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Effect of different levels of monensin with cold process urea molasses mineral block on rumen fermentation *in vitro*

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ABSTRACT

Rumen fluid was collected in 2 periods from rumen fistulated cattle adapted with monensin (50 mg/d) for 0 (period I) and 21 (period II) days for *in vitro* microbial fermentation study using wheat straw (WS) and concentrate mixture (3:2, T_1), wheat straw and cold process urea molasses mineral block (UMMB) (6:1; T_2), in addition different level of monensin i.e., 35 ppm (T_3), 70 ppm (T_4), 100 ppm (T_5), 150 ppm (T_6) and 200 ppm (T_7) as substrates. When rumen fluid was used from steer not adapted with monensin, the digestibility of DM, NDF, ADF, and total gas production was significantly lower in all monensin treatments (i.e. T_3 T_4, T_5 T_6 and T_7) as compared to T_1 and T_2. On the other hand, when rumen liquor was used from monensin adapted steer the digestibility of DM, NDF, ADF, TVFA, and total gas production were not affected but propionate production significantly ($P<0.05$) increased and methane production significantly ($P<0.01$) decreased in all monensin treatments as compared to non-monensin treatments. But no significant difference in propionate proportion and methane production was observed among different levels of monensin treatments. So, it can be concluded that after 21 days of adaptation with monensin fibre degrading ability of rumen microbes is not affected and 35 ppm level of monensin was sufficient to reduce methane production and resulted in an increase production of propionate.

Key words: Animal nutrition, Fermentation, Mineral block, Rumen, Urea

As methane production is negatively correlated with energy utilization in ruminants (Orskov *et al.* 1968), many efforts have been put to inhibit its production and to divert hydrogen to produce more volatile fatty acids (VFA). Many compounds, viz. halogenated methane analogue, 9, 19, anthraquinone, co-enzyme- M analogue have been tested *in vitro* and *in vivo* an methane inhibitors (Czerkawsky and Breckenridge 1972, Martin and Macy 1985, Gracia Lopez *et al.* 1996). Monensin belongs to general class of compound termed polyether. Feeding monensin to cattle and sheep decreases the molar per cent of acetate, butyrate and methane and increase the molar percent of propionate in concentrate based diet (Potter *et al.* 1976, Boling *et al.* 1977, Dinious *et al.* 1978, Chalupa *et al.* 1980, Goodrich *et al.* 1984, Rumpler *et al.* 1986, Badawy *et al.* 1996, Mbanzaamihigo *et al.* 1996, Russel and Martin 1984). The fermentation rate of organic matter (OM) in low quality roughage is slow and fails to provide energy at a rate that could match the metabolic activities of the rumen microbes (Oldham *et al.* 1977). Supplementation of nitrogen, easily fermentable energy and minerals together in a block form, such as urea molasses mineral block (UMMB) lick improves fermentation of organic matter (Tiwari *et al.* 1990, Garg and Gupta 1991). In the present investigation different levels of monensin were added with UMMB to study whether incorporation of monensin in UMMB will improve the rumen fermentation and which level of monensin is optimum.

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MATERIALS AND METHODS

Effect of different levels of monensin (Table 1) with concentrate mixture, urea molasses mineral block and wheat straw on *in vitro* microbial fermentation were evaluated. Rumen fluid was collected from 3 rumen fistulated steers on 2 periods *i.e.* before supplementation of monensin and at 21 days of adaptation with monensin (50 mg/d). Steers were maintained on wheat straw and concentrate mixture (60:40) diet. Ruminal fluid was strained through 4 layer of cheese cloth under anaerobic condition. Rumen fluid collected from 3 steers were pooled and used for *in vitro* study.

In the *in vitro* study (Tilley and Terry 1963) 0.5 g substrate with different levels of monensin were incubated with 40 ml McDougall buffer (McDougall 1948) and 10 ml strained rumen liquor in conical flask fitted with rubber bung having bunsen valve. After passing enough anaerobic grade CO_2 (<2 ppm O_2) into the conical flask, it was kept in water bath having shaker for 48 hr incubation at 39°C. At the end of

Table 1. Substrates (0.5g) for *in vitro* experiment

Treatment	Substrate			
	Concentrate mixture (g)	UMMB (g)	Wheat straw (g)	Level of monensin (ppm)
T_1	0.20	-	0.30	0
T_2	-	0.07	0.43	0
T_3	-	0.07	0.43	35
T_4	-	0.07	0.43	70
T_5	-	0.07	0.43	100
T_6	-	0.07	0.43	150
T_7	-	0.07	0.43	200

incubation, 1 ml of 25% H_2SO_4 was added to arrest microbial fermentation. *In vitro* dry matter (DM), neutral detergent fibre (NDF) and acid detergent fibre (ADF) digestibility was determined in the sample by measuring DM, NDF and ADF content in the sample before and after the *in vitro* digestion (Goering and Van Soest 1970). The difference in the NDF and ADF content was considered as digested. The acidified rumen fluid was analysed for TVF (Barnett and Reid 1957) concentration and molar proportion of VFA by gas chromatography (Erwin *et al.* 1961).

