mL)]; 2) PB [10 g TMR + 23 mL inoculated SLB with T54 (10^9 cfu/mL)]; 3) FO (6% DM flax oil with autoclaved T54) FOPB (6% DM flax oil with T54). In situ DMD was determined at 48 h, and VFA concentrations were measured at 24 h on d 10 to 13. Proportions of individual fatty acids (FA) in feed residues were determined at 48 h and fatty acids profiles in the effluent was measured daily on d 20 to 23. Data were analyzed using the PROC Mixed SAS procedures. Within each run (n = 2), treatments were replicated twice. In situ DMD (%) at 48 h was not affected ($P > 0.10$) by either oil or T54. Flax oil resulted in higher ($P < 0.01$) total VFA production (m*M*) in comparison with non-oil groups. T54 or flax oil alone, or in combination, decreased ($P < 0.02$) acetate production. T54 increased ($P < 0.01$) propionic acid (%) by 14% compared with the control. Oil increased ($P < 0.01$) the percentages of PUFA and C18:3, yet decreased ($P < 0.01$) total saturated fatty acid content (SFA, %) in feed residues. Conversely, in the effluent, oil decreased PUFA (%) and C18:3 (%), while increasing SFA (%). T54 did not affect ($P > 0.05$) BH, except a slight decrease ($P < 0.01$) in PUFA (%) in the oil-based diet. Overall, it was determined that *P. freudenreichii* (T54) does not alter the fatty acid profiles in the effluent through changes in the biohydrogenation patterns of PUFA in flax oil.

Key Words: biohydrogenation, lipids, RUSITEC

TH73 Effects of probiotics supplementation on milk performance of late lactation dairy cows. L. C. Huang[1,3], N. Zheng[1,2], J. Q. Wang*[1,2], J. B. Cheng[1,3], and D. P. Bu[1], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Ministry of Agriculture - Milk and Dairy Product Inspection Center (Beijing), Beijing, China, [3]College of Animal Science and Technology, Anhui Agricultural University, Hefei, China.*

The supplementation of probiotics, including yeast, lactic acid bacteria and *Bacillus subtilis* natto, were evaluated in 48 late lactation dairy cows with similar milk yield (27.34 ± 4.92kg/d) and DIM (230.63 ± 31.59 d). Cows were allocated to 4 treatments, blocked by average daily milk yield in the previous weeks. Four treatments were control (C, no supplemental probiotics), 15 g/(cow·d) of yeast (YC), 1 g/(cow·d) of lactic acid bacteria (LC), and 1 g/(cow·d) of *Bacillus subtilis* natto (NT). The research lasted 9 wk, including 2 wk of diet adaptation and 7 wk of experimental period. The experimental data were analyzed by SAS 8.0 via the mixed model. Milk yield of LC (26.51 kg/d) and NT (25.65 kg/d) was greater than C (24.24 kg/d) and YC (24.68 kg/d) ($P < 0.0001$). LC was also greater than NT, however, YC was not different with C. Fat corrected milk (4%), milk fat and protein yield of LC (25.90 kg/d, $P < 0.0001$; 1019.12 g/d, $P = 0.0046$; 920.14g/d, $P < 0.0001$) and NT (25.56 kg/d, 1016.72 g/d and 905.36 g/d) were greater than C (24.21 kg/d, 970.89 g/d and 849.57 g/d) and YC (24.77 kg/d, 974.37 g/d and 851.66 g/d), while there was no significant difference between LC and NT, YC and C. Milk fat of C (3.99%) was greater than LC (3.83%) and YC (3.85%) ($P = 0.0147$), and NT (3.92%) was greater than LC; however, there was no significant difference between NT and C, NT and YC. Milk protein of C (3.52%) was greater than YC (3.46%) ($P = 0.0023$), furthermore NT (3.55%) was also greater than YC and LC (3.49%), there was no significant difference between LC and C, NT and C, YC and LC. Somatic cell counts (SCC) of C (105.14×10^4/mL), LC (96.60×10^4/ml), NT (96.88×10^4/mL) and YC (99.74×10^4/mL) had no significant difference, but SCC in cows with supplementation tended to decrease. The results indicated that the supplementation of lactic acid bacteria and *Bacillus subtilis* natto can improve milk performance of dairy cows in late lactation, but the supplementation of yeast did not have significant effect.

Key Words: dairy cow, milk performance, probiotic

TH74 Diversity of monensin-sensitive rumen proteolytic bacteria under different nitrogen sources in vitro. Y. F. Lu[1,2], J. Q. Wang*[1], S. G. Zhao[1], D. P. Bu[1], and G. Q. Zhao[2], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Sciences, Beijing, China, [2]College of Animal Science and Technology, Yangzhou University, Yangzhou, China.*

Monensin has been shown to inhibit amino acid-fermenting bacteria in rumen. Objective of this study was to compare the rates of ammonia production with added monensin and the diversity of monensin-sensitive proteolytic bacteria in rumen fluid in vitro under different nitrogen sources. Rumen fluid from the Holstein cows was inoculated media under anaerobic chamber with added different substrates, with or without 5 μ*M* monensin. The tubes were incubated in 3 triplicates at 21 h for 39°C and ammonia was evaluated at 0, 7, 14 and 21 h of incubation. Data were analyzed using SAS. Moreover, MOTHUR was used to assign 16S rDNA sequences to operational taxonomic units (OTUs) based on 97% sequence identity criterion and bacterial diversity. Both the NH_3 production of protein and amino acid were significant decreased ($P < 0.01$) by monensin (P-M, AA-M) after 14 h and 7 h, respectively. NH_3 was formed more than twice as rapidly from amino acid (AA) compared

with protein (P) at 21h. The rate of NH_3 production from protein was on average 56.65 nmol mg of protein^{-1} h^{-1} lower than amino acid ($P < 0.01$). Monensin had a greater effect on the rate of NH_3 production from protein (39.33834 nmol mg^{-1} h^{-1}) compared with amino acid (99.57078 nmol mg^{-1} h^{-1}) ($P < 0.01$). Furthermore, a total of 230 16S rDNA gene sequences were assigned to 67 OTUs which were from the phyla *Firmicutes*, *Bacteroidetes*, *Gammaproteobacteria*. Phylum *Gammaproteobacteria* was only to the AA-M (OTU22) and NN (OTU35). P had the most abundant OTU and had 5 more OTUs than P-M, but AA had 1 less OTU than AA-M. However Simpson and Chao 1 indices indicated P and AA had more diversity of proteolytic bacteria than that with added monensin P-M and AA-M respectively, especially P had the most abundant diversity than other treatments. These results suggest that monensin had a greater effect on the rate of NH_3 production from protein compared with amino acid and monensin can reduce the diversity of proteolytic bacteria, especially using protein as the nitrogen resource.

