334 Chemotaxis toward glucose and xylose by mixed ruminal protozoa and dose-responsive insulin recovery from wortmannin inhibition by entodiniomorphid cultures. H. L. Diaz*[1], J. L. Firkins[1], M. A. Lyons[1], and J. R. Knapp[2], [1]*The Ohio State University*, *Columbus*, [2]*Fox Hollow Consulting, LLC*, *Columbus, OH*.

Isotrichid (IS) protozoa migrate toward sugars after feeding and then sink ventrally. We hypothesized that entodiniomorphids (EN) also sense glucose and xylose (end-products of glucanases and xylanases). Two blood capillary tubes (75 mm) were not filled (positive control, POS) or filled with 0 (negative control, NEG), 1, 10, 100, or 1000 m*M* of glucose or xylose in saline and then placed in 3 replicate beakers with 20 mL of rumen fluid from a dairy cow withheld feed for 12 h or repeated 3 h after feeding. IS counts in the tubes peaked for 1000 m*M* glucose when feed was withheld but 100 m*M* after feeding (interaction $P < 0.01$). There was no interaction ($P > 0.20$) for EN. IS were 3 fold more chemotactic toward xylose than glucose, with the reverse for EN. Combining 2 studies for the fed cow, tubes contained 2.1, 6.5, 25.5, 140.2, 65.7, and 22.1 IS counts for NEG, 1, 10, 100, and 1000 m*M* glucose, and POS, respectively (140.2 > 65.7 > rest, $P < 0.06$); and for EN, 7.0, 12.0, 23.1, 6.4, 33.1, and 115.8 (115.8 > 33.1 > 23.1 > rest, $P < 0.09$). IS depleted the sugar gradient or physically blocked the tube entrance from EN for 100 m*M*. Chemotaxis and phagocytosis by higher eukaryotic cells is mediated by phosphatidylinositol-3-kinase, a component in insulin signaling inhibited by wortmannin (WORT). Cryopreserved *Entodinium caudatum* (Ento) and *Epidinium caudatum* (Epi) were incubated with 0.2 or 2.0 μ*M* WORT 30 min prior to stimulation by insulin. Counts at 24 h (proportion of 0-h counts) for Ento were 1.54, 1.30, 1.12, 1.54, 1.91, 0.94, 0.87, 1.18, and 1.00 for control; 0.2 μ*M* WORT + 0, 0.1, 0.5, and 2.5 μ*M* insulin; and 2.0 μ*M* WORT + 0, 0.1, 0.5, and 2.5 μ*M* insulin. Normalized Epi counts were 1.32, 0.92, 1.22, 1.19, 1.16, 0.84, 0.91, 1.19, and 0.97. Insulin linearly ($P < 0.08$; corrected for unequal spaces) rescued Ento from 0.2 μ*M* WORT, with no effect at 2.0 μ*M*. Insulin recovery for Epi was $P > 0.15$ for 0.2 μ*M* but $P < 0.05$ quadratic for 2.0 μ*M* WORT. These data justify future research to coordinate chemotaxis with nutrient sensing signals that stimulate rumen protozoal growth rate.

Key Words: Rumen Protozoa, Chemotaxis, Glucose

335 Influence of disodium fumarate on ruminal fermentation and microbial growth in sheep fed high-forage diets. Y. W. Zhou*, J. X. Liu, and L. Zhou, *Zhejiang University*, *Hangzhou, P.R. China*.