Total gas production in different samples (0.5g) was measured by the gas tight 100 ml plastic syringe (Menke *et al.* 1979). Measurement of total gas production was done at 4, 8, 12, 24, 30, 36, 42 and 48 hr of incubation by observing the displacement of plunger of syringe. After 48 hr of incubation, proportion of methane in total gas was measured using gas chromatography. The composition of standard gas ran for comparison was ethylene 2%, propylene 1.2%, methane 27.4%, carbon dioxide 7.6%, ethane 1.1% and nitrogen 7.7%.

Experimental results were analyzed for statistical significance between treatments using randomized block design (Snedecor and Cochran 1986).

RESULTS AND DISCUSSION

Chemical composition of concentrate mixture, wheat straw and UMMB used in the present study are presented in Table 2. *In vitro* DM, NDF and ADF digestibility of different treatments at 0 and 21 days of adaptation with monensin has been shown in Table 3. *In vitro* DM, NDF and ADF digestibility of all monensin enriched treatments (T3, T4, T_6 and T_7) were significantly ($P<0.01$) lower, except NDF digestibility in T_3 (35 ppm) which is equivalent to T_1 and T_2, than treatments without monensin (T_1 and T_2) in period I when rumen fluid was taken from animals not adapted to monensin, but there was no significant difference between T_1 and T_2. In period II, when donor animals were adapted to monensin for 21 days, no significant difference in DM, NDF and ADF digestibility was observed between treatments.

Table 2. Chemical composition of different feeds (%DM)

Treatment	Concentrate mixture[1]	Wheat	UMMB[2]
Dry matter	89.62	87.15	84.91
Organic matter	92.93	90.45	71.13
Crude protein	20.10	3.44	38.38
Ether extract	5.38	0.68	0.39
Neutral detergent fibre	50.20	80.07	17.58
Acid detergent fibre	16.17	49.74	7.49

[1]Composition of concentrate mixture; maize 320 g, kg^{-1}, groundnut-cake 350 g kg^{-1}, wheat bran 300 g kg^{-1}, mineral mixture 25 g kg^{-1} and salt 5 g kg^{-1}. [2]Composition of urea molasses mineral block (UMMB): molasses 380 g kg^{-1}, urea 100 g kg^{-1}, salt 50 g kg^{-1}, mineral mixture 60 g kg^{-1}, sodium bentonite 40 g kg^{-1}, calcium oxide 80 g kg^{-1}, deolied rice bran 190 g kg^{-1} and cotton seed-cake 100 g kg^{-1}.

In period I, TVFA production in T_1 and T_2 (Table 4) was apparently, though, statistically not significant, higher than other treatments. In period II, no significant difference in TVFA production was observed among treatments.

Acetate molar per cent was not affected due to treatment in period I (Table 4). In period II, acetate molar per cent in all monensin added treatments was lower, though statistically not significant that T_1 and T_2 molar per cent of propionate was not significantly different among treatments in period I, but this difference became significant ($P<0.05$) when monensin was added to UMMB. Different levels of monensin addition to UMMB did not show any significant difference in molar per cent of propionate among monensin added treatments i.e. T_3 to T_7 molar per cent of butyrate were not affected due to treatment effect in period I as well as in period II.

Gas production after 48 hr of incubation in T_1 was significantly ($P<0.01$) higher as compared to T_3, T_4, T_5, T_6 and T_7 similar to T_2 in period I (Table 4). However, difference between T_2, T_4, T_6; T_3, T_6 and T_7 and between T_4, T_5 and T_7 were not significant. In period II cumulative gas production was not affected due to treatments.

Methane per cent in total gas (Table 4) did not differ significantly among treatment. In period II, methane production was significantly ($P<0.01$) lower in all UMMB treatments (T_2 to T_7) either with or without monensin as compared to concentrate treatment, i.e. T_1 Again among UMMB treatments, methane production was significantly ($P<0.01$) lower in T_3 and T_7 as compared to T_2. However, methane production among all levels of monensin treatments did not differ significantly.