Key Words: monensin-sensitive, proteolytic bacteria, rumen

TH75 Effects of antibacterial agents on ruminal biohydrogenation of unsaturated fatty acid in vitro. Y. H. Jiang[1,2], J. Q. Wang*[1], H.J. Yang[1], D. P. Bu.[1], E. W. Jin[1], H. T. Shi[1], W. H. Bao[1], and P. Sun[1], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]College of Animal Science and Technology, China Agricultural University, Beijing, China.*

The objective of this study was to evaluate the rumen biohydrogenation of unsaturated fatty acid by adding antibacterial agents (containing streptomycin sulfate, Penicillin G, potassium, and chloromycetin) (RA) in vitro. A dual-flow continuous culture system was used with a 2 × 4 factorial design: with unsaturated fatty acid (no fatty acid, 0%-CK; oleic acid, 3%-OL; linoleic acid, 3%-LA; linolenic acid, 3%-LNA on a DM basis) and antibacterial agents (0 mg/mL, RA0; 0.1 mg/mL, RA) and 40 g basic diet as the substrate. Mixed ruminal fluid from 3 lactating Holstein cows fed with 20 kg DM/day (forage to concentrate = 60:40) was withdrawn via the cannula 3 h after feeding and was mixed with buffer in a ratio of 1:4. Twenty-four fermenters were used with a volume of 1000 ± 10 mL, the dilution rate was set at 6%/h. The experiment last 8 d, and all the samples were taken during the last 3 d. Each treatment has 3 replications. Statistical analysis was carried out by ANOVA (GLM) using SAS. The products of biohydrogenation of C18 unsaturated fatty acid were lower in OL, LA, and LNA than CK with adding RA (0.27, 0.25, 0.37 vs. 0.61 g/100 g fatty acid methyl esters, $P < 0.01$). Adding antibacterial agents decreased the concentration of C18:0 and t9C18:1 (0.783 vs. 0.379, 43.1 vs. 11.3g/100 g fatty acid methyl esters, $P < 0.01$), and the proportion of c9C18:1, c9c12 C18:2, and C18:3 were higher in RA than in control ($P < 0.01$). The biohydrogenation rate of OL was sharply decreased by adding RA ($P < 0.01$), and followed by LA ($P < 0.01$) and LNA ($P < 0.05$). Overall, antibacterial agents decreased the extent of biohydrogenation of C18 unsaturated fatty acids in rumen.

Key Words: antibacterial agent, biohydrogenation, rumen simulation system

TH76 Effects of supplemental bupleurum extract on blood parameters, antioxidant status and immune function in heat-stressed dairy cows. X. Z. Sun[1,2], J. Q. Wang*[1], D. P. Bu[1], J. B. Cheng[1,2], L. Pan[1], and W. Liu[1], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China, [2]College of Animal Science and Technology, Anhui Agricultural University, Hefei, China.*

The objective of this study was to investigate effects of bupleurum extract (BE) on blood parameters, antioxidant status and immune function in heat-stressed dairy cows. Forty Holstein cows (75 ± 15 DIM, 37.5 ± 1.8 kg of milk/d) were individually fed and randomly assigned to 1 of 4 treatments (10 cows/group). Treatments consisted of 0 (control), 0.25, 0.5 or 1.0 g BE /kg DM . The experimental lasted 10 wks. Average temperature–humidity index (THI) was 78.20 ± 2.40, 79.72 ± 3.26 and 78.26 ± 3.37 at 6:00h, 14:00h and 22:00h, respectively. Blood samples were collected from all of animals via tail vein before the morning feeding at wk 1, 4, 7, and 10. Data were analyzed by GLM procedure of SAS 9.2. Compared with control, cows supplemented with 0.5 g/kg BE increased the counts of red blood cell (5.97 vs. 5.52 10^{12}/L, $P < 0.05$), hematocrit (27.83 vs. 25.33 10^9/L, $P < 0.05$), and hemoglobin (95.11 vs. 86.85 g/L, $P < 0.05$). Cows supplemented with BE increased ($P < 0.05$) the count of blood platelet, mean corpuscular hemoglobin, but had no effect on creatine kinase activities and alkaline phosphatase activities compared with the controls. Furthermore, the activity of nitric oxide synthase (NOS) in serum was increased for cows fed diets supplemented with BE than for control cows (29.84, 29.98, 28.51 vs. 25.57 U/mL, $P = 0.01$), and the activities of glutathione peroxidase (GSH-PX) were higher in cows fed 0.25 or 0.5 g/kg BE than that in control cows ($P > 0.05$). The superoxide dismutase (SOD) activities were not affected ($P > 0.05$) by supplementing BE. Cows supplemented with BE increased concentrations of interleukin-4 (65.40, 64.07, 70.42 vs. 55.45 pg/mL, $P < 0.05$) and interleukin-6 (140.62, 113.47, 104.26 vs. 94.12 pg/mL, $P < 0.01$) Compared with the controls, whereas concentrations of CD4+ and CD8+ were not influenced. Results indicate that BE supplementation improved health status, increased antioxidant status and immune function in heat-stressed cows.

Key Words: antioxidant status, bupleurum extract, immune function

TH77 Effect of *Saccharomyces cerevisiae* I-1077 feed additive on rumen bacterial diversity in calves. F. Chaucheyras-Durand[1,2], V. Demey[1], F. Ossa[3], and E. Chevaux*[1], *[1]Lallemand Animal Nutrition, Blagnac, France, [2]Institut National de la Recherche Agronomique (INRA), Saint-Genès Champanelle, France, [3]Lallemand Animal Nutrition, Montreal, QC, Canada.*

The effect of live yeast on establishment of rumen bacterial community was studied on 2 groups of 8 calves. A supplemented group (SC) received from the first wk 4 × 10^9 cfu/d of *S. cerevisiae* I-1077 mixed with fibrous concentrate. The Control group did not receive any supplement. For each animal 2 rumen samples were collected by stomach tubing: one at 2 wk of age when calves were fed mainly milk replacer (MR), the other at 8 to 10 wk of age, after they had started consuming solid feed (SF) for at least 6–8 wks. DNA was extracted from these samples and the V6-V8 region of the 16S rRNA gene was PCR-amplified using universal primers (L1401/U968-GC). PCR fragments were separated by temporal temperature gradient gel electrophoresis (TTGE). Scans were analyzed with Gel Compar II software. Whatever the diet, 2 major clusters were obtained: 66% of Control samples fell in one cluster, 71% of SC samples grouped together in the other. The mean number of TTGE bands and the Shannon diversity index (H') were higher in SC than in Control samples regardless of the diet, this being particularly pronounced with MR (Table 1). Only for Control samples number of TTGE bands and Shannon index were slightly increased from MR to SF. However, due to the low number of animals, statistical differences could not be found. Although preliminary, these data suggest that bacterial diversity increases with age and with solid feed distributed to Control calves. They also suggest that diet supplementation with live yeasts may

accelerate microbial diversity in the developing rumen, which could be of importance to achieving a functional rumen ecosystem at weaning.

Table 1. Effects of the nature of feed and of SC supplementation on bacterial diversity in the rumen of calves (n = 8 per group); means (SE) of each parameter are shown.

	MR		SF		Diet		Treatment	
	Control	SC	Control	SC	MR	SF	Control	SC
TTGE bands (no.)	7.9 (1.7)	9.9 (1.9)	8.1 (1.4)	9.4 (2.5)	8.8 (1.8)	8.8 (2.1)	8.0 (1.5)	9.6 (2.2)
Shannon index H'	0.77 (0.1)	0.8 (0.1)	0.81 (0.1)	0.84 (0.1)	0.81 (0.1)	0.82 (0.1)	0.79 (0.1)	0.85 (0.1)

Key Words: calf, live yeast, rumen microbiota

TH78 Effects of cellulase and xylanase levels on the kinetics of in vitro fermentation of corn stover. A. Z. M. Salem*[1], Y. Liu[1], H. Ammar[2], L. M. Camacho[3], M. M. Y. Elghandour[1], H. Gado[4], and Z. Tan[5], *[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma del Estado de Mexico, Mexico, [2]Ecole superieure d'agriculture de Mograne, Mograne, Zaghouan, Tunisia, [3]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Guerrero, Cd. Altamirano, Guerrero, Mexico, [4]Animal Production Department, Faculty of Agriculture, Ain Shams University, Qalubia, Egypt, [5]Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agricultural, the Chinese Academy of Sciences, Hunan, Changsha, China.*

