Table 1. Ingestive behavior (% of the Total Time) of heifers supplied with different sources of lipids and frequency

		Source				
		MEG[1]	SO[2]	SS[3]	Mean[4]	Pr(t)
Daily	Grazing	43.5	51.7	55.1	50.1	0.1
	E. suppl.	3.9	4.8	6.7	5.2	0.9
	Stand	18.4	15.7	13.0	15.7	0.2
	Lay down	20.6	17.8	16.8	18.4	0.3
	D. water	2.6	2.3	2.6	2.5	0.6
3x/Week	Grazing	53.6	44.	48.9	49.0	0.1
	E. suppl.	3.4	3.2	6.5	4.4	0.9
	Stand	13.8	19.4	18.8	17.3	0.2
	Lay down	16.2	25.0	20.5	20.5	0.3
	D. water	2.7	1.9	1.0	1.9	0.6

[1] Megalac-E; [2, 3] Soybean oil and seeds; [4] Mean;

Key Words: tropical pastures, beef cattle, energy supplementation

W262 Degree of dietary fatty acid saturation affects plasma glucose kinetics in growing beef steers. S. E. Cartiff*, V. Fellner, and J. H. Eisemann, *North Carolina State University*, *Raleigh*.

The objective was to determine the effect of type of fatty acid on insulin sensitivity in growing steers. Steers (n=12, initial BW=336.3 kg, SEM=7.7) were adapted to a basal diet that was 70% concentrate mix and 30% orchardgrass hay and contained 13.1% CP and 2.7 Mcal ME/kg DM. Steers were fed a daily amount of 0.26 Mcal ME per kg $BW^{.75}$. The basal diet contained no added fat. After 3 wks steers were transitioned to one of 2 treatment (Trt) diets (n=6 per diet) containing added Ca salts of fatty acids (FA; Virtus Nutrition) at 4% of DM using a source of fat that was enriched in omega-3 fatty acids (StrataG) or a source of fat without omega-3 fatty acids and a greater percentage of C16:0 and C18:1 (EnergII). Three i.v. glucose tolerance tests (IVGTT; 0.9 g glucose/kg $BW^{.75}$) were conducted; one while on the basal diet, and two while on treatment diets at time 1(T1; d3 Trt), and time 2(T2; d38 Trt). Three i.v. insulin tolerance tests (IVITT; 0.45 IU insulin/kg $BW^{.75}$) were conducted the day after each IVGTT. Blood samples were taken at 30, 15, and 5 min before and 2.5, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, and 150 min after infusion. Variables were analyzed for effect of time, diet, and diet*time. Measurements of glucose kinetics on the basal diet were used as covariates. For IVGTT, peak glucose tended to be greater (p=0.06) at T2 (12.4mM) than TI (12.0mM). There was a diet by time interaction (p<0.05) for area under the response curve (AUC). The AUC (mM glucose*50min) at T1 was less (p=0.02) for EnergII (126.2) than StrataG (151.8), AUC at T2 tended to be greater (p=0.07) for EnergII (165.9) than StrataG (146.0). For IVITT, minimum glucose value was less (p=0.02) on StrataG (1.5mM) than EnergII (1.8mM); AUC (mM glucose*150min) was less (P=0.001) for EnergII (203.1) than StrataG (263.6). Results show that the amount of time steers were on diets affected glucose kinetics, and response to insulin was greater in steers supplemented with omega-3 FA compared to more saturated FA.

Key Words: fatty acid, insulin sensitivity

W263 Seminal characteristics in beef bulls supplemented with rumen bypass fat. H. O. Patino*[1], M. M. H. Ramirez[3], J. C. C. Angel[1], K. C. Swanson[2], and R. M. Gregory[3], [1]*Dep. Zootecnia, UFRGS*, *Porto Alegre, RS, Brazil*, [2]*Dept. Animal and Poultry Science, University of Guelph*, *Guelph, ON, Canada*, [3]*Faculdade Veterinaria, UFRGS*, *Porto Alegre, RS, Brazil*.

Twenty Hereford, Angus, Brangus and Braford mature bulls (950 kg average body weight) were used in a completely randomized design to evaluate the effect of bypass fat supplementation on fresh semen characteristics. Bulls were fed diets with similar levels of crude protein and metabolizable energy consisting of green forage and concentrate supplemented with rumen bypass fat (BF) or energy supplement (ES). For 75 days bulls in the BF treatment received Megalac-E® (200 g/day) and bulls in the ES treatment received Cassava meal (750 g/day). Bulls were naturally stimulated by androgenic cows and semen samples were collected with an artificial vagina every 15 days. There were no differences due to treatment on seminal volume (6.38 ml/ejaculate), concentration (983,000/ml) and mass motility of spermatozoa (3.6 ; p>0.05). Semen of bulls supplemented with rumen bypass fat had a 10% increase in individual motility (83.2 vs 75.8%) and a 11% increase in vigor (3.62 vs 3.26) in relationship to semen of bulls supplemented with cassava meal (p<0.05). Energy supplementation in the form of rumen bypass fat resulted in greater individual motility and vigor of spermatozoa in semen from bulls.

Key Words: bypass fat, semen, cassava meal

Ruminant Nutrition: Metabolism

W264 Malate and fumarate enhanced CLA production and reduced methane emission by rumen microbes when incubated with linoleic acid. G. L. Jin*[1], X. Z. Li[2], C. G. Yan[2], R. J. Long[3], and M. K. Song[1], [1]*Department of Animal Science, Chungbuk National University*, *Cheong-ju, Chungbuk, Korea*, [2]*Animal Science department of Agricultrue college, Yanbian University*, *Yanji, Jilin, China*, [3]*International Centre for Tibetan Plateau Ecosystem Management, Lanzhou University*, *Lanzhou, Gansu, China*.

An in vitro study was conducted to investigate the effect of malate or fumarate on production of conjugated linoleic acid (CLA) and methane (CH4) by rumen microbes when incubated with linoleic acid (C18:2). Sixty mg of C18:2 (LA), or C18:2 (60mg) with 24mM malic acid (M-LA) or C18:2 (60mg) with 24mM fumaric acid (F-LA) was added to the 150mL culture solution consisting of 75 ml rumen fluid and 75ml artificial saliva. Culture solution was also prepared without any supplements (Control). Two grams of feed (70% concentrate and 30% ground alfalfa hay, DM) were added to the culture solution. The incubation was made anaerobically in a shaking incubator up to 12 hours at 39 C. Malate (M-LA) or fumarate (F-LA) increased pH (P<0.0001) and total VFA (P<0.032) in culture solution from 3h compared to other treatments. The F-LA increased proportion of C3 from 3h incubation (P<0.001-0.0015) compared to other treatments while M-LA increased (P<0.001) its proportion at 6h and 12h incubation times compared to control and LA. Increased (P<0.0007) total gas production was observed from M-LA or F-LA at 12h but was not influenced by LA. Total CH4 production for 12h incubation was greatly reduced (P<0.0001) by all the supplements and its production from M-LA or F-LA was smaller than that from LA. Malate or fumarate with C18:2 also increased concentrations of c9,t11-CLA (P<0.039 - 0.001) and t10,c12-CLA at 1h (P<0.013), 3h (P<0.036) and 12h (P<0.025) incubation times compared to LA. It

may be concluded that malate and fumarate as propionate precursors act as alternative electron sinks, and are competing with CH4 generation and bio-hydrogenation of C18:2 in the utilization of metabolic H2. The increased CLA concentration at early (1h) incubation stage was accompanied by the reduced propionate proportion.

Key Words: propionate precursors, methane, CLA

W265 Phosphate inhibits in vitro ruminal acetoclastic methanogenesis of maize-rich substrates with lactating Holstein dairy cow rumen liquor. H. J. Yang*[1], D. F. Zhang[1], Y. C. Cao[1], Y. H. Jiang[1], and J. Q. Wang[2], [1]*Department of Animal Nutrition and Feed Science, College of Animal Science and Technology*, *Beijing, P.R. China*, [2]*State key Laboratory of Animal Nutrition, Beijing Institute of Animal Science, China Academy of Agricultural Sciences*, *Beijing , P.R. China*.

