EFFECT OF UNSATURATED FAT AND MONENSIN ON METHANE AND VFA PRODUCTION, IN VITRO

by

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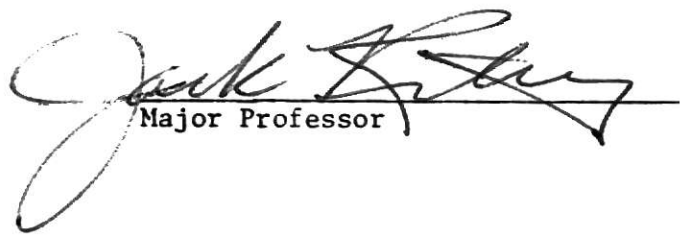

Major Professor

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INTRODUCTION

The addition of long-chain unsaturated fatty acids to the diets of ruminants has been shown to inhibit methane production and alter proportions of volatile fatty acids (VFA) produced by decreasing the acetate to propionate ratio. These effects have been observed *in vivo* (Blaxter and Czerkawski, 1966; Clapperton and Czerkawski, 1969; Czerkawski, 1966; Czerkawski *et al.*, 1966a,b; Robertson and Hawke, 1964a,b) and *in vitro* (Demeyer and Henderickx, 1967; Robertson and Hawke, 1964a). Monensin sodium,[1] a polyether antiobiotic, has produced similar effects on VFA ratios when added to the diets of beef cattle and when introduced into *in vitro* fermentation systems (Dinius and Simpson, 1975; Richardson *et al.*, 1976; McCartor *et al.*, 1976 Raun *et al.*, 1976). Thornton *et al.* (1976) demonstrated a significant reduction in methane yield in steers fed monensin. A shift in molar proportions of VFA toward greater propionate and less acetate and butyrate and accompanying methane inhibition (Van Nevel *et al.*, 1969) represents an energy savings to the animal, as the efficiency of both rumen formation and tissue utilization is greater for propionate than for acetate or butyrate, and because methane losses can account for 6-8 percent of gross energy intake (Armstrong and Blaxter, 1957; Blaxter and Czerkawski, 1966; Czerkawski, 1969; Holter *et al.*, 1970; Hungate, 1966). The purpose of this study was to determine the effect of unsaturated long-chain fatty acids alone or in combination with monensin sodium on VFA proportions and methane production *in vitro*.

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[1]Monensin, trade name Rumensin,® is a product of Eli Lilly and Company.

EXPERIMENTAL PROCEDURE

Measurements of gas production, gas composition, pH, and pre- and post-fermentation volatile fatty acids were made on *in vitro* incubations of strained bovine rumen fluid. Twenty-four fermentation bottles were allotted to treatments according to a 2x4x3 factorial design comparing oil sources (soybean oil and corn oil), oil levels (0, 2, 4, and 6 percent of the substrate), and monensin levels of 0, 16.5, and 33 ppm (0, 15, and 30 g/ton). Thirteen repetitions of the experiment were performed with chemical and statistical analysis carried out on 11, two having been rejected because of low gas production. Data were analyzed by least squares analysis of variance.

Incubation Technique

Substrate used was an 11.5 percent crude protein diet of corn, prairie hay, and soybean meal, ground in a Wiley mill through a 1-mm screen (table 1). Five grams of the substrate were added to each bottle. To facilitate measurement, monensin (Rumensin® 30) was dissolved in absolute ethanol at 0.8333 g/liter and 1.5 and 3.0 ml were added to the bottles for the 16.5 and 33 ppm treatments. Ethanol without Rumensin® was added to the 0 and 16.5 ppm treatments to bring total ethanol in each bottle to 3 ml. The bottles were then placed in a forced air oven overnight at 50 C and the ethanol driven off. A potassium phosphate buffer (table 2) was adjusted to pH=6.80 with concentrated $NaCo_3$ and 100 ml were added to each bottle. The oils, which

TABLE 1

SUBSTRATE

	Int. Ref. No.	% Crude protein, DM basis	% in ration, DM basis	% Crude protein furnished
Corn	4-02-931	11.32	76.15	8.62
Prairie hay	1-07-956	4.80	20.00	.96
SBM	5-04-604	49.92	3.85	1.92
			100.00	11.50