An in vitro gas production technique was used to investigate combination effects of *Salix babylonica* extract (SB) with the exogenous fibrolytic enzymes of cellulase (C) and xylanase (X), or their mixture (1:1, vol/vol) on in vitro fermentation characteristics of a total mixed ration as substrate (208 (CP) and 364 (NDF) g/kg DM). Four levels of extracts (i.e., 0, 0.6, 1.2 and 1.8 mL/g DM) and 4 fibrolytic enzymes (1 μL/g DM; Control, X, C and XC (1:1, vol/vol)) were used in 4 × 4 factorial arrangement. In vitro gas production (GP) was recorded at 2, 4, 6, 8, 10, 12, 24, 36, 48 and 72 h of incubation. After 72 h, the incubation was stopped and supernatant pH was determined and then filtered to determine dry matter degradability (DMD). Fermentation parameters, such as the 24-h gas yield (GY_{24}), in vitro organic matter digestibility (OMD), metabolizable energy (ME), short-chain fatty acid concentrations (SCFA), and microbial protein production (MP) were estimated. Results show that the extract of *S. babylonica* and different kind of enzymes (X and C or XC by 1.1, vol/vol) supplementation influenced ($P < 0.001$) in vitro gas production after 12 h of incubation. Extract × enzymes interaction ($P < 0.001$) occurred for gas production at all incubation times of measures. Addition of *S. babylonica* extract (i.e., SB) at the highest doses (i.e., 1.8 mL/g DM) with absence of any exogenous fibrolytic enzymes, increased ($P < 0.05$) volumes of gas produced (216 vs. 145 mL gas/g DM) after 24 h of incubation. In general and except for values recorded at 12 h of incubation, GP was lowest (184 vs. 116 mL gas/g DM, $P < 0.05$) when the highest SB was combined with C addition. Extract × enzymes interaction ($P < 0.001$) decreased ruminal pH (6.67 vs. 6.63), while at the highest SB doses (1.8 mL/g DM) with the presence of C or X, increased ($P < 0.05$) OMD (60 vs. 46 g/g DM), ME (8.8 vs. 6.7 MJ/kg DM), GY_{24} (242 vs. 161 mL gas/g DMD) and SCFA (4.5 vs. 2.8 mmol/g DM). Data suggested that SB extract, C, and X effectively improved in vitro rumen fermentation, and the combination of enzyme with SB extract at the level of 1.8 mL/g DM was more effective than the other treatments.

Key Words: cellulose, corn stover, in vitro fermentation

TH79 Effect of exogenous glucoamylase enzyme on in vitro fermentation of diet with 25% of maize or sorghum grains. A. Z. M. Salem*[1], H. Ammar[2], L. B. Ortiz[1], H. Gado[3], M. M. Y. Elghandour[1], and G. D. Mendoza[4], *[1]Facultad de Medicina Veterinaria, Universidad Autonoma del Estado de Mexico, Mexico, [2]Ecole superieure dagriculture de Mograne, Mograne, Zaghouan, Tunisia, [3]Department of Animal Production, Faculty of Agriculture, Ain Shams University, Qalubia, Egypt, [4]Universidad Autónoma Metropolitana, Unidad Xochimilco, México.*

The objective of this study was to evaluate the effects of glucoamylase enzyme in the in vitro ruminal fermentation of total mixed rations (TMR) with 25% of maize (M) and other of 25% of sorghum (S) grains. The 2 diets (i.e., M and S) were treated with 0, 1.5 and 3 g of enzyme protein (65% of protein) per kg of grain in diet. In vitro gas production of TMR of M and S grains was measured (quantity of substrate) at 2, 4, 6, 8, 10, 12, 24, 48 and 72 h of incubation in ruminal fluid diluted in buffer solution (1:4, v:v) flushed with CO_2 and maintained at 39°C. The incubation was stopped at 72 h and after measuring the pH and supernatant was filtered to determine pattern of degradation of in vitro organic matter, neutral detergent fiber (NDFD), and acid detergent fiber (ADFD). The results showed that addition of glucoamylase enzyme to maize diet had no significant effect on kinetics of gas production. However, when added to sorghum diet, a high dose of the enzyme (3 g/kg DM) was traduced by a significant increase ($P < 0.05$) of the rate of gas production (c) and the volume of gas produced at 2, 4 and 6 h of incubation, with no statistical differences between the control and the lowest dose enzyme (i.e., 1.5 g/kg DM). Likewise, effect of glucoamylase was no significant either on the in vitro degradation of DM and cell wall (NDFD and ADFD) or on pH values. Effect of the enzyme supply on sorghum diet was significant ($P < 0.05$) only on pH (5.70 vs. 5.64), and no significant differences between both doses were recorded. Irrespectively to enzyme supply, kinetics of gas production and pattern of degradation of maize were generally higher than those of sorghum. A significant effect ($P < 0.0001$) of the diet (maize or sorghum) and the interaction between diet and enzyme were recorded for the volume of gas produced at different incubation times. The use of high doses of glucoamylase enzyme should be tested on the pattern rumen fermentation

Key Words: glucoamylase, in vitro fermentation, maize

TH80 Effects of feeding *Bacillus subtilis* and *Bacillus licheniformis* on performance, health parameters, and a low quality roughage diet intake and digestibility by lambs. E. Martínez*, A. A. Rodríguez, and L. C. Solórzano, *University of Puerto Rico, Mayagüez, Puerto Rico*

The dietary addition of *Bacillus subtilis* and *Bacillus licheniformis* (BSL) during 49 d, phase 1 (P1), and possible residual effects thereof during phase 2 (P2) without the probiotic addition on lamb performance, health parameters, and intake and digestibility of a low quality roughage was evaluated. Ten crossbred lambs (11.2 kg) were randomly assigned to one of 2 dietary treatments, with (T2) or without (T1) daily addition of BSL in P1. The diet consisted of 50% mixed grass hay (71.5% NDF, 5.2% CP) and 50% *Hyparrhenia rufa* hay (77.2% NDF, 4.5% CP) at a daily hay offering level equivalent to 4% of body weight on a dry basis; water was available ad libitum. The bacterial strains were mixed with a calcium carbonate carrier, and included in the additive to provide 1.33×10^9 cfu/g supplying 1.33×10^8cfu/head/daily. The additive was mixed with 225 g of a commercial concentrate (16.3% CP) and fed daily to the lambs during d 49 (P1). Residual effects were measured from d 50 to d 84 (P2). In both phases, lambs were weighed weekly to monitor changes in body weight. Feces and blood sampling and FAMACHA index scoring were carried out every 21 d on individual animals to determine changes in fecal egg counts (FEC), packed cell volume (PCV)

and relative anemia level, respectively. Feed intake and digestibility were determined in the latter part of each phase. Statistical analysis of data within period was performed as a completely randomized design with 5 replicates per treatment. During P1, the addition of BSL improved ($P < 0.05$) feed intake (484 vs. 445 g/d) and NDF digestibility (62.5 vs.58.7%), but did not affect lamb total body weight gain or changes in health parameters such as FEC, PCV and anemia level. During P2 total body weight gain, changes in FEC, PCV, anemia, diet intake (T1 = 588 and T2 = 564 g/d) and digestibility (T1 = 58.5 and T2 = 57.6%) were not affected ($P > 0.05$) by previous treatments. The addition of BSL improved diet intake and cell-wall digestibility in growing lambs, but no beneficial residual effects of the microbial additive were observed.