An in vitro cumulative gas production trial was conducted with mixed rumen microorganisms from cannulated lactating Holstein dairy cow. 25 ml mixed populations of ruminal microbes collected from cannulated cows were incubated together with 50 ml anaerobic modified Menke's basal medium at 39°C for 48 h under a 100% Nitrogen gas phase in 100 ml volume glass bottles preweighted inside with 0.1 g grounded Chinese wildrye grass hay and 0.4 g maize meal. Modified mediums with phosphate addition level of 10, 20, 50, and 100 mM were compared for its in vitro kinetic gas production, volatile fatty acids and methane productions. The control cultures containing citrate buffer instead of phosphate buffer were incubated simultaneously together with treated incubations. Phosphate additions significantly decreased total gas production (GP, ml/g DM) as well as the rate to reach maximum digestion (RmaxG, ml/h) ($P<0.05$). Increasing phosphate inclusion in the cultures decreased the total volatile fatty acids (VFA) production ($P<0.05$), and rumen fermentation shifted from an initially acetate dominated production towards a propionate production as demonstrated by the ratio of non-glucogenic to glucogenic acids (NGR) ($P<0.05$). Although increased phosphate inclusion in the culture fluids did not shift CH_4 proportion in end-product gases, net fractional CH_4 production (ml/g DM), compared to the control, were significantly reduced from those measured in 10, 20, 50, 100 mM phosphate buffers by 6.2%, 11.8%, 18.57%, and 26.2% respectively ($P<0.05$). Therefore, rumen microorganism is indeed sensitive to increased phosphate addition. Methanogen in rumen may be mostly acetotrophic, and phosphate addition inhibits ruminal methanogenesis at the expense of the overall fermentation efficiency of starch-rich substrates. Phosphate level in ration should not be neglected with cares of CH_4 production in ruminant when designing strategies of ruminal methanogenesis inhibition.

Key Words: phosphate buffer, methanogenesis, in vitro cumulative gas production

W266 The effect of concentrate to forage ratios on methanogenes bacteria population in rumen fluid of Holstein steers determined by real-time PCR. A. R. Vakili*[1], M. Danesh Mesgaran[1], A. Heravi Moussavi[1], D. R. Y´ñez Ruiz[3], and C. J. Newbold[2], [1]*Dept. of Animal Science, Ferdowsi University of Mashhad*, *Mashhad, Iran*, [2]*Institute of Biological, Environmental and Rural Sciences, Aberystwyth University*, *Aberystwyth, UK*, [3]*Unidad de Nutrición Animal Estación Experimental del Zaidín (CSIC) Profesor Albareda*, *Spain*.

The objective of the present experiment was to investigate the effect of concentrate to forage ratios on the population of methanogenes bacteria in the rumen fluid of Holstein steers (300±15 kg, body weight) fitted with rumen canolae. Animals were fed experimental diets (7 kg of DM/d) differing in their concentrate (155 g CP/kg DM; 30% maize, 34% barley, 8% soybean meal, 5% sugar beet pulp, 10% wheat bran, 12% cottonseed meal, 0.3% CaCo3, 0.5% mineral and vitamin premix, 0.2% salt) to alfalfa (155 g CP/kg DM) ratios [60:40 (T1), 70:30 (T2), 80:20 (T3), and 90:10 (T4)] in a 4×4 Latin square design (28-day periods). Steers fed the experimental diets as a total mixed ration at 0800 and 2000h. The samples of rumen fluid were taken before the morning feeding and 4 h post feeding. DNA was extracted from the samples using the QIAamp® DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK). Methanogenes bacteria rDNA concentrations were measured by real time PCR relative to total bacteria amplification (ΔΔCt). The 16s rRNA gene-targeted primer sets used in the present study were forward: TTCGGTGGATCDCARAGRGC and reverse: GBARGTCGWAWCCGTAGAATCC. Cycling conditions were 95°C for 10 min, forty five cycles of 94°C for 10s, 55°C for 20s and 72°C for 15s; fluorescence readings were taken after each extension step. Data were analyzed using the GLM procedure of SAS (Y = Mean + Treatment + Animal + Period + Time + Time × Treatment + Animal × Time + residual) and the means compared by the Tukey test ($P<0.05$). The results of the present study demonstrated that increasing the inclusion of concentrate in diets caused a change in the population of methanogenes bacteria in the free rumen fluid taken before and 4 h after the morning feeding [T1= 93 and 84, T2= 60 and 45, T3= 36 and 75, T4= 45 and 81, SEM = 25 and 17 ($\times 10^{-5}$) methanogenes bacteria relative to total bactria, respectively].

Key Words: rumen, PCR, methanogenes

W267 Microbial growth, methane production and fermentation of a high-concentrate diet in Rusitec fermenters as affected by dilution rate and concentrate retention time. M. E. Martínez, M. J. Ranilla*, S. Ramos, M. L. Tejido, C. Saro, and M. D. Carro, *Departamento de Producción Animal, Universidad de León*, *León, Spain*.

Increasing dilution rate (DL) and solids retention time (RT) in ruminal fermenters usually result in significant changes in pH values, making difficult to separate the relative effects of each factor. This study was therefore conducted to investigate the effects of two DL (LDL: 3.78%/h; HDL: 5.42%/h) and two concentrate RT (CRT; T24: 24 h; T48: 48 h) on microbial growth, methane production and fermentation of a 30:70 alfalfa hay:concentrate diet in Rusitec fermenters maintained at similar pH values. Forage RT was 48 h in all fermenters. Differences among treatments were declared at $P < 0.05$. Increasing DL and CRT increased degradability of diet dry matter and neutral detergent fiber (NDF), DL effects being more pronounced in T48 fermenters than in T24 ones. Methane production was not affected by DL, but it was greater in T48 compared to T24 fermenters which was consistent with the greater NDF degradation in T48 fermenters. Increasing DL augmented volatile fatty acid (VFA) production and molar proportions of propionate, isovalerate and valerate. Greater VFA production and molar proportions of acetate and butyrate, and lower propionate, valerate and isovalerate proportions were observed in T48 fermenters compared to T24 ones. Ammonia-N production was greater at HDL compared to LDL which would indicate enhanced deaminative activity, but it was not affected by CRT. Microbial growth was not affected by DL, but was greater in T48 compared to T24 fermenters. Efficiency of microbial growth was augmented by lowering DL and increasing CRT. Fibrolytic activities in ruminal fluid were greater in HDL than in LDL fermenters, but were not affected by CRT. There were DL x CRT interactions for diet apparent disappearance, molar proportions of propionate, butyrate, isovalerate,

and acetate:propionate ratio, indicating that effects of DL on these variables were influenced by CRT.

Key Words: dilution rate, retention time, RUSITEC

W268 Effect of diets supplemented by sucrose and/or starch on *Ruminococcus albus* populations in the rumen fluid of Holstein steers determined by real time-PCR. F. Rezaii, M. Danesh Mesgaran*, A. Vakili, A. Heravi Moussavi, and S. Ghovvati, *Dpt. of Animal Science (Exellence Center for Animal Science), Ferdowsi University of Mashhad, Iran*.

The objective of this work was to investigate the effect of diets containing different types of non-fiber carbohydrates (NFC) on *Ruminococcus albus* populations in the rumen fluid of Holstein steers as determined by real-time polymerase chain reaction (RT-PCR). Four steers (body weight= 280±15 kg) were assigned to a 4 X 4 Latin square with 21 days in each period. A basal diet (BD) was formulated containing alfalfa hay, barley grain, soybean meal and sugar beet pulp (400, 290, 190 and 50 g/Kg DM, respectively). Sucrose (Su) and/or starch (St) or a 1:1 mixture of sucrose and starch (Su+St) was added to the basal diet at the rate of 70 g/Kg DM. Diets were offered at 2-2.5 times maintenance requirements (7 Kg DM/d). Rumen fluid samples were collected before and 4 h after the morning feeding on the last day of each period. Samples were stored in liquid nitrogen until used for *Ruminococcus albus* quantitation by qPCR. DNA was extracted from the samples using the QIAamp® DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK). *Ruminococcus albus* rDNA concentrations were measured by RT-PCR relative to total bacteria amplification. The 16s rRNA gene-targeted primer sets used in the present study were forward: CCCTAAAAGCAGTCTTAGTTCG and reverse: CCTCCTTGCGGTTAGAACA. Cycling conditions were 95°C for 5 min, forty cycles of 95°C for 15 s, 60°C for 15 s and 72°C for 30 s. Data were expressed relative to quantification of the total bacterial population, and analyzed statistically using mixed procedure of SAS (2003). model was: Y = Mean + Treatment + Animal + Period + residual and the means compared by the Tukey test ($P < 0.05$). Present experiment results indicated that the source of NFC added to the basal diet did not cause a significant change in the ruminal *Ruminococcus albus* population relative to the total bacterial population before and 4 h after the morning feeding (BD= 0.0048 and 0.0175, Su= 0.0125 and 0.0242, St= 0.0062 and 0.0107, SuSt= 0.0093 and 0.0178, SEM= 0.0068 and 0.0130, respectively).