TABLE 2

BUFFER

	g/liter H_2O
KH_2PO_4	4.08
$MgSO_4$	.20
NaCL	.50
$CaCl_2 \cdot 2H_2O$	.05

contained 0.3 percent Tween® 80[2] emulsifier, were added at 0.1, 0.2, and 0.3 ml for the 2, 4, and 6 percent levels. Fifty ml of strained fluid collected 16 hours post-feeding from a ruminally cannulated steer fed 7.2 kg alfalfa hay and 4.5 kg concentrate mix (table 3) per day were added. A 50-ml rumen fluid sample was diluted to 150 ml and frozen for pre-fermentation VFA analysis. Anaerobiosis in the fermentation bottles was obtained by flushing them with CO_2, after which fermentation was carried out six hours in a water bath at 39.5 C. Bottles were shaken and gas production by water displacement read at 30-minute intervals.

After completion of the 6-hour incubation, gas samples were taken in 15-ml evacuated blood tubes through rubber septa. Post-fermentation fluid was frozen and reserved for VFA analysis.

Gas Analysis

Partitioning of gases was accomplished using a Varian Aerograph Series 200 with thermal conductivity detector. The column used was Poropak Q and argon served as the carrier gas. Oven temperature was set at 60 C and gas flow rate at 45 ml/min. Samples of 500 µl were drawn from the blood tubes and injected with a gas-tight syringe. Peak area values were automatically calibrated to pure standards of each gas fractionated, and concentrations of gases in the samples were adjusted by calculation to correct for dilution by CO_2 added to the system.

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[2]Polyoxyethylene (20) sorbitan monooleate, Fisher Scientific Co.

TABLE 3

CONCENTRATE MIX, DONOR STEER

	Int. Ref. No.	%, as fed
Grain Sorghum	4-04-383	66.8
Corn	4-02-935	20.0
Soybean meal	5-04-604	5.0
Starea 70		5.7
Dicalcium phosphate	6-01-080	2.0
Trace-mineralized salt		.5
Vit. A & D supplement		.1

VFA Analysis

Four ml of 5N HCl and 36 ml of the post-fermentation sample were placed in centrifuge tubes and spun at 19,000 x g for 25 min. One-µl samples were injected into a Beckman GC-4 gas chromatograph, flame ionization detector. Column used was 1/8 in. x 8 ft., packed with Chromosorb 101, 8100 mesh, and maintained at 200 C. Correction factors calculated from peak areas of a standard injected every fourth sample were used to adjust peak area to concentration. All samples and standards were run in at least duplicate. VFA production was determined by differences in concentration between pre- and post-fermentation samples.

RESULTS AND DISCUSSION

Figure 1 shows the nearly linear relationship between time and percent of gas production at each half hour interval during the six hour fermentation. Points represent averages from 21 observations of the 0 oil and 0 monesin controls. The line indicates that fermentation progressed without initial lag time and did not run to completion before termination at six hours.

Gas production data from these trials show a negative value for CO_2, while methane is in excess of the total volume of gas produced. Conversion of CO_2 to methane (Hungate, 1966) consumed more CO_2 than was produced, utilizing some of the 130 ml CO_2 used to flush each bottle. Gas production values for each observation were obtained from the formulas:

(Gas production, ml + 130 ml added CO_2)(CO_2 conc. in sample)- 130 = CO_2 produced, ml.

(Gas production, ml + 130)(Conc. of N_2, H_2, or CH_4 in sample) = N_2, H_2, or CH_4 produced, ml.