Key Words: *Bacillus* spp., health, lamb performance

TH81 Use of organic acids and polyphenols to mitigate induced ruminal acidosis in dairy heifers. R. De Nardi*[1], S. Segato[1], J. C. Plaizier[2], S. Li[2], E. Khafipour[2], I. Andrighetto[1,3], and G. Marchesini[1], *[1]Department of Animal Medicine, Productions and Health, University of Padova, Legnaro (Padova), Italy, [2]Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada, [3]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (Padova), Italy.*

The aim of this study was to determine the effects of supplementation of dairy heifers with organic acids or polyphenol-essential oil mixtures on their ability to cope with induced subacute ruminal acidosis (SARA). In each of 3 periods heifers were fed a low starch (LS) diet (NDF 39.8%, NSC 36.4%, starch 24.0% DM) for 2 wk followed by a high starch (HS) diet (NDF 33.6%, NSC 43.2%, starch 30.0% DM) for 8 d. To induce SARA top dressed barley meal was given (0.5–1.5 kg) in the last 5 d of the study. During the HS diet 6 Holstein heifers were randomly assigned to 1 of 3 dietary treatments in a 3 × 3 factorial design: no supplement (Ct), a daily dose of 60 g of fumarate and malate mixture (Fm) or 100 g of polyphenol and essential oil mixture (Pol). Reticular pH values were continuously measured using wireless sensors and compared with pH measurements obtained by rumenocentesis. Fecal pH was measured at 0800, 1400 and 2100 h. Fm led to a lower DMI due to its possible lower palatability. The correlation coefficient comparing the pH values obtained using the 2 methods was 0.83 ($P < 0.001$). Although the mean and maximum daily reticular pH were not affected by the treatment, the nadir pH resulted the lowest in Ct treatment, confirming the effectiveness of both the supplements in mitigating the pH drop. This result was confirmed also by the greatest mean time spent daily by the control-fed heifers below the 5.6 pH threshold compared with Fm and Pol. The fecal pH was not affected by treatment, and its values were 6.43, 6.69, and 6.74, at 0800, 1400, and 2100 h, respectively ($P < 0.001$). This study showed that both the addition of fumarate-malate and polyphenol-essential oil mixtures could help in the prevention of SARA in cattle.

Table 1. Effect of supplementation on DMI and pH

	Ct	Fm	Pol	SEM	*P*-value
DMI,[1] kg/d	14.4	13.6	14.7	0.58	0.079
Reticular nadir pH[1]	5.48[b]	5.66[a]	5.60[a]	0.040	0.027
Reticular mean pH[1]	6.07	6.09	6.08	0.020	0.653
Reticular maximum pH[1]	6.58	6.56	6.53	0.038	0.448
Time spent below 5.6 pH,[2] min	120	16	21	—	0.091

[a, b]Means with different superscripts within a row differ ($P < 0.05$).
[1]Linear mixed model.
[2]Nonparametric Kruskal-Wallis test;

Key Words: organic acid, polyphenol, subacute ruminal acidosis

TH82 Effects of exogenous enzymes on in vitro gas production kinetics and degradation of wheat dried distillers grains with solubles and barley silage. Z. X. He*[1,2], S. Ding[1], L. Xu[1,3], K. A. Beauchemin[1], and W. Z. Yang[1], *[1]Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, [2]Key Laboratory of Agro-Ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, Hunan, China, [3]College of Food Science and Engineering, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China.*

In vitro batch cultures were conducted to examine the effects of exogenous enzymes (EE) on gas production (GP) kinetics and ruminal fermentation of wheat dried distillers grains with solubles (DDGS) and barley silage. Seven EE were obtained from several manufacturers (E1, E2, E3, E4, E5, E6 and E7) that contained a range of xylanase, endogulcanase, exoglucanase and protease activities. Each was evaluated at 6 doses (0, 0.5, 1, 2, 4, 8 μL/g DM). The GP was recorded at 3, 6, 9, 12, 24, 36 and 48 h for DDGS, and until 72 h for barley silage. After 48 h (DDGS) or 72 h (barley silage) of incubation, degradability of DM (DMD) and NDF (NDFD) were determined. To estimate kinetic parameters of GP, results (mL/g DM) were fitted using the NLIN option of SAS using equation $A = b \times (1\text{-}e^{-c(t-L)})$. For DDGS, there was an interaction ($P < 0.01$) between EE and enzyme dosage (ED) on rate of GP and GP. Rate of GP (/h) linearly ($P < 0.05$) increased with increasing ED, except for E6, and quadratic effects were rarely observed. Additionally, GP linearly ($P < 0.05$) increased with increasing ED mostly at 6, 9 to 12 h, except for E5 and E6. For some EE, DMD and NDFD linearly ($P < 0.01$) increased with ED. Maximum improvements ($P < 0.05$) in DMD and NDFD were, respectively: E2 (51 to 53%; 40 to 44%), E3 (51 to 53%; 40 to 45%), E4 (51 to 52%; 40 to 42%), and E6 (51 to 55%; 40 to 47%). For barley silage, there was overall no interaction between EE and ED for rate of GP and GP. A linear increase ($P < 0.05$) in GP with increasing ED was only observed during early incubation times (3 to 9 h) for most of the EE tested. The DMD and NDFD were generally not affected by the EE although NDFD numerically ($P = 0.10$) increased for E2 (0 vs. 1 or 2 mL/g DM; 49 vs. 55%), E3 (0 vs. 0.5 mL/g DM; 49 vs. 56%), and E6 (0 vs. 1 mL/g DM; 49 vs. 54%). These results demonstrate the potential of EE to improve ruminal degradability of DDGS, but the improvements in degradability of barley silage using EE are less pronounced. Furthermore, extending the incubation time to 72 h could negate the beneficial effects of EE on degradability.

Key Words: exogenous enzyme, gas production, wheat DDGS

TH83 Selenium-enriched tall wheatgrass hay as a substitute for sodium selenite in diets of dairy cattle. G. S. Cun*[1,2], P. H. Robinson[2], and S. E. Benes[1], *[1]California State University, Fresno, Fresno, [2]University of California, Davis, Davis.*

California's San Joaquin Valley (SJV) is one of the most agriculturally productive areas in the United States. However, due to strict environmental restrictions, re-use of agricultural drainage and tail water to irrigate salt-tolerant forage crops is attractive. 'Jose' tall wheatgrass (TWG) is a Se-accumulating salt tolerant forage suitable for such cropping systems, and as a ruminant feed, which re-uses agricultural drainage and tail water as an irrigant. Utilization of TWG as a Se supplement for dairy cattle would reduce importation of 'new' Se into the SJV of California as sodium selenite (NaSe), a common dietary supplement of dairy cattle, in the eastern SJV where Se levels are low in soils and forages. Our study utilized Se-enriched (~5 mg/kg of DM) TWG hay as a Se source for lactating dairy cows and determined Se accumulation patterns in blood, urine and feces to determine its bioavailability. Three pens of ~310 cows each were fed a similar total mixed ration in a 3 × 3 Latin square design with 4-wk periods, except that the supplemental Se source differed (i.e., none; from TWG; from NaSe).

The chemical composition of the diets was the same, except for Se which was higher ($P < 0.01$) in the TWG and NaSe diets (0.53 and 0.65 mg/kg DM) versus 0.35 mg/kg DM in the control diet. Feeding Se-enriched TWG increased blood Se by 6.4% over control, whereas NaSe increased it 4.8%, suggesting slightly higher bioavailability for Se in TWG hay versus NaSe. In contrast, the amount of dietary Se that was apparently digested increased from 47 to 58% with NaSe, but with TWG supplementation there was no increase over the control, suggesting lower bioavailability for TWG compared with NaSe. As Se outputs in the urine did not differ ($P = 0.07$) among treatments, the metabolizability of Se for the NaSe diet was higher than that for the Control and TWG diets. Overall, the similar metabolizability of Se in the TWG diet to that in the base diet suggests that Se-enriched TWG hay can be a value-added Se feed for cattle producers in the eastern SJV who are currently challenged by environmental regulations to reduce use of 'new' (to the SJV) trace minerals in their cattle rations.

