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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

NOTE: ***Megasphaera elsdenii* strain CH4 = *Megasphaera elsdenii* NCIMB 41125**

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