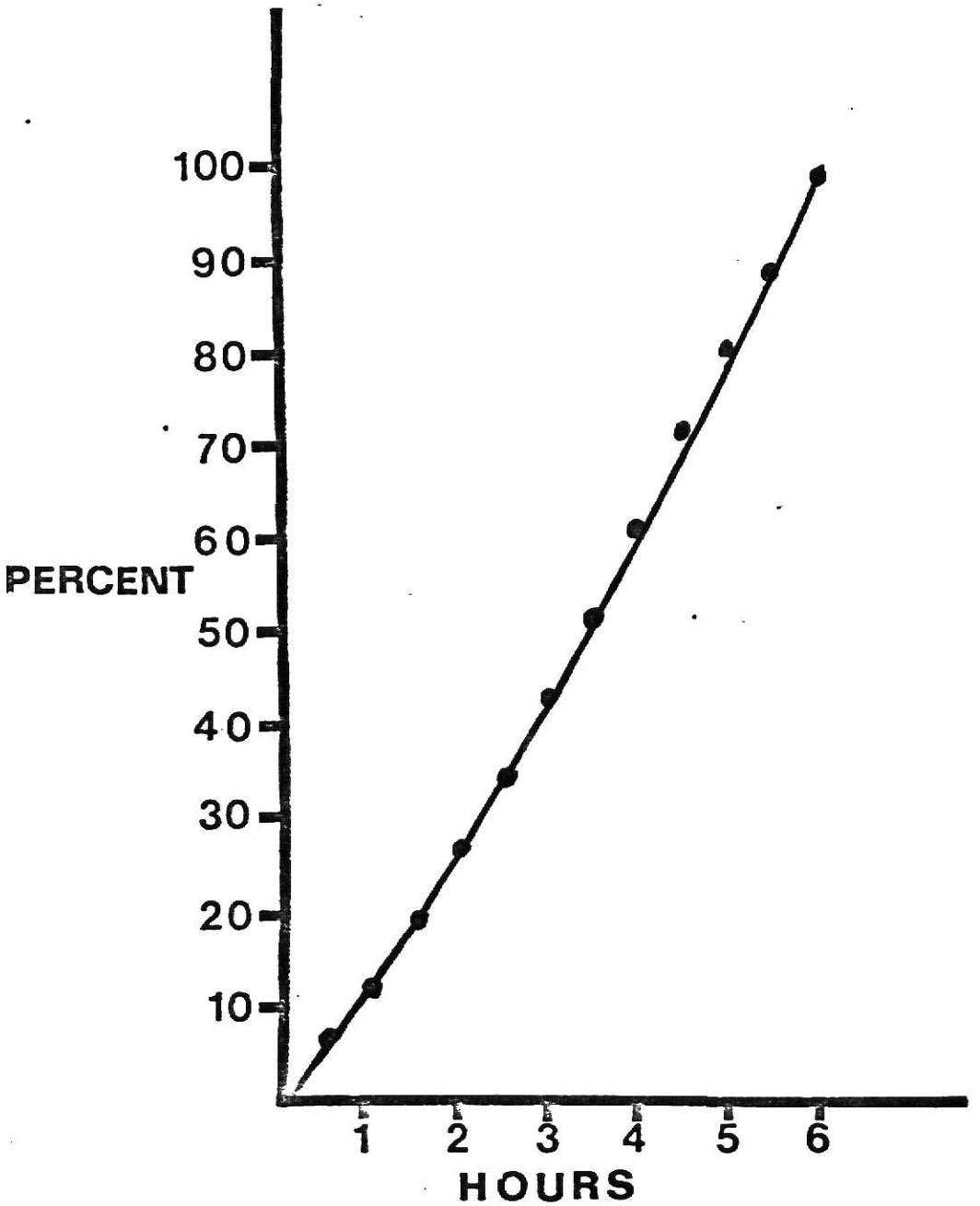

FIG.1. CUMULATIVE GAS PRODUCTION, % OF TOTAL.

Soybean oil treatments were significantly lower in total gas and methane production than corn oil treatments (table 4). Since the difference in total gas was nearly the same as the difference in methane production between oil sources, the difference in gas production could be attributed to greater methane reduction by the soybean oil. This trend is in agreement with the *in vivo* findings of Czerkawski *et al.* (1966b) and *in vivo* work of Demeyer and Henderickx (1967) who demonstrated greater methane inhibition with increasing degree of unsaturation of the C^{18} fatty acids used. Both groups of workers concluded that methane reduction was due, in part, to saturation of the fatty acids by hydrogen which would otherwise combine with CO_2 to form methane, although there was no direct relationship between number of double bonds and methane reduction. The drop in methane production was, according to these authors, far greater than can be attributed to hydrogenation alone, indicating a possible toxic effect of fatty acids on the methanogenic bacteria.

Level of oil had no significant effect on methane production (table 5). These results, *in vitro*, are inconsistent with those of Czerkawski (1966) where it was found that methane production decreased with increasing level of linseed oil fatty acids in the diets of sheep. Levels of oil used were 0, 2, 4, 6, 8, and 10 percent of the concentrate mix, or 0, 1.7, 3.4, 5.1, 6.9, and 8.6 percent of the total diet. Methane production for the high oil diet was more than 30 percent lower than for the control.