Key Words: fortified forage, ruminant

TH84 The effect of different sources of zinc on some blood mineral of finishing lambs. M. Mallaki*, M. A. Norouzian, and A. A. Khadem, *The University of Tehran, Tehran, Iran.*

The effect of different sources of zinc on serum blood concentration of zinc, copper, iron, calcium and phosphor were studied. Eighteen male Zandi lambs (21.28 ± 0.85 kg BW and 70 ± 5 d of age) were randomly allocated to 1 of 3 dietary treatments in a randomized design. Animals in group 1 were treated as control (no zinc supplementation), whereas animals in groups 2 and 3 were supplemented with 25 mg of zinc/kg DM from either zinc peptide (ZnP) or zinc sulfate monohydrate (ZnS), respectively. Lambs were blooded from jugular vein at began of study (d 0) and d 35 and 69 of experiment for determination of serum mineral. The Zn serum concentration for ZnP (155.2 μg/dL) and ZnS (149.7 μg/dl) groups were greater than the control (134.6 μg/dL) group ($P < 0.05$). Lambs fed diets containing ZnP had lower concentration of serum Cu and Fe (76.17, 92.08, 96.58 and 146.5, 157.4, 182.4 μg/dL for ZnP, ZnS and Control, respectively; $P < 0.05$). There was no significant difference between experimental groups for serum Ca and P (11.59, 11.16, 11.38 and 8.86, 9.21, 9.88 mg/dL for ZnP, ZnS and Control, respectively). These results indicate source of Zn supplementation in diets of finishing lambs affect mineral profile of blood and increase concentration of Zn.

Key Words: blood mineral, finishing lamb, Zinc

TH85 Effects of supplemental niacinamide on lactation performance and rumen fermentation of Holstein cows under heat stress. L. Pan[2], D. P. Bu[2], J. Q. Wang*[1,2], J. B. Cheng[2], X. Z. Sun[2], and W. Liu[2], *[1]Agronomy College of Heilongjiang August First Land Reclamation University, Heilongjiang, China, [2]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Niacinamide (NAM) has been used to reduce heat stress in dairy cows. However, effects of NAM on relieving heat stress are not consistent. This experiment was conducted to study effects of NAM on lactation performance and rumen fermentation of heat-stressed cows. Twenty Holstein cows (DIM = 78.8 ± 11.0; milk production = 37.7 ± 1.8 kg/d; parity = 1.7 ± 0.3) were divided randomly into 2 groups and were individually fed a basal diet (control) or the basal diet with 8 g/head/d NAM. The experiment lasted for 10 wk in hot summer. Ambient temperature and humidity were daily measured 3 times. Milk yields and feed intake were recorded twice a week, and milk samples were collected every 10 d. Respiration rates (RR) and rectal temperatures (RT) were measured for 2 consecutive days every week. Rumen fluid samples were collected at wk 6 and 10 of the trial. Data were analyzed by repeated measurements using Proc Mixed procedure of SAS. During the experiment, average ambient temperature and temperature-humidity index were respectively 27.52 ± 1.54°C, 29.77 ± 1.89°C and 28.13 ± 1.69°C, and 78.20 ± 2.74, 79.72 ± 3.26 and 78.26 ± 3.37 at 6:00h, 14:00h and 22:00h. RR was decreased (69.27 vs. 76.31 breaths/min, $P < 0.01$; 71.64 vs. 77.15 breaths/min, $P < 0.01$), while RT was not different (39.18 vs. 39.17°C, $P = 0.92$; 39.53 vs. 39.44°C, $P = 0.27$) at 06:00 and 14:00 compared with control. There was no significant difference in milk yield or dry matter intake between treatments. However, 4% fat-corrected milk yields (28.82 vs. 27.27 kg/d, $P < 0.01$) and energy-corrected milk yields (31.28 vs. 29.63 kg/d, $P < 0.01$) were increased. There was no treatment effect on milk protein concentration or milk fat concentration, while milk fat yield was increased in NAM group compared with control (1.08 vs. 1.00 kg/d, $P < 0.01$). Rumen pH, ammonia-N concentration and total volatile fatty acid concentration were not different between groups. In conclusion, while indicators of heat stress were ameliorating for cows fed NAM, this slight increase in cow comfort did not result in improving lactation performance or rumen fermentation significantly.

Key Words: cow, heat stress, niacinamide

TH86 Effect of Rumensin and Amaferm on performance of heifers fed in dry lot and on wheat pasture. H. Gray*[1], P. Beck[1], K. Glaubius[2], and B. Stewart[1], *[1]University of Arkansas, Hope, [2]BioZyme Incorporated, St. Joseph, MO.*

Previous studies have found adding Rumensin to diets will increase cattle performance by improving ruminal efficiency. Results have shown that by adding Amaferm to the diet, fungal activity in the rumen will increase, allowing more surface area for bacteria to attach to and maximize the digestion of high fiber forages. The objective of this research was designed to test the effects of adding Rumensin and Amaferm on performance of growing beef heifers fed high roughage mixed diets in dry lot and while grazing wheat pasture. Angus influenced cross-bred heifers (n = 72; BW = 220 ± 5.5 kg) were placed in 12 (7 × 3 m) dry lot pens located in Hope, AR. Calves were fed ad libitum a diet of corn stalk hay (50% as fed) and corn distiller's grains, and corn as the primary concentrate energy sources for 83-d beginning in early November. Heifers were then placed on 12, 0.8-ha cool-season annual pastures keeping heifers within their original feeding groups, feeding Amaferm and/or Rumensin in 0.9 kg soybean hulls/heifer daily. Data were analyzed as a complete randomized design with 2 × 2 factorial arrangement of treatments using mixed procedure of SAS. Pen (or pasture) treatment was the experimental unit and heifer the sampling unit. There was no Rumensin by Amaferm interaction in dry lot or pasture performance ($P \geq 0.72$). In dry lot, total BW gain, ADG, feed intake and feed efficiency were not affected by treatment ($P \geq 0.16$). On pasture there, however, was a tendency ($P = 0.10$) for Amaferm to increase BW gain by 7.9 kg and ADG by 0.10 kg/d. These results indicate Rumensin and Amaferm did not affect performance of heifers fed of these high roughage low energy diets but Amaferm inclusion provided improved performance on high-quality pasture.

Key Words: Amaferm, growing heifer, Rumensin

TH87 Effects of salinomycin and virginiamycin supplementation on ruminal fermentation and blood characteristics of Nellore steers fed a high concentrate diet. A. J. C. Nuñez*[1], V. V. Almeida[2], J. P. Schoonmaker[3], I. E. Borges[1], F. Pinese[1], F. T. Mercado[1], E. M. Ferreira[4], A. V. Pires[4], P. R. Leme[1], and J. C. M. Nogueira Filho[1], *[1]FZEA/USP, Pirassununga, SP, Brazil, [2]FCAV/UNESP, Jaboticabal, SP, Brazil, [3]Purdue University, West Lafayette, IN, [4]ESALQ/USP, Piracicaba, SP, Brazil.*

This experiment was conducted to evaluate the effects of the supplementation with salinomycin (SL), virginiamycin (VM), or their combination on ruminal fermentation and blood characteristics of Nellore steers receiving an 80% concentrate diet (DM basis). Eight ruminally cannulated Nellore steers (322 ± 26 kg of initial BW) were allotted to a 4 × 4 replicated Latin square design with 16-d periods. Treatments were arranged as a 2 × 2 factorial, with 2 SL levels (0 and 13 ppm) and 2 VM levels (0 and 15 ppm) in the diet DM. Animals were housed in individual pens and fed once daily at 0800 h. On d 13 of each period, ruminal fluid samples were collected at 0, 2, 4, and 8 h post-feeding to determine pH and concentrations of VFA and lactate. Blood samples were collected by jugular venipuncture at 2 h post-feeding on d 14 of each period to determine pH and concentrations of lactate and bicarbonate. Statistical analyses were conducted using the MIXED procedure of SAS. No interactions between SL and VM levels, nor between time of collection and SL or VM levels were observed. The inclusion of VM in the diets decreased ($P = 0.01$) ruminal concentrations of acetate (63.4 and 59.4 ± 1.1 m*M* for 0 and 15 ppm VM, respectively), butyrate (12.0 and 10.5 ± 0.3 m*M* for 0 and 15 ppm VM, respectively), and lactate (0.42 and 0.39 ± 0.01 m*M* for 0 and 15 ppm VM, respectively), and increased ($P = 0.01$) ruminal concentrations of propionate (21.5 and 23.6 ± 0.6 m*M* for 0 and 15 ppm VM, respectively). There was a tendency ($P = 0.08$) for increased ruminal pH (6.42 and 6.48 ± 0.03 for 0 and 15 ppm VM, respectively) in VM-treated steers. There were no effects of VM level on blood pH and bicarbonate concentrations, but lactate concentrations were higher (0.57 and 0.79 ± 0.06 m*M* for 0 and 15 ppm VM, respectively; $P = 0.05$) in the blood of steers receiving the antibiotic. No effects of SL were observed for any analyzed variable. The inclusion of VM in high concentrate diets for Nellore steers improves ruminal fermentation by increasing propionate and decreasing lactate production.