Key Words: non-fiber carbohydrate, *Ruminococcus albus*, real-time PCR

W269 Synergistic fibrolysis by cellulolytic *Ruminococcus flavefaciens, Fibrobacter succinogenes,* and non-cellulolytic *Prevotella ruminicola* and *Prevotella bryantii*: study in semi-defined cultures. J. Chiquette* and K. Lauzon, *Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada*.

The objective was to investigate the occurrence of synergistic fibrolysis when cellulolytic bacteria are co-cultured with non-cellulolytics in a semi-defined medium in vitro. Cellulolytic bacteria were: *Fibrobacter succinogenes* GC5 and *Ruminococcus flavefaciens* NJ and the non-cellulolytics were: *Prevotella bryantii* 25A and *Prevotella ruminicola* 19189. Avicel, timothy hay and alfalfa hay were used as substrates to measure dry matter (DM) disappearance with time. Cellulolytic bacteria were grown at 37°C for 72 h in basal medium containing 1% (w/v) Avicel (NJ) or 0.3% cellulose filter paper (GC5). After 5 passages in Avicel or cellulose filter paper, fiber was removed and the bacterial pellet was recovered and suspended in an anaerobic dilution solution which OD_{660} was adjusted to 0.5. Non-cellulolytic bacteria were grown in basal medium containing 0.5% (w/v) cellobiose as a sole carbon source until the end of the log phase (12h). The bacterial pellet was treated as mentioned previously. The OD-adjusted inocula were added in monocultures (0.2 ml) or in cocultures (0.2 ml each for cellulolytics and non-cellulolytics) to tubes containing 10 ml of basal medium with 100 mg of each fiber substrate. After 3 days of incubation, a greater ($P \leq 0.001$) disappearance of Avicel was observed when *flavefaciens* was cocultured with *ruminicola* (11.0%) or *bryantii* (10.5%) compared with the monoculture of *flavefaciens* (6.7%). No synergy was recorded in the cocultures with timothy hay or alfalfa hay. Oppositely, after 3 days of incubation, a greater ($P \leq 0.01$) disappearance of alfalfa hay DM was observed when *succinogenes* was cocultured with *ruminicola* (12%) or *bryantii* ($P \leq 0.08$) (9.9%), compared with the monoculture of *succinogenes* (3.1%). No synergy was observed with timothy hay. All of these synergistic effects were observed after 3 days of incubation but not after 7 days. The presence of *ruminicola* and *bryantii* seem to accelerate fiber digestion by the cellulolytic species *succinogenes* and *flavefaciens*. The synergistic effect is substrate dependent.

Key Words: fiber digestion, rumen bacteria, synergism

W270 Role of inulin as a modifier in rumen fermentation. H. D. Umucalilar[1], N. Gulsen[1], A. Hayirli*[2], and M. S. Alatas[1], [1]*Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Medicine, Selçuk University, Konya, Turkey*, [2]*Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey*.

Few studies dealing with inulin fermentation by rumen microbes are available, although its prebiotic effects in the hindgut of humans, nonruminant farm animals and pet animals have been extensively investigated. The objective of this *in vitro* experiment was to examine the effects of inulin (0, 2 and 4%) (Frutafit® TEX, Sensus, The Netherlands) at different forage:concentrate ratio (F:C, 20:80, 40:60 and 60:40) on rumen fermentation variables. Ruminal fluids were collected from two Holstein steers and incubated with the mixtures for 48 hours. Rumen variable data were subjected to 2-way ANOVA using the MIXED Procedure. Ruminal fluid pH (from 6.71 to 6.77) and NH_3-N concentration (from 11.72 to 15.71 mmol/L) increased linearly, whereas lactate concentration (from 28.11 to 26.29 mmol/L) decreased quadratically as the forage proportion increased ($P < 0.0001$ for all). Inulin did not affect ruminal fluid pH and concentrations of NH_3-N and lactate. Increasing F:C ratio was associated with a linear increase in the proportion of acetate from 53.39 to 55.41% ($P < 0.0001$), no change in the proportion of propionate, a linear decrease in the proportion of butyrate from 19.74 to 17.59% ($P < 0.002$). Total VFA concentration quadratically decreased with increasing the forage proportion ($P < 0.006$). Inulin had no effect on VFA proportions. There was no F:C ratio by inulin level interaction effect on rumen variables, except for total VFA concentration. Increasing inulin level increased total VFA concentration at high concentrate, whereas decrease it at low concentrate ($P < 0.02$). Total gas production linearly decreased from 41.14 to 33.74 mL with increasing the forage proportion ($P < 0.0001$), whereas it linearly increased from 36.76 to 38.25 mL with increasing inulin level ($P < 0.005$). However, there was no interaction effect on gas production. In conclusion, our data do not support that inulin may play a modifier role in rumen fermentation.

Key Words: inulin, rumen fermentation, gas production

W271 Role of lactulose as a modifier in rumen fermentation. N. Gulsen[1], H. D. Umucalilar[1], A. Hayirli*[2], and O. B. Citil[1], [1]*Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Medicine, Selçuk University, Konya, Turkey*, [2]*Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey*.

Lactulose is a prebiotic and resistant to acidic hydrolysis and enzymatic digestion, but fermented to volatile fatty acids (VFA) by the colonic bacteria in humans and monogastric animals. This *in vitro* experiment was conducted to examine the effects of lactulose at 0, 2, and 4% (667 mg/ml, Osmolak® Solüsyon, Biofarma Ílaç Sanayi ve Ticaret A. S., Ístanbul, Turkey) on ruminal fermentation of the mixtures (230 mg) differing in forage:concentrate ratio (F:C; 20:80, 40:60 and 60:40). Ruminal fluids collected from two Holstein steers were incubated with the mixtures for 48 hours. Data were analyzed using 2-way ANOVA. Increasing F:C ratio caused a linear increase in ruminal fluid pH (from 6.71 to 6.76; $P < 0.0001$), a linear decrease in NH_3N concentration (from 15.84 to 11.77 mmol/L; $P < 0.0001$), and a quadratic increase in lactate concentration (from 25.51 to 26.91 mmol/L; $P < 0.04$), but did not affect total VFA concentration. There were also linear increases in acetate (from 53.89 to 55.69%; $P < 0.0002$) and valerate (from 3.05 to 3.25%; $P < 0.003$) proportions and a linear decrease in butyrate (from 19.74 to 17.32% ($P < 0.0001$) proportion. There were no main effect of lactulose and F:C ratio by lactulose level interaction effect on fermentation pattern. Increasing forage portion linearly decreased cumulative gas production from 41.14 to 33.54 mL ($P < 0.0001$), whereas increasing lactulose level did not alter cumulative gas production. In conclusion, lactulose failed to alter fermentation pattern of the basal mixtures consisting of different F:C ratios, suggesting that fermentation modifying role of lactulose in the ruminant animal is negligible.

Key Words: lactulose, rumen fermentation, gas production

W272 Lactic acid modulates DM degradation kinetics of barley grain in the rumen and decreases the risk of acidosis in dairy cows. S. Iqbal, Q. Zebeli*, A. Mazzolari, S. M. Dunn, and B. N. Ametaj, *University of Alberta, Edmonton, AB, Canada*.