Methanogenesis was significantly ($P<.0001$) reduced by the addition of monensin to the substrate (table 6). Although there was no difference in methane production between the 16.5 and 33.0 ppm monesin levels,

TABLE 4

EFFECT OF OIL SOURCE ON IN VITRO GAS PRODUCTION

ml	Soybean Oil Mean	SE	Corn Oil Mean	SE	Prob.	LSD[1] P<.05	LSD[1] P<.10
Total gas production	82.80[a]	1.08	86.53[b]	1.07	.0155	3.00	2.52
H_2 prod.	1.18	.15	1.16	.15	.9179	----	----
N_2 prod.	41.87	1.09	41.81	1.08	.9728	----	----
CH_4 prod.	117.40[a]	1.02	120.87[b]	1.01	.0162	2.82	2.36
CO_2 prod.	- 78.45	.66	- 78.39	.66	.9541	----	----

[a,b] P<.05

[1]LSD given only where significant differences (P<.10) between means exist.

TABLE 5

EFFECT OF OIL LEVEL ON IN VITRO GAS PRODUCTION

Level, % of Substrate	-0-		-2-		-4-		-6-			LSD[1]	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Prob.	P<.05	P<.10
ml											
Total gas production	84.09	1.51	86.08	1.50	84.39	1.50	84.10	1.59	.7551	----	----
H_2 "	.98	.21	1.09	.21	1.44	.21	1.18	.22	.4450	----	----
N_2 "	45.24[a]	1.52	41.78[a,b]	1.51	40.14[b]	1.51	40.19[b]	1.60	.0648	4.26	3.57
CH_4 "	116.61	1.42	120.44	1.41	118.88	1.41	120.61	1.49	.1730	----	----
CO_2 "	- 79.76	.92	- 78.34	.91	- 77.06	.92	- 78.53	.98	.2323	----	----

[a,b] P<.05

[1]LSD given only where significant differences (P<.10) between means exist.

TABLE 6

EFFECT OF MONENSIN LEVEL ON IN VITRO GAS PRODUCTION

Level, ppm		-0-		-16.5-		-33-			LSD[1]		
ml		Mean	SE	Mean	SE	Mean	SE	Prob.	P<.0001	P<.05	P<.10
Total gas production		90.73[a]	1.35	82.29[b]	1.32	80.99[b]	1.29	.0000	----	3.68	3.08
H_2	"	1.18	.19	1.03	.18	1.30	.18	.5535	----	----	----
N_2	"	42.29	1.36	42.90	1.33	40.33	1.30	.3504	----	----	----
CH_4	"	125.32[A]	1.27	115.95[B]	1.24	116.14[B]	1.21	.0000	5.85	3.45	2.89
CO_2	"	- 78.60	.83	- 78.79	.81	- 77.87	.79	.6874	----	----	----

[a,b] P<.05

[A,B] P<.0001

[1]LSD given only where significant differences (P<.10) between means exist.

calorimetric determination on post-fermentation residues revealed that the 7.4 percent reduction in methane loss between the 0 and 16.5 ppm monensin treatments amounted to 2.8 percent of energy digested during incubation. Analysis of respired gases from steers with face masks has shown monensin feeding to reduce methane production from 8.99 to 7.5 liters/hr/$kg^{.75}$, energy loss as methane from 85.5 to 71.4 kcal/hr, and methane loss as percent of total heat production from 17.5 to 13.1 percent. (Thornton et al., 1976).

The only significant two- or three-way interaction for all gases and VFA measured was the combined effect of oil source and monensin level on methane production (table 7). The combination of soybean oil and 16.5 ppm monensin was the most effective methane inhibitor ($P<.05$). Soybean oil or corn oil in combination with 33 ppm monensin was not as effective ($P<.05$) as the soybean oil, 16.5 ppm monensin group, but was significant over the corn oil, 16.5 ppm monensin combination ($P<.05$). Corn oil with 16.5 ppm monensin reduced methane production ($P<.05$) compared to either oil group without monensin.