Key Words: antibiotics, beef cattle, ionophore

TH88 Concentrate level and combined use of ionophore and virginiamycin on ruminal fermentation and blood characteristics of Nellore steers fed high grain diets. A. J. C. Nuñez*[1], V. V. Almeida[2], J. P. Schoonmaker[3], F. Pinese[1], I. E. Borges[1], F. T. Mercado[1], E. M. Ferreira[4], A. V. Pires[4], P. R. Leme[1], and J. C. M. Nogueira Filho[1], *[1]FZEA/USP, Pirassununga, SP, Brazil, [2]FCAV/UNESP, Jaboticabal, SP, Brazil, [3]Purdue University, West Lafayette, IN, [4]ESALQ/USP, Piracicaba, SP, Brazil.*

Eight ruminally cannulated Nellore steers (434 ± 35 kg initial BW) were used in a 4 × 4 replicated Latin square design (21-d periods) to evaluate the effects of concentrate and virginiamycin (VM) levels in diets containing salinomycin (SL) on ruminal fermentation and blood characteristics of Zebu cattle. Treatments were arranged as a 2 × 2 factorial arrangement, with 2 concentrate levels (70C and 90C diets had 70 and 90% concentrate, respectively) and 2 VM levels (0 and 15 ppm) in the diet DM. Steers were fed once daily at 0800 h and all diets included the ionophore SL (13 ppm). On d 18 of each period, ruminal fluid samples were collected at 0, 2, 4, and 8 h post-feeding to determine pH and concentrations of VFA and lactate. Blood samples were collected by jugular venipuncture at 2 h post-feeding on d 19 of each period to determine pH and concentrations of lactate and bicarbonate. Statistical analyses were performed using the MIXED procedure of SAS. There were no interactions between time of collection and concentrate or VM levels for any analyzed variable. Ruminal concentrations of acetate were lower ($P = 0.01$) for the 90C treatment in comparison with the 70C group. Acetate concentrations were also lower ($P < 0.01$) for steers receiving VM and SL in comparison with those fed only SL. The inclusion of VM increased ($P < 0.01$) ruminal concentrations of propionate in steers from the 90C treatment, whereas VM decreased ruminal butyrate concentrations ($P < 0.01$) for animals fed the 90C diet. Ruminal pH and lactate concentrations were not affected by VM inclusion. Increasing dietary concentrate level increased ruminal concentrations of lactate ($P < 0.01$) and decreased ruminal pH ($P < 0.01$). Blood pH and bicarbonate concentrations were lower ($P < 0.01$) in steers from the 90C treatment, whereas blood lactate concentrations did not differ between concentrate levels. Blood characteristics were not affected by VM inclusion. These results indicate that the combined use of SL and VM has positive effects on ruminal fermentation, especially when dietary concentrate levels are greater.

Key Words: antibiotics, beef cattle, salinomycin

TH89 Effects of different amino acid patterns on the expression of four major milk protein genes in primary cultured bovine mammary epithelial cells. X. F. Zhang[1], C. J. Ao*[1], M. Gao[2], E. Khas[1], H. Zhang[1], and L. W. Song[1], *[1]Department of Animal Science, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China, [2]Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot, Inner Mongolia, China.*

The objective of this study was to determine whether different amino acid (AA) patterns could affect milk protein gene (α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein) expression in bovine mammary epithelial cells. Mammary tissues were collected from a 3 years old lactating Chinese Holstein dairy cow (DIM 100-d), Primary mammary epithelial cells were isolated by modifications of the methods of Miranda et al., (2009). A completely random design was used with 4 AA patterns as treatments (Table 1), each treatment has 3 replicates, and all experiments were repeated 3 times. Data were analyzed by the ANOVA using the GLM procedure of SAS (9.0). The concentration of total AA in each medium was 534 mg/L. The results showed that different patterns of AA can induce the expression of αs1-casein, β-casein and κ-casein genes differently ($P < 0.05$), in contrast, the expression of αs2-casein gene was not significantly affected ($P = 0.26$). The milk pattern, casein pattern and combination pattern of AA increased the expression of αs1-casein and κ-casein genes significantly ($P < 0.05$) compared with blood AA pattern. The milk pattern, casein pattern and blood pattern of AA upregulate β-casein gene expression compared with combination AA pattern ($P < 0.05$). In conclusion, the milk pattern might increase major milk protein genes expression, perhaps, an appropriate AA pattern will play a very important role in milk protein synthesis.

Table 1. The percentage of amino acids in different patterns[1]

AA, %	Blood	Milk	Combination	Casein
Tyr	2.18	5.67	6.30	6.63
Ala	11.66	3.78	2.91	2.77
Gly	29.77	2.27	0.40	0.48
Glu	10.5	29.3	25.0	26.4
Ser	8.31	6.05	5.93	6.03
Cys	4.15	0.76	1.16	0.48
Phe	2.29	6.05	6.54	6.63
Leu	5.75	10.97	18.52	17.37
lle	3.94	5.30	6.17	6.27
His	1.28	3.78	2.32	2.41
Lys	2.72	10.21	7.46	7.24
Thr	6.76	5.67	5.60	5.55
Met	0.80	2.27	3.65	3.74
Try	1.65	1.89	1.80	1.57
Val	8.25	6.05	6.22	6.39
Tyr	2.18	5.67	6.30	6.63

[1]Blood pattern comes from Weekes et al. (2009). Milk pattern and Casein pattern come from Martin et al. (1944). Combination pattern (80% casein pattern plus 20% lactoalbumin pattern) also comes from Martin et al. (1944).