The aim of this study was to evaluate the effects of barley grain treated with lactic acid (LA) on *in vivo* diurnal fermentation patterns and *in situ* DM degradation kinetics in the rumen of dairy cows. In the *in vivo* trial, 8 rumen-fistulated Holstein cows (~200 DIM) were fed once daily a TMR containing rolled barley grain (27% in DM) steeped for 48h in equal quantity of water (CTR) or with 0.5% LA (v/v; TRT) in a crossover design with two 21-d periods. Rumen fluid samples were collected at the last day of each period at 0, 2, 4, 6, 8, 10, and 12h post-feeding, and analyzed for pH and VFA concentration. The *in situ* trial consisted in ruminal incubation (n = 6 cows) of dublicate samples of 3 different substrates (CTR, TRT at 0.5% or 1.0% LA; v/v) for 0, 2, 4, 8, 12, 24, 48, and 72h. Data were analyzed statistically by PROC MIXED of SAS using a model for repeated measures. Degradation kinetics parameters were estimated by fitting the *in situ* data using NLIN PROC of SAS. Results of *in vivo* fermentation patterns showed an interaction between diet and hour of measurement for total VFA and pH of the ruminal fluid (P<0.05). The analysis indicated lower concentration of VFA particularly at 2 (P=0.03) and 4h (P=0.01) post-feeding for cows fed the TRT-diet compared to control group. Interestingly, the group of cows fed the TRT-diet had higher pH readings at 10 (P=0.02) and 12h (P=0.04) post-feeding. The latter effect was associated with a shorter duration time in which ruminal pH was below 5.8 for the TRT-diet (2.4h) compared to the CRT diet (3.9h; P=0.04). The *in situ* data indicated a lower (P=0.05) fractional DM degradation rate (kd=12.3%/h) and effective degradability (ED=81.5%) for barley grain treated with 0.5% LA compared to other two substrates (kd=18.6 and 21.2%/h and ED =82.4 and 83.6% for control- and 1.0% LA-substrates, respectively). In conclusion, treating barley grain with 0.5% LA modulated *in vivo* diurnal rumen fermentation patterns and *in situ* DM degradation kinetics suggesting a potential role for the LA-treatment of barley grain to decrease the risk of rumen acidosis in dairy cows.

Key Words: dairy cow, lactic acid, rumen fermentation

W273 Effect of condensed tannins and maceration on in vitro ruminal degradation of protein in legume hay. G. A. Broderick* and J. H. Grabber, *U.S. Dairy Forage Research Center, Madison, WI*.

Protein in alfalfa hay and most forages is extensively degraded in the rumen. Condensed tannins in birdsfoot trefoil (BFT) and polyphenol oxidase in red clover act differently to reduce protein degradation. Maceration (extensive mechanical conditioning) increases field-drying rate and decreases proteolysis in the swath, which may further reduce degradation. Alfalfa (ALF), low tannin BFT (LTBFT, 0.6% tannin), high tannin BFT (HTBFT; 1.5% tannin), and red clover (RC) were cut and conditioned using either rolling (ROLL) or maceration (MAC), field-dried and harvested as hay. Hay was ground (2 mm screen), analyzed for chemical composition and incubated with ruminal inocula in the Michaelis-Menten inhibitor in vitro method to quantify degradation (Broderick and Clayton, Brit. J. Nutr. 67:27-42, 1992). Extent of degradation was estimated from net release of ammonia N plus amino N determined using an assay that detects only free AA or one that detects both AA and small peptides. Results were analyzed by proc mixed in SAS; LS means are in the table. Quantifying peptide release increased ($P < 0.01$) degradation rate from 0.31 to 0.35/h. There was no effect of forage source or conditioning on CP. However, RC had lower NDF and NPN, higher NDIN, lower degradation rate and greater estimated escape. MAC gave a small increase in NDF but had large effects on NPN, NDIN, degradation rate and escape. Tannin slightly reduced NPN and increased ADIN but had little effect on degradation. Results indicated that maceration reduced protein degradation and that greater amounts of protein in RC hay would escape the rumen.

Table 1.

	Forage source					Conditioning		
Item	ALF	LTBFT	HTBFT	RC	P > F	ROLL	MAC	P > F
Composition								
CP, % of DM	23.9	23.3	22.6	22.8	0.07	23.4	22.8	0.18
NDF, % of DM	43.1^{a}	41.1^{ab}	42.4^{a}	39.6^{b}	0.02	40.1	43.0	0.04
NPN, % of N	26.4^{a}	27.4^{a}	24.2^{b}	19.6^{c}	<0.01	29.6	19.2	<0.01
NDIN, % of N	12.1^{b}	13.9^{b}	14.5^{b}	25.9^{a}	<0.01	11.4	21.9	<0.01
ADIN, % of N	2.6^{c}	3.0^{bc}	4.0^{a}	3.4^{b}	<0.01	3.2	3.2	0.67
Protein degradability								
Rate, /h	0.36^{a}	0.34^{a}	0.34^{a}	0.27^{b}	<0.01	0.40	0.26	<0.01
Est. escape, %	10.9^{c}	10.7^{c}	12.3^{b}	20.3^{a}	<0.01	6.7	20.5	<0.01

$^{a-c}$Forage sources with different superscripts differ (P < 0.05).

Key Words: tannins, forage conditioning, protein degradability

W274 Shift in in vitro microbial fermentation in response to condensed tannin supplementation in mixed ruminal cultures. C. M. Dschaak, J.-S. Eun*, Y.-M. Kim, F. H. Bhushan, and A. J. Young, *Utah State University, Logan*.

This study investigated the dose response of quebracho condensed tannins (CT) supplemented to high concentrate, lactating dairy TMR diet on ruminal pH, fermentation, and methane production in mixed ruminal cultures. A dual-flow continuous culture system consisting of 4 fermentors at liquid dilution rate of 10%/h was used in a 4 × 4 Latin square design. The diet used in the study consisted of 33% alfalfa hay, 7% corn silage, 40% rolled barley grain, and 20% concentrate mix. Water-soluble quebracho extract (QE) was used as the source of CT (99% solubility; Chemtan Company Inc., Exeter, NH). The 4 treatments were 1) control = TMR without QE; 2) TMR with 2% QE (LCT); 3) TMR with 4% QE (MCT); and 4) TMR with 6% QE (HCT). Filtered ruminal contents were allowed 5 d of adaptation to the treatments followed by 3 d of data collection. Data were analyzed using the MIXED procedure of SAS. Although ruminal pH linearly ($P < 0.01$) decreased with increasing CT supplementation, actual difference between control and HCT was only less than 0.1 (average pH 6.26). Methane production linearly ($P < 0.01$) increased in response to increasing CT, whereas ammonia-N concentration linearly ($P < 0.01$) decreased. Total VFA linearly ($P = 0.02$) increased by increasing CT supplementation. While molar proportion of acetate linearly ($P < 0.01$) increased, propionate proportion tended to decrease linearly ($P = 0.07$), resulting in increased acetate to propionate ratio ($P = 0.02$) when CT supplementation increased. The increased acetate to propionate ratio corresponded to the increased methane production. The supplementation of CT resulted in accelerated microbial production, as was seen in increased VFA and methane production and decreased ammonia-N. Supplementing CT to barley based-high concentrate dairy TMR had no negative impact on in vitro microbial fermentation, but sizably shifted its fermentation patterns due possible to the stimulation of cellulolytic bacteria.

Key Words: condensed tannins, continuous culture, methane production

W275 Deglycosylation of steroidal saponin to sapogenin by mixed rumen microbes and their enzymes. Y. Wang* and T. A. McAllister, *Agriculture & Agri-Food Canada Research Centre, Lethbridge, AB, Canada*.