VFA production was affected by oil source, according to table 8. Corn oil, as compared to soybean oil, reduced production of acetate ($P<.10$), propionate, butyrate, iso-valearte, and total VFA ($P<.05$). The ratio of acetic to propionic acid was greater ($P<.10$) for the corn oil than for the soybean oil treatments. On a molar percent basis, corn oil increased ($P<.05$) the proportion of acetic acid in VFA produced over soybean oil and reduced the proportion of propionate ($P<.10$) and iso-valerate ($P<.05$). Heat of combustion and calorimetric efficiency values for the VFA (Blaxter, 1967) predict an 8.9 percent improvement in metabolizable energy derived from VFA production by soybean oil over

TABLE 7

EFFECT OF OIL SOURCE AND MONENSIN LEVEL ON CH_4 PRODUCTION

Oil Source	Soybean Oil						Corn Oil					
Monensin, ppm	-0-		-16.5-		-33-		-0-		-16.5-		-33-	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CH_4 prod.	124.34[a]	1.81	111.90[d]	1.75	115.96[c]	1.73	126.30[a]	1.77	120.00[b]	1.77	116.31[c]	1.70

Prob. = .0674

a,b,c,d $P<.05$

TABLE 8

EFFECT OF OIL SOURCE ON IN VITRO VFA PRODUCTION

	Soybean Oil		Corn Oil				
	Mean	SE	Mean	SE	Prob.	P<.05	P<.10
μM/ml							
Acetic	33.32[A]	.67	31.49[B]	.67	.0546	1.86	1.56
Propionic	16.02[a]	.34	14.18[b]	.34	.0002	.95	.80
Iso-Butyric	.44	.12	.29	.12	.3857	----	----
Butyric	10.57[a]	.21	9.59[b]	.21	.0014	.60	.50
Iso-Valeric	1.78[a]	.07	1.49[b]	.06	.0037	.19	.16
Valeric	1.80	.07	1.66	.07	.1712	----	----
Acetic/ Propionic	2.20[A]	.06	2.34[B]	.06	.1181	.18	.15
Total VFA	63.91[a]	1.23	58.72[b]	1.23	.0032	3.44	2.88
Molar percent							
Acetic	52.19[a]	.46	53.76[b]	.46	.0160	1.27	1.07
Propionic	25.09[A]	.30	24.33[B]	.30	.0716	.82	.69
Iso-Butyric	.62	.17	.44	.17	.4679	----	----
Butyric	16.60	.19	16.29	.19	.2432	----	----
Iso-Valeric	2.74[a]	.11	2.43[b]	.11	.0521	.31	.26
Valeric	2.77	.10	2.74	.10	.8647	----	----
pH	5.46	.02	5.47	.02	.8312	----	----

[a,b] P<.05

[A,B] P<.10

[1] LSD given only where significant differences (P<.10) between means exist.

corn oil treatments (table 9). Oil level had no significant effect on VFA production (table 10), nor was there an interaction effect between oil source and level. Demeyer and Henderickx (1967), however, demonstrated altered VFA production with the addition of 15 μmoles linolenic acid to an *in vitro* system containing 250 μmoles glucose substrate. Difference in μmoles VFA formed per 100 μmoles of substrate fermented for the acid treatment as percent of control were, in one experiment: acetic,-28.6; butyric,-30.9; propionic,-19.5. When linseed oil fatty acids were added to incubations with pyruvate as the substrate, percent of end product hydrogen as propionic acid increased as much as 43.5 percent, accompanied by a 36.8 percent drop in hydrogen as methane.

Monensin at 33 ppm lowered production of acetic and iso-valeric acid ($P<.05$, table 11). Production of butyric acid and the ratio of acetic to propionic were lowered with increasing monensin level ($P<.05$). Concentration of propionic acid was greater ($P<.05$) than controls at the higher monensin level. Total VFA production was depressed by 33 ppm monensin compared with controls. The significant shifts in proportions of acetic, propionic, and butyric acids on a molar percent basis would support trends observed *in vitro* by Richardson *et al.* (1976) and *in vivo* by McCartor et al. (1976), Potter *et al.* (1976), Raun *et al.* (1976) Dinius *et al.* (1976), and Richardson *et al.* (1976) for both concentrate- and pasture-fed cattle. However, no changes in total VFA with addition of monensin occurred in trials by Potter *et al.* (1976), Dinius *et al.* (1976), McCartor *et al.* (1976), Richardson *et al.* (1976) on feedlot cattle, and Raun *et al* (1976) on cattle fed 49 and 70 percent concentrate rations. Richardson *et al.* (1976) observed an increase in total VFA over control with addition of monensin *in vitro*. The trend toward