Key Words: amino acid, bovine mammary epithelial cell, gene expression

TH90 Effects of tributyrin supplementation in milk replacer on performance and gut development of Holstein calves. G. Araujo*[1], M. Terré[1], A. Mereu[2], I. Ipharraguerre[2], and A. Bach3,1, *[1]Department of Ruminant Production, IRTA, Caldes de Montbui, Spain, [2]Lucta S.A., Barcelona, Spain, [3]ICREA, Institut de Recerca i Estudis Avançats, Barcelona, Spain.*

Sodium butyrate (SB) is often used as an additive for milk replacers (MR) to improve calf performance. Tributyrin (TRB) is a triglyceride containing equivalent amount of butyrate than SB but in a more stable form. The aim of this study was to evaluate the effects of supplementing a MR with TRB on performance and development of the gastro-intestinal tract (GIT) of suckling calves. Thirty-six Holstein calves (46 ± 5.9 kg BW and 12 ± 3.0 d age) were fed 4 L/d of MR at 12.5% DM dilution and water and starter feed ad libitum. Calves were randomly distributed in 2 groups. Milk replacer was either unsupplemented (CTR) or supplemented with 3 g of TRB per kg of DM (TRB). Five calves per group were feed-restricted at 200 g/d of starter and killed on d 42 of study to measure rumen VFA, pH and GIT weights. Starter and MR intakes were recorded daily on an individual basis and calves were weighed every 14 d. Data for VFA, pH and GIT weights were analyzed using ANOVA and performance parameters were analyzed using ANOVA with time as a repeated measure. Milk replacer intake was greater ($P < 0.01$) in CTR than in TRB calves (591.7 vs. 585.4 ± 39.02 g of DM/d; respectively). The CTR calves tended ($P = 0.06$) to have a greater starter feed intake than TRB calves, and total DMI was greater ($P < 0.01$) in CTR than in TRB calves (358.2 vs. 260.8 ± 1.15 g of DM/d; respectively). Moreover, BW and ADG were greater ($P < 0.01$) for CTR calves (68.2 ± 0.94 kg and 0.53 ± 0.024 kg/d, respectively) compared with TRB (63.8 ± 0.94kg and 0.42 ± 0.024kg/d; respectively). Gain to feed ratio was also greater ($P < 0.05$) for CTR calves compared with TRB (0.57 vs. 0.48 ± 0.026; respectively). Rumen pH, VFA profile, and GIT weights were not affected by treatments. Nevertheless, CTR calves tended ($P = 0.07$) to have a heavier abomasum than TRB calves, whereas TRB calves tended ($P = 0.08$) to have a heavier duodenum than CTR calves. In conclusion, these results provide no evidence that butyrate addition to MR in the form of TRB at 3 g/kg of DM has positive effects on performance or GIT development of pre-weaned calves.

Key Words: calf, performance, tributyrin

TH91 Casein and whey protein as delivery methods for synthetic vitamin B12 to increase intestinal absorption in lactating dairy cows. V. M. Artegoitia*[1], M. J. de Veth[2,3], F. Harte[1], D. R. Ouellet[4], and C. L. Girard[4], *[1]Department of Food Science and Technology, University of Tennessee, Knoxville, [2]Department of Animal Science, University of Tennessee, Knoxville, [3]Balchem Corporation, New Hampton, NY, [4]Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

Improving vitamin B12 absorption is important for optimal performance in dairy cows and for increasing vitamin B12 concentrations in milk for human consumption. However, 80% of a supplement of synthetic vitamin B12, cyanocobalamin (CN-CBL), is degraded in the rumen of dairy cows and only 25% of the amount escaping destruction in rumen disappears in the small intestine. The objective of this study was to evaluate the efficacy of casein and whey protein as delivery methods for CN-CBL to increase intestinal absorption of vitamin B12 in cows. Four multiparous lactating Holstein cows (237 ± 17 DIM) equipped with a rumen cannula and catheters in the portal vein and a mesenteric artery were used in a randomized crossover design. They were fed 12 equal meals/d to maintain steady-state. On experimental days, they received an abomasal bolus of: 1) CN-CBL alone (100 mg; CA), 2) CN-CBL (100 mg) + casein (10 g; CC) or 3) CN-CBL (100 mg) + whey proteins (10 g; CW). After the bolus, blood samples were taken simultaneously from the 2 catheters every 30 min during the first 4 h and then every 2 h until 24 h post-bolus. Milk yield, DMI, and vitamin B12 porto-arterial concentration differences (P-A) were analyzed using the MIXED procedure of SAS. Milk yield and DMI were not affected by treatments ($P > 0.8$). Overall, vitamin B12 P-A was positive during the first 90 min after the abomasal bolus but negative or not different from 0 until the end of the sampling period ($P = 0.007$). On average for the 24-h period after the abomasal bolus of CN-CBL, vitamin B12 P-A was negative for CA ($P = 0.008$) and CW ($P = 0.06$) but not different from 0 ($P = 0.7$) for CC. There was a trend for a treatment effect ($P = 0.08$) for P-A with CA being lower from CC ($P = 0.03$; −21.43 pg/mL, SEM = 5.8 vs. 2.28 pg/mL, SEM = 6.3) whereas CW (−12.76 pg/ml, SEM = 5.9) did not differ from the 2 others treatments. The present results suggest that CN-CBL given with casein increases vitamin B12 absorption as compared with CN-CBL given alone. For practical applications of our findings, development of a casein-based formulation may improve CN-CBL absorption in dairy cows.

Key Words: bioavailability, cyanocobalamin, vitamin

TH92 The effects of propyl-propylthiosulphonate and capsicum addition on ruminal fermentation and animal performance of lactating dairy cows. A. Foskolos*[1], A. Siurana[1], A. Ferret[1], L. Castillejos[1], D. Bravo[2], and S. Calsamiglia[1], *[1]Universitat Universitat Autònoma de Barcelona, Bellatera, Spain, [2]Pancosma, Geneva, Switzerland.*

The last decade the social concern on food safety stimulated research on plant extracts (PE) as alternatives to antibiotics. However, data from in vivo experiments is limited. We conducted an in vivo study to test the addition of propyl-propylthiosulphonate (PTSO), a stable compound of garlic, and capsicum (CAP), the active compound of hot peppers, on ruminal fermentation and animal performance of lactating dairy cows. Six Holstein dairy cows fitted with cannulas in the rumen were assigned randomly to one of 3 treatments in a duplicate 3x3 Latin Square design. Treatments were: control (CTR; no addition of PE), CAP (0.5 g/animal/day of capsicum oil; Pancosma), and PTSO (0.25 g/animal/day of PTSO; Garlicon, Pancosma). Each experimental period lasted 4 weeks, the first 3 for adaptation and the fourth for sampling. Cows were milked twice daily and milk samples were analyzed for their chemical composition and fatty acid (FA) profile. Ruminal samples were collected at 8 h intervals on 4 consecutive days with a 2 h shift between days, providing 12 samples, one from every even hour of the 24 h day. Rumen liquid was analyzed for pH, ammonia-N and volatile fatty acid concentrations. Significance of was declared at $P < 0.05$. The addition of PE compounds did not affect dry matter intake, milk production and ruminal fermentation profile. The addition of PTSO increased milk concentration of monosaturated (29.9 vs. 26.7 for PTSO and CTR, respectively) and unsaturated FA (38.2 vs. 34.7 for PTSO and CTR, respectively) in expense of saturated FA (61.8 vs. 65.3 for PTSO and CTR, respectively). Moreover, PTSO addition decreased the concentration of C13:0 and C15:0 and increased the concentration of C16:1, C17:1, C18:1 and C18:2. The addition of CAP increased milk protein (3.39 vs. 3.29% for CAP and CTR, respectively) and fat (3.88 vs. 3.62 for CAP and CTR, respectively) concentration and reduced somatic cell counts (215 vs. 621 × 10^3 cells/mL for CAP and CTR, respectively). Results indicate that supplementation of CAP improved milk quality and PTSO improved the fatty acid profile of milk fat.