Inconsistent effects of steroidal saponins on ruminal fermentation may arise from rumen microbes adapting to and/or deactivating saponins by deglycosylation. It is not known whether this process is intra- or extracellular in nature. To study this further, ruminal fluid (RF) was collected from two heifers that were non-adapted or saponin-adapted (i.e., fed powdered *Yucca schidigera* (YS) for 14 d). The RF was centrifuged at 20,000 × *g* (yielding clarified RF, cRF) or at 500 × *g* (whole RF, wRF), and incubated for 4 h with saponins extracted from YS. Four of 8 replicates were assayed directly (measuring soluble saponin + deglycosylated, insoluble sapogenin); the other four were centrifuged (12,000 × *g*; 20 min) and supernatant was assayed (measuring saponin only). Added YS saponins were all accounted for in directly assayed wRF and cRF (99.1 and 100.6%, respectively), whereas saponin recovery was greatly reduced ($P < 0.001$) in centrifuged wRF compared with centrifuged cRF (58.5 vs. 98.7%). Saponin recoveries did not differ between adapted (59.2 and 99.0%) and non-adapted cattle (57.3 and 99.3%). In Exp. 2, wRF prepared from non-adapted RF was centrifuged at 20,000 × *g*. Half of the supernatant was used as a source of mixed extracellular enzymes (ECE). The remainder was autoclaved, added back to the bacterial pellet, and the re-suspension was sonicated, yielding a source of mixed cell-bound enzymes (CBE). The ECE and CBE fractions were incubated with YS saponins for 24 h at 39°C. Subsamples were assayed directly, for saponin + sapogenin, and after centrifugation, for soluble saponin. In ECE, >92% of the added saponin was detected in supernatant fraction (i.e., as saponin), whereas in CBE, <30% was detected in supernatant, and >70% in the sapogenin fraction. These findings clearly demonstrate that mixed rumen bacteria deglycosylate steroidal saponin to sapogenin, irrespective of prior exposure to YS, but they were unable to degrade the sapogenin core structure. Deglycosylation activity was attributable primarily to cell-bound enzymes.

Key Words: steroidal saponin, mixed rumen bacteria, enzymes

W276 Starch fermentation kinetics in rumen fluid and synthesis of end products. J. W. Cone*[1] and P. M. Becker[2], [1]*Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands*, [2]*Animal Sciences Group of Wur, Lelystad, the Netherlands*.

In this study, fermentation kinetics in rumen fluid of 16 starchy feedstuffs, differing in starch content, were correlated with the amount of synthesized volatile fatty acids (VFA-s) and microbial mass (MM). The fermentation kinetics were determined using a gas production technique (GPT). Rumen fluid was collected from two non-lactating rumen cannulated cows, receiving 1 kg of standard compound feed (150 g/kg starch) in the morning and ad libitum hay in the morning and the afternoon. After 0, 4, 8 and 12 h of incubation, individual and total VFA-s were determined. Gas production profiles were modeled and the incubation time at which the proportional rate of gas production was maximal (tRmax) was determined. This is the time point at which MM is maximal and the substrate is to be exhausted. At tRmax VFA-s were determined as well as the purin content as a measure of the MM. Gas productions were corrected for blank gas productions, buffered rumen fluid without sample, and VFA and MM were corrected for contents at 0 h incubation. At all incubation times, there proved to be a rather good relationship between gas production and synthesis of total VFA ($r2 = 0.88$), acetic acid ($r2 = 0.88$), propionic acid ($r2 = 0.79$) and butyric acid ($r2 = 0.84$). The relationship between gas production at all incubation times and the non-glucogenic:glucogenic ratio (NGGR) was rather poor ($r2 = 0.34$). However, for each incubation period separately, there was a linear relationship between gas production and NGGR ($r2 = 0.77$), which was also the case at tRmax. There proved to be a linear relationship between tRmax for the different samples and the amount of synthesized MM ($r2 = 0.58$). A fast fermentation caused a high amount of MM. At tRmax there was a linear relationship between NGGR and synthesized MM. It was concluded that both the NGGR and amount of MM are largely determined by the rate of starch fermentation.

Key Words: gas production technique, rumen fermentation, starch

W277 Empirical prediction of oxygen consumption by portal-drained viscera in ruminants: Meta-analysis approach. C. Loncke*[1], I. Ortigues-Marty[1], S. Amblard[1], J. Vernet[1], S. Léger[2], H. Lapierre[3], D. Sauvant[4], and P. Nozière[1], [1]*Institut National de la Recherche Agronomique - UR 1213, Theix, France*, [2]*Université de Clermont Ferrand II - Laboratoire de Mathématiques, Aubière, France*, [3]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada*, [4]*Institut National*

de la Recherche Agronomique - AgroParisTech - UMR 791, Paris, France.

In Ruminant nutrition, the evolution of feed evaluation systems towards nutrient-based systems is an important challenge. Net portal appearance of volatile fatty acids, glucose, β-hydroxybutyrate, lactate and α-amino N can be estimated with response equations based on ration intake and composition (Loncke et al., 2008, Renc. Rech. Rum. 15:285; Loncke et al., 2009, J. Anim. Sci. 87:253-268) characterized according to INRA Feed Tables for Ruminants and derived from a meta-analysis (Sauvant et al., 2008, Animal 2:1203-1214) on the FLORA (FLux of nutrients through Organs and tissues in Ruminant Animals) database (Vernet and Ortigues-Marty, 2006, Reprod. Nutr. Dev. 5:527-546). The present objective was to extend the meta-analysis to oxygen (O_2) consumption by portal-drained viscera (PDV) in relation with changes in intake and dietary composition. Data on PDV O_2 consumption in sheep and cattle (n = 38) were selected from FLORA database. Animals of the publications consumed on average 20.2 ± 7.2 g DM/kg BW/d with an average proportion of 25.6 ± 31.7 g concentrate/100 g DM. The best adjustment was obtained with a variance covariance model, common to both sheep and cattle, that included only DMI as predictor and a within experiment effect (R^2 = 0.78; RMSE = 0.4 mmol/kg BW/d; P < 0.001). Adding other parameters such as diet composition (ME, NDF and CP concentration) did not improve significantly the equation. The model showed that an increase of 1g DMI/kg BW/d induces a predicted increase of 0.081 ± 0.013 mmol/kg BW/d of O_2 consumption by the PDV, independently of the nature of diet. It is coherent with the two known factors of variation of PDV O_2 consumption, i.e. physical (digesta mass) and chemical (nutrients) components (Han et al., 2002, J. Anim. Sci. 80:1362-1374).

Key Words: meta-analysis, portal-drained viscera, oxygen consumption

W278 Plasma acetate, glucose and leucine turnover rates and whole body protein synthesis in growing lambs. H. Sano, K. Chiba, A. Saito, K. Shibuya, and M. Al-Mamun*, *Iwate University, Morioka, Iwate, Japan*.

The aim of the present experiment was to determine plasma acetate, glucose and leucine metabolism and whole body protein synthesis in growing lambs. The experiment was performed using crossbred (Corriedale x Suffolk) growing lambs (n=7; 3 male and 4 female) at 2 growth stage; 2 month old (2M, 18±3 kg of initial BW) and 6 month old (6M, 25±6). The lambs were offered mixed hay (metabolizable energy (ME) 1.79 kcal/g) and concentrates (ME 2.62 kcal/g) 33 g/kg$^{0.75}$/d each once a day at 14:00 for a period of 21 day with ad libitum water access. The animals were kept in individual pens for the first 14 day preliminary period in an animal shed and then moved to a controlled environmental house at a temperature of 23±1°C. Three isotope dilution methods using [1-^{13}C]Na-acetate, [U-^{13}C]glucose and [1-^{13}C]leucine were performed simultaneously as a primed continuous infusion for 4 h on the 21st day of the experimental period for each growth stage. Turnover rates of plasma acetate, glucose and leucine were determined from the isotopic enrichments of [1-^{13}C]Na acetate, [U-^{13}C]glucose and [1-^{13}C]leucine and α-[1-^{13}C]keto isocaproic acid (α-KIC), respectively using GC/MS. Whole body protein synthesis was determined using nitrogen balance test and leucine turnover rate calculated from α-KIC. Plasma concentration of NEFA was numerically lower (P = 0.13) in 6M than 2M. Plasma acetate concentration was higher (P = 0.01) and turnover rate was numerically higher (P = 0.15) in 6M than 2M. Plasma glucose concentration was lower (P = 0.05) and turnover rate tended to be lower (P = 0.10) in 6M than 2M. Plasma concentration of α-KIC tended to be lower (P = 0.10) and turnover rate of leucine remained comparable (P = 0.83) between age groups. The present findings laid an idea that the higher plasma acetate and lower plasma glucose metabolism might be attributed to increased microbial activity with the development of rumen. However, protein metabolism was not influenced with the development.