The present study was conducted to examine the effects of disodium fumarate (DF) addition on fermentation and microbial populations in the rumen of sheep fed high-forage diets. In Experiment 1, six Hu sheep fitted with ruminal cannulae were randomly allocated to a 2 × 2 cross design involving two dietary treatments: added with 0 or 20g DF daily. Animals were maintained in individual pens with basal diet (concentration/forage=30:70) and free access to water. Each period lasted for 15d. Rumen samples were taken for determination of fermentation parameters, and microbial populations in fluid and solid samples were analyzed using real-time PCR method. Ruminal pH decreased sharply in the DF group compared with the control (P<0.05). Total volatile fatty acid (P<0.001) and acetate (p<0.05) were increased significantly, but butyrate decreased (P<0.01) by adding DF. Addition of DF tended to decrease ammonia N production (P=0.081). The populations of methanogens, protozoa, fungi and *R. flavefaciens* decreased (P<0.001) in fluid samples by DF addition, whereas *R. albus* populations increased both in fluid (P=0.011) and solid (P<0.001) samples. Experiment 2 was conducted to observe the dynamics of DF addition on rumen fermentation and bacterial growth. Three cannulated sheep were fed continuously for 42 d on a diet (concentration/forage=30:70) added with 20g/d DF. Ruminal samples were collected every 7 d after the first 14d for adaptation. No apparent effects were observed (P>0.05) of DF on pH, acetate and butyrate. Addition of DF produced dynamic changes in propionate (P=0.043), ammonia N (P=0.0006), and growth of fluid-and-solid-associated microorganisms. The populations of methanogens and *R. flavefaciens* decreased (P<0.0001) linearly in fluid samples, but the *R. albus* populations increased in both fluid and solid samples (P<0.0001). These results demonstrated the ability of DF addition to improve *in vivo* rumen fermentation by increasing total VFA production, and to influence bacterial populations of the rumen in sheep under high-forage diets in a positive way by increasing *R. albus* and decreasing methanogens.

Key Words: Disodium Fumarate, Ruminal Metabolism, Microbial Populations

336 Extract from *Larrea tridentata* reduces growth of rumen bacteria. J. Browne-Silva, S. L. Lodge-Ivey*, J. Petersen, R. Reyna-Islas, and M. B. Horvath, *New Mexico State University*, *Las Cruces*.

Larrea tridentata plant extract (CBPE) has antioxidant and antimicrobial properties. The use antimicrobials for growth promotion in livestock has been criticized and resulted in an interest in the use of natural plant extracts as rumen modifiers. The effect of the consumption of CBPE on rumen bacteria and fermentation has not been documented. An in vitro experiment was conducted to evaluate the effect of CBPE on growth of pure cultures of rumen bacteria and microbes found in whole rumen fluid from a cow. Varying levels of CBPE were dissolved in ethanol and added to pure culture incubations at 0, 5, 10, 50, and 100 μg/mL. Similiarly, CBPE was added to growth medium with microbes from whole rumen fluid at 0, 10, 20, 30, 40, 50, 60, 70, 80, 100, 150, 200, 250, 300, 400, 500 μg/mL. Growth was monitored by optical density (OD) at 600 nm. Independent of species, additions of (0 vs 100 μg/mL) to pure cultures of rumen bacteria caused a 35 fold decrease in OD (0.71 vs 0.02; $P < 0.001$). Addition of 10 μg/mL CBPE caused a 62% decrease in OD compared with 0;\mu;g/mL. Ethanol control was similar (0.57 vs 0.71) to 5 μg/mL CBPE (0.51; $P > 0.10$). Growth of *Butyrivibrio fibrisolvens* H17E, *Prevotella ruminicola* GA33, and *Ruminococcus albus* 8 was most affected by CBPE compared to *P. ruminicola* 118B, *R. flavefaciens* FD1, and *Streptococcus bovis* JB1 (0.01, 0.20, 0.10 vs 0.59, 0.58, 0.65, respectively). Growth of microbes in whole rumen fluid was not affected until 100 μg/mL of CBPE was added to the growth medium ($P > 0.05$). There was a 133% reduction in OD between 0 vs 100 μg/mL CBPE (0.60 vs 0.08) and a 50% reduction when comparing 50 vs 100 μg/mL CBPE ($P < 0.001$). Addition of CBPE greater than 100 μg/mL did not result ($P > 0.05$) in a decrease in OD. These data suggest that CBPE is toxic to rumen bacteria. Level of toxicity is dependent upon species and mixture of bacteria present. The bioconversion of CBPE by rumen bacteria is currently under investigation.