Key Words: capsicum, dairy cow, propyl-propylthiosulphonate

TH93 The effect of feed additives on in vitro volatile fatty acid production. A. Duncan*, A. Woldeghebriel, and M. Worku, *North Carolina Agricultural and Technical State University, Greensboro.*

The study was conducted to determine the effects of feed additives on in vitro production of VFA. Ruminal fluid was collected from 2 fistulated dairy animals (a cow and a steer averaging 650 kg) that were fed 11.4 kg of TMR consisting of equal amounts of soybean meal, whole cottonseed, and ground corn once a day with free access to hay. Feed grab sample was oven-dried, coarsely pulverized in a regular kitchen blender for one minute, and separated into 3 particle-sizes (PS; 0.85, 1.00 and 1.40 mm). The feed additives used were nitrate, fumarate and combination at 1:4 feed ratios. The experimental design was a 3 × 4 factorial (3 PS and 4 feed additive combinations). The treatments used were (1) feed only, as control (CON); (2) CON + nitrate; (3) CON + fumarate; and (4) CON + a 50/50 nitrate-fumarate mix. A 4-g sample from each PS for each treatment was weighed in triplicates and transferred to 500-mL flask. Each flask received 400 mL of rumen fluid-buffer mixture according to the Tilley and Terry procedures and incubated at 37°C for 48 h. Volatile fatty acid concentration from the rumen fluid was measured by GC. Data collected were analyzed using the GraphPad Prism software and means were compared using the Student's *t*-test ($P < 0.05$). Results of the study shows that the most abundant VFA found in the CON were acetate ($C_{2;}$ 53.4%), propionate ($C_{3;}$ 20.5%), and butyrate ($C_{4;}$ 16.4%) in a 2.6:1.0 C_2:C_3 ratio. However, the addition of nitrate alone, or in combination with fumarate increased the ratio of the VFA (65.2, 23.4, and 5.3, and 54.1, 28.1, and 10.4%, respectively). Addition of fumarate on the other hand increased the concentration of propionate by as much as 29.1% resulting in 2:1 C_2:C_3 ratio compared with the CON. The lower acetate to propionate ratio with fumarate may indicate a fundamental shift in the microbial population as indicated by the shift in the VFA profile.

Key Words: feed additive, in vitro, VFA

TH94 Anionic diets with chromium or methionine for transition cows on hormone and metabolic profile. I. R. F. M. Veiga[1], B. N. de Faria[1], T. L. Resende[1], A. B. D. Pereira[2], and R. B. Reis*[1,3], *[1]Veterinary School, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [2]University of New Hampshire, Durham, [3]FAPEMIG, Minas Gerais, Brazil.*

Seventy-two multiparous Holstein dairy cows were housed 25 ± 8 d before calving and allocated in 6 treatments, Control (C): basal prepartum diet (DCAD = +16.38 mEq/100gDM); Chromium (CC): control plus 7.56mg of organic chromium MiCroPlex (Zinpro Corporation); Methionine plus Chromium (CCM): control plus 7.56mg of organic chromium and 13.24g of DL-methionine hydroxy analog MFP (Novus International Inc.; Anionic (A): (DCAD = −15.69mEq/100gDM); Anionic plus Chromium (AC): Anionic plus 7.56mg of organic chromium; Anionic plus Chromium and Methionine (ACM): anionic plus 7.56mg of organic chromium and 13.24g of DL-methionine hydroxy analog. The completely randomized design with split plot was used and analyses for orthogonal contrasts were performed. Plasma levels of insulin, cortisol, glucose, nonesterified fatty acids (NEFA) and serum albumin were determined on days −21, −14, −7, −1, 1, 7, 14 and 21 relative to calving. The NEFA levels increased as the parturition approached with a peak for all treatments at first day postpartum. The main difference among treatments occurred during postpartum when treatment A showed higher levels of NEFA ($P = 0.051$; 0.010; 0.052; respectively for 1, 7 and 14 d postpartum) compared with AC diet. The insulin levels decreased until the first week postpartum when the lower values were found (5.95µg/dl). The variation among treatments appeared on 7 and 14 d after calving when treatment A had higher values of insulin compared with AC diet ($P = 0.049$; 0.018, respectively). On the other hand, the addition of chromium to the control diet increased the insulin levels during the postpartum compared with the control diet. There was no effect of A diets or the association with chromium and methionine on glucose and cholesterol during the transition period. The use of chromium associated with anionic diets could possibly alter the mobilization of body reserves of high producing dairy cows, but more research is necessary to prove this.

Key Words: insulin, mobilization of body reserves, nonesterified fatty acids

TH95 The effecs of anionic diets with chromium or methionine for transition cows on blood mineral levels. I. R. F. M. Veiga[1], B. N. de Faria[1], T. L. Resende[1], A. B. D. Pereira[2], and R. B. Reis*[1,3], *[1]Veterinary School, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [2]University of New Hampshire, Durham, [3]FAPEMIG, Minas Gerais, Brazil.*

Seventy 2 multiparous Holstein dairy cows were housed 25 ± 8 d before calving and allocated in 6 treatments, Control (C): basal prepartum diet (DCAD = +16.38mEq/100gDM); Chromium (CC): control plus 7.56mg of organic chromium MiCroPlex (Zinpro Corporation); Methionine plus Chromium (CCM): control plus 7.56mg of organic chromium and 13.24g of DL-methionine hydroxy analog MFP (Novus International Inc.); Anionic (A): (DCAD = −15.69mEq/100gDM); Anionic plus Chromium (AC): Anionic plus 7.56mg of organic chromium; Anionic plus Chromium and Methionine (ACM): anionic plus 7.56mg of organic chromium and 13.24g of DL-methionine hydroxy analog. The completely randomized design with split plot was used and analyses for orthogonal contrasts were performed. Plasma concentrations of calcium (Ca), phosphorous (P), magnesium (Mg), potassium (K) and 25 OH Vitamin D were determined on days −21; −14, −7; −1; 1; 7; 14 and 21 relative to calving. The Ca concentrations remained stable until calving and decreased on day one postpartum reaching the lowest level (9.31 mg/dL). It returned to values similar to prepartum (10.47 mg/dL) on d 14, and didn't differ among treatments. At the first day after calving the Mg concentration was different ($P = 0.038$) between A and C diets (2.52 mg/dL and 2.22 mg/dL, respectively). The P concentrations were above normal for dairy cattle at the beginning of the experiment for all anionic diets and control and returned to normal levels (4.23 to 7.02 mg/dL), after parturition. The treatment A had higher values of P from the first through the third week after calving compared with treatment C (6.31 vs. 4.98 mg/dL, $P < 0.05$). The 25 OH vitamin D concentrations had different pattern of variation along the experimental period among treatments. Two weeks after calving the 25 OH vitamin D was higher for treatment A (94.98 ng/mL) compared with C (77.03 ng/mL; $P = 0.047$) and AC (75.12 ng/mL; $P = 0,028$). Anionic diets did not change the precalving Ca concentrations on high producing dairy cows; however, the P levels were higher compared with the control diet. This could suggest a different mineral mobilization which could not be proven.

Key Words: calcium, postpartum

TH96 *Shigella* isolation, phylogeny and identification, with potential for cellulose hidrolysis in the rumen. L. Luna-Rodríguez, D. Hernández-Sánchez, M. Cobos-Peralta, H. Silva-Rojas, C. Cortez-Romero, S. S. González-Muñoz*, and R. Pinto-Ruiz, *Colegio de Postgraduados, Montecillo, Estado de México, México.*

The objective of this experiment was to isolate and cultivate a ruminal cellulolytic bacterium under anaerobic conditions, to carry out its phy-

logenetic and biochemical identification. From a ruminal fluid sample, a strain of *Shigella* nov. sp. was isolated under anaerobic conditions (39°C and pH 6.8), and cultivated in a selective medium using cellulose as the carbon source. The isolated bacterium is a coccobacillus, gram-negative, showing cellulolytic activity when red Congo was used in 10-d growth colonies. The phylogenetic analysis indicates that it is located in the monophyletic group pertaining to the *Shigella*, with 98% similarity to other species of the taxa. Therefore, this microorganism is a non-recognized species. The utilization of sugars and alcohol by the isolated strain was determined using kit API 50 CH and software API WEB. In agreement with the results, fermentation substrates were glycerol, ribose, xylose, sucrose, galactose and glucose, suggesting a potential for cellulose hydrolysis in the rumen. The isolated bacterium was not identified using biochemical tests, which is related to not being listed in the database. Besides, this result is in agreement with the fact that it is a non-recognized species.

Key Words: cellulolysis, isolation and identification, *Shigella*