Key Words: nutrients metabolism, stable isotope, growing lambs

W279 Mammary cell signaling responses to abomasal starch and casein infusions in lactating dairy cows. A. G. Rius*[1], J. Escobar[2], O. Becvar[3], D. Kirovski[4], and M. D. Hanigan[1], [1]*Dept. of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg*, [2]*Dept. of Animal Science, Virginia Polytechnic Institute and State University, Blacksburg*, [3]*College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg*, [4]*Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia*.

The objective of this study was to evaluate the effects of abomasal infusions of starch and casein on cell signaling pathways in the mammary gland of lactating cows. We hypothesized that abomasal infusions of starch or casein could activate independently the mammalian target of rapamycin (mTOR) cell signaling cascade. Six primiparous, mid-lactation, ruminally cannulated Holstein cows were randomly assigned to casein (C) and starch (S) treatments using a 2 x 2 factorial arrangement of treatments. The design was a replicated incomplete 4x4 Latin-square. All animals received the same basal diet (17.6% CP and 1.58 Mcal NEL/kg DM) throughout the study. Cows were restricted to 70% of ad libitum intake and abomasally infused for 36 h with S (3 kg/d), C (1.3 kg/d), the combination (3kg/d S + 1.3kg/d C), or water using peristaltic pumps. Blood samples were collected during the last 8 h of treatment. At the end of the infusion, biopsy samples were collected and western blot analysis of signaling proteins was conducted. The main effects of S, C, or the interaction of both was analyzed using the Proc Mixed procedure of SAS. Casein increased plasma concentration of Phe (P < 0.01), Leu (P < 0.01), Ile (P < 0.01), and Met (P < 0.01). However, S decreased concentration of Lys (P < 0.01), Val (P < 0.01), and His (P < 0.01). Starch increased (P < 0.05) the phosphorylation of mTOR and ribosomal protein S6. There was a positive interaction between S and C infusion for the phosphorylation of protein kinase B (P < 0.01) and a negative interaction for the unphosphorylated form of mTOR (P < 0.01). Thus, the mTOR pathway in the mammary gland can be regulated by amino acids and energy substrates.

Key Words: amino acids, cell signaling, mammary gland

W280 Meta-analysis for the prediction of net portal absorption of amino acid nitrogen in ruminants. R. Martineau*[1], D. Sauvant[2], D. R. Ouellet[1], J. Vernet[3], I. Ortigues-Marty[3], and H. Lapierre[3], [1]*Agriculture and Agri-Food Canada, Stn Lennoxville, Sherbrooke, QC, Canada*, [2]*AgroParisTech INRA, Paris, France*, [3]*UHR INRA Clermont-Ferrand, Theix, St-Genès Champanelle, France*.

The objective of this meta-analysis was to determine a prediction equation for net portal absorption of amino acid-N (NPA-AAN) based on diet intake and composition. Selection of publications was based on availability of NPA-AAN (as α-amino-N or individual AAN), N intake (NI), BW, and feeding treatments. Some treatments were excluded because of fasting, use of metabolism modifiers, infusion in the gut or abnormal feeding conditions. The final database included 65 publica-

tions (203 treatments). Cattle treatments were aggregated (beef, n=68; dairy, n=22) and the meta-analysis was conducted on a total of 87 experiments (sheep, n=47; cattle, n=40). The diet characteristics were estimated using nutrient composition of feed ingredients from NRC (2001) tables, correcting only for N and NDF when reported. Fluxes of α-amino-N were multiplied by 1.396 to convert to fluxes of total AAN assuming that only the α-N is detected by the α-amino-N analysis. Daily NPA-AAN and NI averaged 0.216 ±0.125 and 0.465 ±0.199 g/kg BW, respectively. Linear and quadratic relationships between NPA-AAN and NI were tested inter- and intra-experiments, treating experiment as a fixed effect. In addition, other potential covariates were tested: DMI (%BW), forage:concentrate, TDN_{1X}, NDF and CP (%DM), NE_L (Mcal/kg), RDP and RUP (%CP), and digestible NFC (%NFC). The best fit was a linear model (R^2=0.916; RMSE=0.042) that included NI (P<0.001), NDF (P<0.001), and species (P=0.004). No outliers were detected on 191 observations in the post-model analysis. The overall equation was [coefficient (±SE)]: NPA-AAN = 0.120 (0.034) + 0.464 (0.040) NI – 0.0032 (0.0007) NDF with a species effect on the intercept: Δ = – 0.071 for cattle and 0.071 for sheep. There was no species × covariate interaction indicating that the variations of NPA-AAN were independent of species. Our results show that the magnitude of NPA-AAN was highest in sheep suggesting that they absorbed more AAN than cattle at similar NI and NDF. This indicates that sheep could transfer N ingested to NPA-AAN with a higher efficiency than cattle, likely due to a higher urea recycling into the rumen.

Key Words: amino acids, portal absorption, ruminants

W281 Acute fasting-induced changes in motilin, luteinizing hormone and metabolites in goat wethers. O. Gazal[1], B. Kouakou*[2], W. Mboko[1], S. Bialka[1], and J. H. Lee[2], [1]*St. Cloud State University, St. Cloud, MN*, [2]*Fort Valley State University, Fort Valley, GA*.

In monogastrics the secretion of motilin, a peptide hormone produced by cells in the gastrointestinal tract of many mammals, increases during fasting. Similarly, the secretion of luteinizing hormone (LH) is suppressed by fasting in monogastrics and other animals. Although different mechanisms of undernutrition-induced suppression of gonadotropin secretion have been proposed, the possible role of motilin in this process remains unclear. In this study, we tested the hypothesis that acute fasting induced changes in plasma motilin secretion in wethers and that changes in plasma motilin and LH secretion are correlated. Six wethers in high body conditions were fed ad-libitum and then fasted for 48 hours. Blood samples were obtained from an indwelling catheter for 4 hours at 10 minute intervals during each feeding regimen. Results indicate that motilin is secreted in a pulsatile manner and that fasting tended to increase plasma motilin secretion (P=.06). Acute fasting induced a paradoxical increase in plasma LH (P<.001). Fasting caused a significant decrease in plasma glucose (P<.02) but increased plasma urea nitrogen (P<.0001) and beta-hydroxybutyrate (P<.001). However, there was no effect of acute fasting on plasma non-esterified fatty acids (P=0.8). Plasma motilin levels were negatively correlated with plasma LH in the fed state (P<.001) but this correlation was not significant in the fasted state. These results indicate that the suppressive effect of acute fasting on LH secretion in goats may be dependent upon testosterone and body condition. Furthermore, fasting for 48 hours may be insufficient to cause a significant increase in plasma motilin in ruminants.

Key Words: goats, acute fasting, motilin

W282 Effect of diet and the $SGLT_1$ inhibitor phlorizin on net intestinal glucose absorption in Holstein steers. A. L. Ballou*, S. W. El-Kadi, and D. L. Harmon, *University of Kentucky, Lexington*.

Previous studies have shown that adult ruminants have a limited capacity for small-intestinal glucose absorption. This is thought to be the result of very low activity by the sodium glucose co-transporter, $SGLT_1$. However, attempts to induce increases in $SGLT_1$ have not been successful in cattle. We hypothesized that if $SGLT_1$ is the primary pathway of transcellular absorption then we should be able to decrease net glucose absorption by phlorizin infusion and this response may differ depending on diet. The objective of this study was to determine net glucose absorption in steers fed forage or grain-based diets in the absence and presence of phlorizin. Steers (n=3) were fed two isocaloric diets, high-forage and high-concentrate, at 1.5xNE_m over two periods of 14 days each. On d 14 of each period, repeated samplings were taken, representing two periods with two different abomasal infusions. For the first infusion, glucose was infused abomasally at a rate of 20g/h for four hours. The second four-hour infusion consisted of glucose infused at 20g/h with the addition of phlorizin at 400μmol/h. Para-aminohippuric acid was infused continuously into a mesenteric vein and six samples of portal venous and mesenteric arterial blood were collected over three hours of each infusion. Steers fed high-concentrate had higher ($P < 0.03$) portal blood flow, net glucose absorption ($P < 0.01$) and net portal glucose recovery ($P < 0.01$) of infused glucose (76 vs. 38%) than steers fed high-forage. Phlorizin infusion did not affect ($P > 0.05$) portal blood flow, net glucose absorption or net portal glucose recovery. These results show that while diet affects net glucose absorption, $SGLT_1$ may not be the primary mechanism through which this occurs.