Key Words: Rumen Bacteria, Antibiotics, Plant Extract

337 Effects of a combination of feed additives on methane production, diet digestibility and animal performance in lactating dairy cows. S. M. van Zijderveld*[1,2], B. C. J. Fonken[1,2], J. R. Newbold[3], W. B. Fokkink[3], J. Dijkstra[2], W. J. J. Gerrits[2], and H. B. Perdok[1], [1]*Provimi B.V.*, *Rotterdam, the Netherlands*, [2]*Animal Nutrition Group, Wageningen University*, *Wageningen, the Netherlands*, [3]*Provimi Research and Innovation Centre*, *Brussels, Belgium*.

An experiment was conducted to study the effects of a mixture of lauric acid (C12:0), myristic acid (C14:0), linseed oil and calcium fumarate on methane production, diet digestibility and milk production. Inclusion rates of the additives were 0.4, 1.2, 1.5 and 0.7% of DM, respectively. The basal diet comprised (DM basis) 37.1% grass silage, 37.1% corn silage, 1.7% wheat straw and 42.0% concentrate. The experiment was designed as a randomized block design and conducted using 20 lactating Holstein-Friesian dairy cows (FPCM production 32.8 ± 4.9 kg/d, 176 ± 76 DIM at the start of the experiment). Cows were assigned to either the control treatment (CON) or the treatment receiving the additives (ADD) for treatment periods of 22 days. In the ADD ration, rumen-inert fat from palm oil was substituted for lauric acid, myristic acid and linseed oil to maintain diets isolipidic. Cows were housed in 2 identical, open-circuit, indirect climate respiration chambers (2 cows per chamber) during experimental observations in the third week. As a consequence of restricted feeding, DMI did not differ between treatments (16.7 and 16.5 kg DM/ day for CON and ADD, respectively). Apparent digestibility of OM, N, starch and sugar were unaffected, apparent fat digestibility was higher for ADD (65.6 vs 75.6%, P= 0.01). Daily milk yield did not differ between treatments (27.8 vs. 27.2 kg/ day, P=0.70). Milk fat concentration tended to be lower (P = 0.06) in ADD (41.0 g/ kg) than in CON (46.3 g/kg). FPCM production was lower for ADD as a result of the lower fat content for this treatment (29.4 vs 27.4 kg/d, P= 0.02). MUN levels were significantly lower for ADD (10.3 vs. 8.0 mg/dl, P=0.02), possibly reflecting a defaunating effect of the additives, with a consequentially lower rumen ammonia production. Methane production was lower for ADD relative to CON (362 vs. 326 g methane/ cow/day, P = 0.02).

Key Words: Methane, Digestibility, Dairy Cows

338 Ruminal parameters of cattle drenched with a placebo or live cultures of *Megasphaera elsdenii* strain CH4. M. R. McDaniel*[1], J. J. Higgins[1], J. M. Heidenreich[1], M. K. Shelor[1], G. L. Parsons[1], P. H. Henning[2], and J. S. Drouillard[1], [1]*Kansas State University*, *Manhattan*, [2]*KK Animal Nutrition*, *Centurion, South Africa*.

A metabolism study was conducted to evaluate ruminal parameters in cattle intraruminally dosed with 0, 1×10^{10}, 1×10^{11}, or 1×10^{12} CFU of *Megasphaera elsdenii* strain CH4 following an abrupt change from an all-forage diet to a 66% concentrate diet. Angus steers (n=20; average BW=253 kg) fitted with ruminal fistulas were blocked by BW and assigned randomly to treatments. Cattle were allowed free access to alfalfa hay and water, which were removed for 12 h prior to administering treatments. *Megasphaera* treatments were dosed via the rumen cannula as a liquid suspension containing 10^9 viable cells/mL of *M. elsdenii* strain CH4. The placebo consisted of 100 mL of autoclaved culture. On the morning of the diet change, cattle were administered their treatments and then allowed free access to a diet consisting of 34% alfalfa hay and 66% concentrate. Ruminal pH and concentrations of lactate and VFAs were monitored following introduction of the concentrate diet. Ruminal lactate concentrations increased in response to the diet change (P<0.05), but concentrations were lower for cattle that received *M. elsdenii* compared to the placebo group (P<0.05). Compared to the placebo group, cattle administered *M. elsdenii* maintained higher ruminal pH 24 h after feeding the concentrate diet (P<0.05). Dosing cattle with *M. elsdenii* before introduction of a concentrate diet may reduce the risk of acidosis by preventing accumulation of lactic acid and avoiding severe depressions in ruminal pH.