TABLE 9

EFFECT OF OIL SOURCE ON PREDICTED METABOLIZABLE ENERGY PRODUCTION

Oil	Acid	Millimoles/ml, Table 8	Heat of combustion, cal/millimole Blaxter (1962) Table 1	cal/ml	Calorimetric efficiency Blaxter (1967), Table 52	Metabolizable cal/ml	
	Acetic	.03332	209.4	6.98	.592	4.13	
Soybean	Propionic	.01602	367.2	5.88	.865	5.07	
	Butyric	.01057	524.3	5.54	.764	4.23	
				18.40	.85[1]	13.43	15.64
	Acetic	.03149	209.4	6.59	.592	3.90	
Corn	Propionic	.01418	367.2	5.21	.865	4.50	
	Butryic	.00959	524.3	5.03	.764	3.84	
				16.83	.85[1]	12.24	14.30

[1]Average value for four combinations of the three VFA.

TABLE 10

EFFECT OF OIL LEVEL ON IN VITRO VFA PRODUCTION

	Oil Level, % of substrate								
	-0-		-2-		-4-		-6-		
μM/ml	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Prob.
Acetic	32.77	.95	32.02	.93	32.41	.96	32.42	.94	.0546
Propionic	15.64	.49	14.71	.48	15.06	.49	14.99	.48	.5832
Iso-Butyric	.51	.17	.29	.17	.21	.17	.44	.17	.6066
Butyric	10.19	.31	9.99	.30	10.17	.31	9.97	.30	.9346
Iso-Valeric	1.73	.10	1.65	.10	1.58	.10	1.58	.10	.6566
Valeric	1.78	.10	1.79	.09	1.70	.10	1.64	.10	.6670
Acetic/ Propionic	2.25	.09	2.26	.09	2.32	.09	2.27	.09	.9561
Total VFA	62.59	1.75	60.46	1.72	61.18	1.77	61.03	1.74	.8464
Molar Percent									
Acetic	52.51	.65	52.66	.64	53.09	.65	53.64	.64	.5995
Propionic	25.10	.42	24.51	.41	24.73	.42	24.53	.42	.7348
Iso-Butyric	.67	.24	.46	.24	.35	.25	.63	.24	.7800
Butyric	16.31	.26	16.66	.26	16.64	.26	16.16	.26	.4412
Iso-Valeric	2.67	.16	2.74	.16	2.43	.16	2.47	.16	.4312
Valeric	2.74	.15	2.96	.15	2.75	.15	2.57	.15	.3093
pH	5.47	.02	5.47	.02	5.47	.03	5.46	.20	.9769

TABLE 11

EFFECT OF MONENSIN LEVEL ON IN VITRO VFA PRODUCTION

	Monensin Level, ppm								
	-0-		-16.5-		-33-			LSD[1]	
µM/ml	Mean	SE	Mean	SE	Mean	SE	Prob.	P<.05	P<.10
Acetic	34.15[a]	.82	32.85[a]	.82	30.22[b]	.81	.0027	2.28	1.91
Propionic	14.05[a,A]	.42	15.17[a,b,B]	.42	16.08[b,B]	.42	.0029	1.17	.98
Iso-Butyric	.51	.15	.29	.15	.29	.15	.5059	----	----
Butyric	11.18[a]	.26	9.96[b]	.26	9.10[c]	.26	.0000	.73	.62
Iso-Valeric	1.78[a]	.08	1.61[a,b]	.08	1.52[b]	.08	.0771	.23	.19
Valeric	1.65	.08	1.82	.08	1.71	.08	.3592	----	----
Acetic/ Propionic	2.53[a]	.08	2.30[b]	.08	1.99[c]	.08	.0000	.22	.18
Total VFA	63.35[a]	1.51	61.66[a,b]	1.52	58.93[b]	1.50	.1131	4.22	3.53
Molar Percent									
Acetic	54.15[a]	.56	53.25[a]	.56	51.52[b]	.56	.0036	1.56	1.31
Propionic	22.20[a]	.36	24.64[b]	.36	27.32[c]	.36	.0000	1.01	.84
Iso-Butyric	.74	.21	.42	.21	.41	.21	.4608	----	----
Butyric	17.67[a]	.23	16.23[b]	.23	15.43[c]	.23	.0000	.63	.53
Iso-Valeric	2.71	.14	2.54	.14	2.49	.14	.4997	----	----
Valeric	2.52[a]	.13	2.92[b]	.13	2.82[b]	.13	.0743	.36	.30
pH	5.42[a]	.02	5.94[b]	.02	5.94[b]	.02	.0279	.06	.05