Key Words: glucose, ruminant nutrition, $SGLT_1$

W283 Plasma concentration of glucose-dependent insulinotropic polypeptide is negatively correlated with respiratory quotient in lactating dairy cows. A. E. Relling*[1], L. A. Crompton[2], S. C. Loerch[1], and C. K. Reynolds[2], [1]*The Ohio State University, Wooster*, [2]*University of Reading, Reading, UK*.

In dairy cows, an increase in plasma concentration of GIP was associated with an increase in ME intake (MEI), but the role of GIP in energy partitioning of dairy cattle is not certain. The objective of our study was to examine the relationship between plasma GIP concentrations and energy metabolism. Four mid-lactation, primiparous, rumen-fistulated Holstein-Friesian cows were fed a control diet of 50% forage and 50% concentrate (DM basis) in a 4 X 4 Latin square design with 4-wk periods. The four treatments were: 1) a control diet fed at 1000 and 1600 h; the control diet plus one dose (1.75 kg DM basis at 0955 h) into the rumen of supplemental vegetable proteins (Amino Green, SCA, NuTec) and fed: 2) once (1000 h); 3) twice (1000 and 1600 h); or 4) 4 times (1000, 1600, 2200 and 0400 h). Measurements of respiratory exchange and energy balance were obtained over 4 d during the last week of each period while cows were housed in open-circuit respiration chambers. Blood was collected from the jugular vein every 30 min for 12 h using indwelling catheters and starting at 0800 h on d 20 of each period. Plasma GIP concentration was measured in samples pooled over each 5 consecutive blood samplings. Data were analyzed as repeated measures using mixed models testing random effects of animal and period and fixed effect of treatments, time and their interaction. The relationships between plasma GIP and MEI, heat production (HP), respiratory quotient (RQ) and milk yield (MY) were analyzed using linear correlation procedures, with MEI as partial variant. There was no effect (P> 0.2) of treatment on DMI (18.6 kg/d) and MY (27.6 kg/d). Plasma GIP

increased over time during the day ($P < 0.01$), but it did not change due to treatment ($P > 0.7$). Plasma GIP concentration was not correlated with MEI or HP ($P > 0.4$), but was positively correlated with MY ($r^2 = 0.42$, $P < 0.11$) and negatively correlated with RQ ($r^2 = -0.72$, $P < 0.01$). The correlations between GIP and RQ or milk yield do not imply causality, but suggest there may be a role for GIP, or associated factors, in the regulation of energy metabolism in dairy cows.

Key Words: GIP, energy partitioning, RQ

W284 Gluconeogenesis and carbon recycling in beef steers is modulated by energy-substrate supply. B. J. Bequette*[1], J. Sumner-Thomson[1], J. A. Moorefield[1], D. Hucht[2], M. Niland[2], and R. L. Baldwin VI[2], [1]*Department of Animal and Avian Sciences, University of Maryland, College Park*, [2]*Bovine Genomic Laboratory, Animal and Nutrition Resources Institute USDA-ARS, Beltsville, MD*.

To identify important rate-controlling sequences in regulation of gluconeogenesis by macronutrient supply, 19 beef steers (272.5 ± 17.6 kg initial BW) were fed a forage-based diet and infused per abomasum with either water (Control, n = 4), Casein (n = 5) or Starch (n = 5), or fed Na-propionate (n = 5) for 42 d. Treatments were administered on an equal energy basis (40 kcal/kg metabolic BW). On day 42, [U-^{13}C] glucose (1.25 g/h) was continuously infused into a jugular vein for 12 h. Over the last 5 h of tracer infusion, plasma glucose isotopic enrichment was determined by gas chromatography-mass spectrometry followed by ^{13}C-mass isotopomer distribution analysis. Data were analyzed by ANOVA and differences between Control and nutrient infusion treatments were assessed using Dunnett's *t*-Test. Steers receiving nutrient infusion gained more weight (3.6 to 5.2%, $P < 0.05$) over the 42-d experiment compared to Control. Apparent glucose production (g/kg empty BW/d) was greater (25 to 30%, $P < 0.05$) for steers receiving Starch, Casein, and Na-propionate compared to Control. Steers receiving Casein tended ($P < 0.10$) to have greater (40%) rates of glucose recycling (g/d) compared to Control. Steers receiving Starch and Na-propionate had greater (26 to 31%, $P < 0.05$) rates of gluconeogenesis plus glucose absorption. Despite different substrate compositions of the treatments, gluco-control was maintained and this involved different mechanisms. For example, steers receiving Casein increased glucose recycling whereas steers receiving Starch and Na-propionate had increased gluconeogenesis and/or glucose absorption.

Key Words: steer, gluconoegenesis, stable isotope

W285 First-pass glucose uptake (FPU) in the intestine of kids fed casein- or soy protein-based milk diets. U. Schönhusen, A. Flöter, P. Junghans, C. C. Metges, and H. M. Hammon*, *Research Institute for the Biology of Farm Animals (FBN), Dummerstorf, Germany*.

Soy protein alters the intestinal morphology with consequences for absorptive function. Supplementation of soy protein with indispensable amino acids (AA) seems to ameliorate mucosal growth retardation and stimulates the glucose transport capacity in the mucosa. We investigated effects of feeding soy protein with or without AA supplementation on FPU in the intestine. Goat kids (14 d of age) were fed milk diets, in which 50% of the crude protein was either casein (CA), soy protein isolate (SP) or soy protein isolate with supplementation of those AA known to be lower concentrated in soy protein than in casein (SPA) for 43 d (n=8/group). A single bolus dose of D-[U-$^{13}C_6$]glucose (10 mg/kg BW) was given with the morning diet, and simultaneously, a bolus D-[6,6-$^2H_2$]glucose (5 mg/kg BW) was injected into a jugular vein. Blood samples were collected between -30 and +420 min relative to the tracer administration to measure plasma glucose enrichments, resulting in separate pools for D-[6,6-$^2H_2$]glucose and D-[U-$^{13}C_6$] glucose, respectively. FPU was calculated from the rate of appearance of differentially labeled glucose tracer in plasma. Glucose oxidation was calculated from $^{13}CO_2$ enrichment in blood. In addition, plasma concentrations of glucose, insulin, and glucagon were measured. Data were evaluated by using the Mixed Model and GLM procedures of SAS. Before feed intake, plasma glucose concentration tended to be higher ($P < 0.1$) in CA than SPA, whereas insulin was higher ($P < 0.05$) in CA than SP and SPA, and glucagon was higher ($P < 0.05$) in CA than SPA. Plasma glucose and insulin concentrations increased ($P < 0.05$) during first hour after feeding, whereas plasma glucagon increased immediately after feeding and after 1 h of feeding. The [6,6-$^2H_2$]glucose pool size tended to be higher ($P < 0.1$) in CA than SPA. FPU and glucose oxidation were not affected by diet. Feeding milk diets with soy protein isolate seems to impair glucose status in kids, but has no effect on FPU and glucose oxidation.

Key Words: goat kids, soy feeding, glucose uptake

W286 Plasma leptin, feed intake and body fat reserves in ruminants. An updated overview. E. González-García*[1], N. Debus[1], Y. Chilliard[2], and F. Bocquier[1], [1]*INRA, Montpellier, France*, [2]*INRA, Theix, St-Genes-Champanelle, France*.