Table 1. Ruminal VFA, lactate, and pH of cattle intraruminally dosed with 0, $1x10^{10}$, $1x10^{11}$, or $1x10^{12}$ CFU of *M. elsdenii* strain CH4.

	Colony forming units of *M. elsdenii* strain CH4								
	0		$1x10^{10}$		$1x10^{11}$		$1x10^{12}$		
Hours post-challenge	0	24	0	24	0	24	0	24	SEM
Acetate, mM	24.5	29.3	26.0	34.0	26.6	32.6	22.5	40.3	6.03
Propionate, mM	4.6	17.4	5.0	17.1	5.6	28.1	3.9	19.7	3.93
Butyrate, mM	2.5	13.3	2.0	9.6	3.2	16.0	2.1	19.3	2.77
Lactate, mM	0.0	49.8	0.0	24.6	0.1	3.5	0.0	3.0	7.57
pH	7.4	5.3	7.4	6.0	7.3	5.7	7.4	6.0	0.18

Key Words: *Megasphaera elsdenii*, Lactate, VFA

339 Quantitative detection of bacterial genomes following intraruminal dosing of cattle with *Megasphaera elsdenii* strain CH4. M. R. McDaniel*[1], J. J. Higgins[1], J. M. Heidenreich[1], M. K. Shelor[1], G. L. Parsons[1], P. H. Henning[2], and J. S. Drouillard[1], [1]*Kansas State University*, *Manhattan*, [2]*KK Animal Nutrition*, *Centurion, South Africa*.

Angus steers (n=20; average BW=253 kg) fitted with ruminal fistulas were used to quantify changes in bacterial populations following intraruminal dosing with *Megasphaera elsdenii* strain CH4. Treatments consisted of inoculation with a placebo (100 mL of autoclaved culture) or $1x10^{10}$, $1x10^{11}$, or $1x10^{12}$ CFU of *M. elsdenii* strain CH4. Cattle were blocked by initial BW, assigned randomly to treatments, placed into individual pens, and allowed *ad libitum* access to alfalfa hay, salt, and water for a 3-wk adaptation period. Treatments were administered via the ruminal cannula following 12 h of feed and water deprivation. Immediately after dosing, steers were given *ad libitum* access to a diet consisting of 34% roughage and 66% concentrate. Ruminal samples were collected at 0, 2, 4, 6, 8, and 24 h after feeding for quantitative rt-PCR detection of native and introduced strains of *M. elsdenii*, as well as total bacterial genomes. Capacity for metabolism of lactic acid was evaluated by inoculating 0.2 mL of strained ruminal fluid into anaerobic culture tubes containing 15 mL of semi-defined lactate media. Tubes were incubated at 39C, and turbidity changes were determined by measuring absorbance at 2-h intervals for 12 h. Total number of bacterial genomes 24 h after inoculation was unaffected by intraruminal dosing of *M. elsdenii* strain CH4 (P>0.05). Populations of total *M. elsdenii* and *M. elsdenii* strain CH4 increased to 3.6×10^8 and 2.4×10^8 genomes/mL, respectively, by 24 h after inoculation (P<0.05). Turbidity of cultures containing lactate media increased in response to *M. elsdenii* administration (P<0.05), suggesting a greater capacity for lactate utilization

in inoculated cattle compared to the placebo group. Inoculating cattle with *M. elsdenii* is effective in bolstering populations of ruminal lactate utilizers, and may be useful in preventing ruminal lactate accumulation in grain-fed cattle.