[a,b,c] P<.05
[A,B,C] P<.10

[1]LSD given only where significant differences (P<.10) between means exist.

decreasing VFA production with increasing monensin level (table 11) is in contrast to the above findings and is sufficient to more than offset the expected gain in predicted metabolizable energy production (table 12) due to the significant drop in acetate: propionate ratio with increasing monensin. Performing the same calculations, but using VFA molar percentages and holding total VFA production equal to the control level for the monensin treatments in accordance with the results of the above trials, one might expect .53 and 1.97 percent improvement in predicted metabolizable energy production for the 16.5 and 33 ppm monensin treatments, respectively.

TABLE 12

EFFECT OF MONENSIN LEVEL ON PREDICTED METABOLIZABLE ENERGY PRODUCTION

Monensin, ppm	Acid	millimoles/ml, Table 10	Heat of Combustion cal/millimole Blaxter (1962), Table 1	cal/ml	Calorimetric efficiency, Blaxter (1967), Table 52	Metabolizable cal/ml		Percent of Control
	Acetic	.03415	209.4	7.15	.592	4.23		
0	Propionic	.01405	367.2	5.16	.865	4.46		
	Butyric	.01118	524.3	5.86	.764	4.48		
						13.17		100.0
				18.17	.85[1]		15.44	100.0
	Acetic	.03285	209.4	6.88	.592	4.07		
16.5	Propionic	.01517	367.2	5.57	.865	4.82		
	Butyric	.00996	524.3	5.22	.764	3.99		
						12.88		97.8
				17.69	.85[1]		15.02	97.3
	Acetic	.03022	209.4	6.33	.592	3.75		
33.0	Propionic	.01608	367.2	5.90	.865	5.11		
	Butyric	.00910	524.3	4.77	.764	3.64		
						12.50		94.9
				17.00	.85[1]		14.45	93.6

[1]Average value for four combinations of the three VFA.

SUMMARY

The effects of soybean oil or corn oil at four levels (0, 2, 4, and 6 percent of the substrate) in combination with monensin sodium levels of 0, 16.5, and 33 ppm on *in vitro* gas production and composition, pH, and volatile fatty acid (VFA) production were studied. Total gas and methane production were lower ($P<.05$) for soybean oil than for corn oil treatments. Oil level had no significant effect on methane production, but methanogenesis was reduced ($P<.0001$) by the addition of monensin to the substrate. The combination of oil source and monensin level most effective ($P<.05$) in methane inhibition was that of soybean oil with 16.5 ppm monensin. Reduced production of acetate ($P<.10$), propionate, butyrate, iso-valerate, and total VFA was observed for the corn oil treatments compared with soybean oil ($P<.05$) and acetate: propionate ratio was greater ($P<.10$) with corn oil. Monensin at 33 ppm lowered production of acetic and iso-valeric acid and increased propionic acid production compared with controls ($P<.05$). Acetate: propionate ratio and butyric acid production were lowered with increasing monensin level ($P<.05$).

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EFFECT OF UNSATURATED FAT AND MONENSIN ON METHANE AND VFA PRODUCTION, IN VITRO

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The effects of soybean oil or corn oil at four levels (0, 2, 4, and 6 percent of the substrate) in combination with monensin sodium levels of 0, 16.5, and 33 ppm on *in vitro* gas production and composition, pH, and volatile fatty acid (VFA) production were studied. Total gas and methane production were lower ($P<.05$) for soybean oil than for corn oil treatments. Oil level had no significant effect on methane production, but methanogenesis was reduced ($P<.0001$) by the addition of monensin to the substrate. The combination of oil source and monensin level most effective ($P<.05$) in methane inhibition was that of soybean oil with 16.5 ppm monensin. Reduced production of acetate ($P<.10$), propionate, butyrate, iso-valerate, and total VFA was observed for the corn oil treatments compared with soybean oil ($P<.05$) and acetate: propionate ratio was greater ($P<.10$) with corn oil. Monensin at 33 ppm lowered production of acetic and iso-valeric acid and increased propionic acid production compared with controls ($P<.05$). Acetate: propionate ratio and butyric acid production were lowered with increasing monensin level ($P<.05$).