We aimed to update knowledge on relationship between plasma leptin (PL), feed intake (FI) and body fat reserves (BF) in ruminants. A literature review was done, using papers published (n = 31; 1998-2008) in international scientific journals. Studies were done under controlled conditions in dairy (3) or beef (9), goats (1) or sheep (22). Database contained gender, physiological status, breed, age, feeding level and body fatness. Underfeeding decreased PL, basically in fat individuals, while refeeding increased it. The PL depended on physiological status, also being higher in fat vs. leans whereas it decreased during nutritional restriction, following BCS. PL was related to adipose cell size and was modulated by short-term energy intake (EI) in interaction with long-term regulations (i.e. nutritional history). Rather than type of energy, amount of EI and BF are regulators of PL. The PL was positively related to BW changes, feeding level, plasma glucose, β-OH-butyrate, IGF-I and insulin, and negatively related to plasma NEFA. During nutritional restriction, insulin did not correlate with PL. Metabolic effects of PL are thought to be mediated via neuronal systems that possess PL receptors rather than via peripheral effects. There is evidence for a dissociation of PL effects on appetite and neuroendocrine function. Hence, FI and BW increased under long photoperiod and were associated with increases in PL, which was also modulated by daylength. The last have a physiological significance for body fat deposition, which naturally occurs during long days when feed is abundant. The breed had a small effect although PL differences were probably related to breed differences in body composition. Carcass fat can be estimated using PL with the same accuracy than using ultrasound fat thickness. There are positive phenotypic correlations between PL and ultrasound backfat thickness and marbling score, fat depth between 12-13 rib, kidney, pelvic, and heart fat, lean meat yield, and yield grade. Performance (ADG, DMI, and gain:feed) and PL were poorly correlated. There are opportunities to use PL information for optimizing ruminant feeding efficiency and carcass quality.

Key Words: body reserves, leptin, ruminants

W287 Variation of basal expression of a sodium-dependent phosphate transporter between sections of cattle small intestine. A. P. Foote*[1], B. D. Lambert[1,2], and J. A. Brady[2], [1]*Tarleton State University, Stephenville, TX*, [2]*Texas AgriLife Research, Stephenville*.

Phosphorus (P) nutrition in cattle is increasingly becoming an important topic with the growing concern over the role of production animals in surface water pollution. Excess P in the diet of dairy and beef cattle is excreted in the manure and can be washed into surface water causing increased algal growth and eutrophication. P transporters have been characterized in other species and homologous genes have been found to be expressed in bovine cell cultures. However, no other information is available regarding the active transport of phosphate in cattle. The objective of this study was to determine the patterns of expression of a known phosphate transporter, NaPi-IIb, in four sections of the small intestine of cattle. RNA was isolated from the duodenal, proximal jejunal, distal jejunal, and ileal mucosa of 20 harvested cattle. Relative amounts of NaPi-IIb mRNA expressed were determined using real-time RT-PCR. Expression of NaPi-IIb was highest in the two distal sections ($P < 0.0001$) and almost absent in the proximal sections. Expression did not differ between the two proximal sections ($P = 0.67$) or the two distal sections ($P = 0.3$). The data suggest that a sodium-dependent secondary active P transport system is not responsible for P absorption in the proximal portion of the bovine small intestine while it does contribute to the P absorbed in the distal sections of the bovine small intestine.

Key Words: phosphorus absorption, gene expression, NaPi-IIb

W288 Insulin and essential amino acids have significant but independent effects on protein synthesis signaling in bovine mammary epithelial cells in-vitro. A. L. Bell*, J. A. D. R. N. Appuhamy, J. Escobar, and M. D. Hanigan, *Virginia Polytechnic Institute and State University, Blacksburg*.

A better understanding of the regulation of milk protein synthesis could help improve the nitrogen efficiency of dairy cows. Protein synthesis responds to signals from hormones, energy substrate, and amino acid supply through several signaling proteins such as protein kinase B (Akt), mammalian target of rapamycin (mTOR), p70 ribosomal protein S6 kinase 1 (S6K1) and ribosomal protein S6 (rpS6) . This study investigated the effects of essential amino acids (EAA) and insulin on phosphorylation status (PS) of these signaling proteins using Mac-T bovine mammary epithelial (BME) cells. Cells were deprived of EAA and insulin overnight and then cultured with complete or EAA-deprived DMEM/F12 with and without 1 μg/ml of insulin (2x2 factorial design). After 1 h incubation, BME were lysed in the presence of protease and phosphatase inhibitors. Cell lysates were analyzed by Western immunoblotting with antibodies against phosphorylated mTOR (Ser^{2448}), Akt (Ser^{209}), rpS6 ($Ser^{235/236}$), and S6K1 (Thr389). Membranes were stripped and reprobed for the total form of each protein. The ratio of phosphorylated:total constitutes the PS of each signaling protein. Both EAA and insulin had significant effects on mTOR, S6K1, and S6 ($P<0.05$). EAA deprivation reduced PS by 55%, 47%, and 54%, respectively, and insulin deprivation reduced PS by 27%, 42%, and 46%, respectively. Akt PS was not affected by EAA status and markedly increased by insulin addition ($P<0.05$). There were no significant interactions between insulin and EAA on PS for any of the signaling proteins. Insulin and EAA appear to exert independent effects on cell signaling pathways in BME cells. If the same is true for intact mammary tissue, milk protein synthesis should be regulated independently by AA supply and insulin status.

Key Words: amino acid, cellular signaling, insulin

W289 Evaluation of the effects of ozonated water on the microbial ecology of the rumen *in vitro* and digestion of corn and alfalfa hay *in situ*. K. L. Neuhold*, S. K. Williams, K. K. Nightingale, and S. L. Archibeque, *Colorado State University, Department of Animal Sciences, Fort Collins*.

The overall goal of this work was to evaluate the effects of ozone treated water on the microbial ecology of the bovine rumen and the subsequent effect on Enterohemmorhagic *Escherichia coli* serotype O157:H7, and *Salmonella* Typhimurium populations *in vitro* and digestibility parameters *in vitro* and *in situ*. To accomplish the objective, rumen contents were collected from three fistulated beef steers consuming a high concentrate diet and provided with control water (non chlorinated well water; 0.05 ppm ozone) or ozone treated water (0.30 ppm ozone) in a cross-over design for at least 15 d. On d 16 ruminal samples were obtained 2-h post feeding. Following rumen fluid collection, we simulated the rumen environment, *in vitro* and evaluated two treatments; 1) Control water; and 2) Ozonated water. The supplemented rumen contents were then inoculated with rifampicin resistant *Escherichia coli* O157:H7, or *Salmonella* Typhimurium. To determine the effect of treatments on the total bacterial population after inoculation, samples were removed at 0, 1, 4, 8, 12, 24 and 48 h post inoculation for analyses. *In vitro* bags were prepared with corn and alfalfa samples and placed in the steers at 0, 2, 6, 12, 24, 48 and 72 h. There was no effect of water treatment on enumerations of *Escherichia coli* O157:H7, or *Salmonella* Typhimurium populations *in vitro*. However, ozone treated water did increase ($P = 0.027$) *in vitro* dry matter digestibility of ground corn. Water treatment had no effect on *in situ* DM disappearance of corn ($P = 0.64$) or alfalfa hay ($P = 0.27$). These data indicate that while ozonated water may have little effect on pathogen viability, ozonated water may improve the digestibility of corn grain in the ruminal contents of finishing beef steers.

Key Words: ozone, pathogen, digestibility

Ruminant Nutrition: Vitamins and Minerals

W290 The influence of feeding chelated trace minerals on dairy cattle performance and colostrum quality. A. Formigoni[1], S. Emanuele*[2], C. Sniffen[3], G. Biagi[1], and M. Fustini[1], [1]*DIMORFIPA-University of Bologna, Bologna, Italy*, [2]*Balchem, New Hampton, NY*, [3]*Fencrest LLC, Plymouth, NH*.

Dairy cow diets are often formulated to exceed NRC 2001 guidelines for Zn and Cu by up to 50%. Trial objective was to determine the effect of chelated trace elements (KeyShure® Zn, Cu and Mn) on dairy cattle performance and colostrum quality when diets were formulated to NRC 2001 guidelines for Zn and Cu. The experiment was conducted at the Pasetto farm, Verona, Italy. There were 2 treatments and 2 pens per treatment with 148 animals per treatment. Experimental period started at dry-off and continued through 150 DIM. Average dry period length was 60 ±11.5 days. The dry cow and lactation diets consisted of a 1-group TMR and were fed ad libitum once daily. The mineral premix for the dry period and lactation control diets contained 100% of the Zn, Mn and Cu as inorganic sulfates. The mineral premix for the dry period KeyShure®