Key Words: *Megasphaera elsdenii*, Lactate, Acidosis

340 Bacterial population shifts in the rumen of lactating dairy cows within and across feeding cycles. D. G. Welkie[1], D. M. Stevenson[2], and P. J. Weimer*[1,2], [1]*University of Wisconsin*, *Madison*, [2]*USDA-ARS*, *Madison, WI*.

While species composition of the ruminal microflora is thought to change during the feeding cycle due to variations in feed intake and ruminal environmental conditions, no studies have systematically characterized these purported population shifts. We used PCR amplification and automated ribosomal intergenic spacer analysis (ARISA) of bacterial DNA from bulk liquid and solid samples to profile changes in bacterial community composition (BCC) in 2 rumen-cannulated lactating cows over 4 successive 12-h feeding cycles. Cows were fed a TMR based on corn silage, alfalfa haylage, dry corn, and soybean meal. Ruminal samples were collected 2, 4, 6, 9, and 12 h post-feeding within each cycle. Cows did not differ in ruminal pH patterns and displayed only slight differences in VFA profiles, but displayed considerable differences in BCC. On average, samples contained 119 phylotypes (unique PCR amplicon lengths), of which 82 exceeded 1% of the peak height of the most abundant amplicon on capillary electrophoresis. Mean number of phylotypes did not differ (P>0.05) by sample type (solid or liquid), cycle number, or sampling time across cycles. Of 257 total phylotypes detected, only 19 were unique to a one cow. Between cows, 29 phylotypes were detected only in the liquid phase, and 24 of these were common to both cows. By contrast, only 5 phylotypes were detected only in the solid phase, 2 of which were common to both cows. Principal component analysis revealed that bacterial population shifts within and across cycles were much greater in liquid samples than in solid samples. Bacterial populations generally returned to near their pre-feed compositions by the end of each cycle, suggesting that feeding resets BCC.

Key Words: Bacteria, Rumen

341 Effect of lauric acid and coconut oil on ruminal fermentation, digestion, ammonia losses from manure, and milk fatty acid composition in dairy cows. A. N. Hristov*[1], M. Vander Pol[1], M. Agle[1], S. Zaman[1], C. Schneider[1], P. Ndegwa[2], V. K. Vaddella[2], K. Shingfield[3], and K. Johnson[2], [1]*University of Idaho*, *Moscow*, [2]*Washington State University*, *Pullman*, [3]*MTT Agrifood Research Finland*, *Jokioinen*.

Six multiparous Holstein cows were used in a replicated 3 × 3 Latin square design trial to investigate the effect of lauric acid (LA) or coconut oil (CO) on ruminal fermentation, nutrient digestibility, and ammonia losses from manure in dairy cows. Treatments consisted of intra-ruminal doses of 240 g/d stearic acid (SA; control), 240 g LA, and 530 g CO administered once daily, before feeding. Between periods, cows were inoculated with ruminal contents from donor cows and allowed a 7-d recovery period. Treatment did not affect ($P = 0.56$ to 0.82) DMI (26.4 kg/d), milk yield (30.2 kg/d), or milk composition. Ruminal pH was slightly increased ($P = 0.04$) by CO compared with the other treatments and ruminal ammonia concentration was decreased ($P = 0.03$) by LA and CO compared with SA (7.9, 7.9, and 10.1 mM, respectively). Protozoal counts were decreased ($P < 0.01$) by LA and CO relative to SA (26.1, 20.0, and 75.4×10^4, respectively). Methane production rate in the rumen was lowered ($P = 0.05$) by CO compared with LA and SA (2.5, 7.1, and 6.4 g/h, respectively). Total tract apparent digestibility of DM, OM, N, and NDF was not affected ($P = 0.37$ to 0.66) by treatment. Compared with SA, cumulative (15 d) *in vitro* ammonia losses from manure were reduced ($P < 0.01$) by LA, but not by CO ($P = 0.35$). Milk fat 12:0 concentration was increased ($P < 0.001$), while 16:0, 18:0, and total cis 18:1 content was decreased ($P < 0.01$) by LA and CO compared with SA. CO also enhanced ($P < 0.001$) milk 14:0 concentration relative to SA and LA. Treatments had no effect ($P = 0.15$) on milk fat CLA content. Current data confirmed the antiprotozoal activity of LA and CO in the rumen, which was accompanied by decreased ammonia concentration, and for CO reduced methane production. Addition of LA and CO in the rumen significantly altered milk fatty acid composition.

Key Words: Lauric Acid, Coconut Oil, Dairy Cow

342 Effect of esterified linolenic acid addition on methanogenesis, fermentation and microbes in the rumen of sheep fed diets with different forage to concentrate ratios. C. M. Zhang*, J. X. Liu, Z. P. Yuan, X. W. Yi, W. T. Li, and Y. Q. Guo, *Zhejiang University*, *Hangzhou, P.R. China*.

This study was conducted to investigate the effect of esterified linolenic acid (ELA) addition on methane production, fermentation characteristics and ruminal microbes in the rumen of sheep fed diets with different forage to concentrate ratio (F/C). The experimental design was a 4 × 4 Latin square with 2 × 2 factorial arrangement of treatments. Four groups of sheep were fed a forage-rich diet without (F/C=70:30, DM basis) or with ELA (F/C=70:25, 5% ELA), a concentrate-rich diet without (F/C=30:70) or with ELA (F/C=25:70, 5% ELA). Methane emission was reduced by addition of ELA markedly. A significant interaction was observed among the basal diet, ELA addition and methane production. Diet type had minor effect on total volatile fatty acids, while ELA addition decreased total volatile fatty acid significantly (P<0.05). Inclusion of ELA decreased molar proportion of acetate and butyrate, and increased molar proportion of propionate in concentrate-rich diet (P<0.05), but had little effect on the fermentation pattern in forage-rich diet (P>0.05). Methanogen and protozoa populations were decreased markedly by ELA addition, but not affected by F/C or their interaction. Growth of fungi was inhibited by the reducing F/C ratio, but little affected by ELA addition and their interaction. Addition of ELA promoted the growth of *R. flavefaciens* and *R. albus* markedly, but had little effect on *F. succnogen* numbers. Reducing F/C ratio decreased *R. albus* population markedly, but had minor effect on growth of *R. flavefaciens* and *F.succnogen*. There were no interactions between F/C and ELA addition on all the determined microbes. It is inferred that interactions of fat with the basal diet have to be taken into consideration to develop effective feeding strategies against ruminal methanogenesis.

Key Words: Esterified Linolenic Acid, Methanogenesis, Rumen Microbes

343 Summary of the effect on ruminal fermentation of Protein Edge® supplementation in continuous culture experiments. C. S. Mooney*, H. M. Dann, C. S. Ballard, K. W. Cotanch, and R. J. Grant, *William H. Miner Agricultural Research Institute*, *Chazy, NY*.

The objective of this analysis was to determine the effect of feeding bacteria and fungal fermentation extracts, Protein Edge® (PE; Agriformulations, Inc., Waddington, NY), on in vitro fermentation across six continuous culture experiments conducted at Rumen Fermentation Profiling Laboratory (West Virginia University, Morgantown, WV). The six experiments varied in liquid dilution rate (12.0, 12.0, 12.0, 12.0, 13.0, and 8.6%/h), solids dilution rate (3.6, 3.6, 3.6, 3.6, 4.6, and 3.3%/h), measured dietary crude protein (14.6, 14.3, 14.4, 16.5, 18.4, and 14.0%), and measured dietary neutral detergent fiber (35.1, 40.3, 36.8, 38.1, 32.1, and 39.5%), respectively. Within each experiment, treatments were a basal diet (control) and a basal diet supplemented with PE (equivalent to 14.2 to 42.5 g/animal/d) with the number of replicates being ≥3 per treatment. The dataset was analyzed with Analyze Model function of JMP® using fixed effect of experiment, fixed effect of treatment, and experiment by treatment interaction. Supplementation with PE did not affect ($P > 0.14$) digestibility of carbohydrate fractions, chemical composition of the microbial mass exiting the fermenter, or molar proportion and daily production of most volatile fatty acids. However, PE supplementation increased crude protein digestibility (81.7 vs. 74.3%; $P < 0.01$) when compared to control. Overall, PE supplementation affected N metabolism by decreasing bypass N in effluent (0.5 vs. 0.7 g/d; $P < 0.01$) while increasing microbial N (MN) in effluent (1.9 vs. 1.7 g/d; $P < 0.01$) indicating greater capture or retention of N within the microbial mass. Supplementation with PE increased ($P < 0.01$) MN production efficiency (g MN/kg substrate) +10% per kg dry matter, +13% per kg organic matter, and +14% per kg carbohydrate. Across experiments, PE altered N metabolism leading to greater microbial N production efficiency in a continuous culture system. Based on this result, adjustment of microbial efficiency constants within ration balancing programs may be warranted.

Key Words: Nitrogen Efficiency, Continuous Culture, Feed Additive

344 Effect of controlled *in vitro* pH on fermentative activity of ruminal contents from finishing cattle adapted to supplemental dried distiller's grains. S. Uwituze*, J. M. Heidenreich, T. G. Nagaraja, J. J. Higgins, and J. S. Drouillard, *Kansas State University*, *Manhattan*.

Ruminal pH typically is lower in cattle fed flaked grain diets compared to cattle fed rolled grain diets; leading us to hypothesize that low ruminal pH may restrict digestion of dried distiller's grains with solubles (DDG) in flaked grain diets. A study was conducted to investigate effects of pH on *in vitro* fermentative activity of ruminal contents from cattle adapted to a finishing diet containing 25% (DM basis) DDG. The study was a randomized complete block design with a 3 × 4 factorial treatment arrangement. Factors were pH (5, 5.5, or 6) and incubation time (6, 12, 24, and 48 h). A 50:50 mixture of DDG and dry-rolled corn was used as substrate. Fermentations consisting of a 2:1 mixture of McDougall's buffer and ruminal fluid were adjusted to target pH using citric acid. Fermentations were duplicated on each of 3 d (6 observations/treatment). Concentrations of VFA and in vitro disappearance of DM (IVDMD) were measured. There was an interaction ($P<0.01$) between pH and incubation time with respect to concentrations of acetate, propionate, valerate, total VFA, and A:P ratio. VFA concentrations were higher for pH 5.5 and 6 fermentations after 6 and 12 h, but were higher for pH 5 fermentations after 24 and 48 h. IVDMD increased with increasing pH (Lin, $P<0.01$; Quad, $P<0.01$) and incubation time. These results may help to explain decreases in cattle performance and diet digestibility when distiller's grains are substituted for steam-flaked grains.

Table 1. *In vitro* fermentation of a 50:50 dry rolled corn:DDG substrate by ruminal contents from cattle adapted to DDG

	Incubation time, h				Contrasts P-Values[a]			
Item	6	12	24	48	1	2	3	4
Total VFA, mM					0.55	0.01	<0.01	0.35
pH 5	49	103	167	170				
pH 5.5	91	115	154	158				
pH 6	93	123	152	136				
IVDMD,%					<0.01	<0.01	0.23	<0.01
pH 5	34	38	48	54				
pH 5.5	37	46	48	59				
pH 6	56	58	61	67				

[a]Contrasts are 1 = effect of pH; 2 = effect of incubation time; 3 = time × pH interaction; and 4 = pH 5 vs average of pH 5.5 & 6

Key Words: Distiller's Grains, Ruminal pH, Digestibility