In vitro studies indicated that UMMB was supplemented with wheat straw, DM, NDF and ADF digestibility were at par to that of concentrate mixture supplemented wheat straw. *In vitro* study using rumen fluid obtained from animal not previously exposed to monensin i.e., period I showed marked

Table 3. Effect of different levels of monensin on *in vitro* DM, NDF and ADF digestibility at different days of adaptation

	T_1 (0 ppm)	T_2 (0 ppm)	T_3 (35 ppm)	T_4 (70 ppm)	T_5 (100 ppm)	T_6 (150 ppm)	T_7 (200 ppm)	Level of significance
Period I (not adapted with monensin)								
Digestibility (%)								
DM	66.00^{b}	65.33^{b}	52.00^{a}	50.67^{a}	50.67^{a}	51.33^{a}	53.33^{a}	**
	±3.46	±1.33	±0.00	±2.40	±1.33	±2.40	±1.33	
NDF	56.33^{b}	57.01^{b}	52.90^{b}	44.90^{a}	44.68^{a}	43.79^{a}	41.34^{a}	**
	±1.38	±1.54	±0.89	±0.58	±1.54	±0.89	±3.08	
ADF	49.21^{d}	51.04^{d}	41.39^{c}	40.35^{bc}	41.39^{c}	35.53^{ab}	30.69^{a}	**
	±2.58	±1.20	±0.00	±0.75	±2.39	±2.39	±2.39	
Period II (21 days of adaptation with monensin 50 mg/d)								
Digestibility (%)								
DM	46.00	46.00	44.67	48.67	44.00	44.67	49.33	NS
	±0.00	±2.00	±1.76	±1.76	±4.62	±2.91	±2.67	
NDF	57.86	56.25	56.25	59.79	53.94	56.10	52.20	NS
	±0.00	±3.33	±3.33	±1.60	±5.59	±3.33	±1.69	
ADF	52.62	51.07	50.79	54.20	51.94	52.33	48.77	NS
	±2.39	±1.96	±1.99	±0.72	±2.30	±2.30	±2.21	

DM, Dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; NS, nonsignificant; **P<0.01; a,b,c,d, values bearing different superscript in a row differ significantly.

Table 4. Effect of different levels of monensin on *in vitro* TVFA(m mol/dl), total gas (ml/0.5g substrate/48 hr), methane (%) and proportion of individual VFA production

	T_1 (0 ppm)	T_2 (0 ppm)	T_3 (35 ppm)	T_4 (70 ppm)	T_5 (100 ppm)	T_6 (150 ppm)	T_7 (200 ppm)	Level of significance
Period I (not adapted with monensin)								
TVFA	9.35	9.80	8.25	8.51	8.50	8.03	7.63	NS
	±0.28	±0.60	±0.45	±0.53	±0.45	±0.90	±0.20	
Acetate	57.57	61.81	60.74	59.93	58.73	60.16	57.99	NS
	±0.98	±1.65	±0.30	±0.09	±1.20	±0.37	±1.95	
Propionate	35.06	31.39	34.00	33.63	34.82	34.21	35.34	NS
	±0.79	±1.21	±0.46	±0.21	±1.66	±1.26	±1.22	
Butyrate	7.37	6.80	5.27	6.44	6.44	5.64	6.67	NS
	±0.19	±0.44	±0.68	±0.12	±0.96	±1.05	±0.74	
Total gas	49.50^{d}	45.00^{cd}	34.00^{ab}	39.67^{bc}	29.33^{a}	39.67^{c}	33.50^{ab}	**
	±0.29	±0.58	±1.53	±3.33	±1.33	±3.18	±3.04	
Methane	25.84	24.70	19.81	24.16	22.42	21.02	21.06	NS
	±0.75	±0.06	±0.84	±2.08	±1.17	±3.49	±0.48	
Period II (21 days of adaptation with monensin 50 mg/d)								
TVFA	10.82	10.33	10.33	9.82	9.22	8.77	9.67	NS
	±0.07	±0.88	±0.83	±1.07	±0.39	±0.39	±0.65	
Acetate	57.48	55.39	51.53	50.28	50.13	49.86	48.39	NS
	±1.75	±1.48	±0.64	±1.45	±0.06	±4.39	±2.39	
Propionate	33.85^{a}	36.67^{ab}	41.03^{bc}	40.88^{bc}	42.15^{bc}	44.77^{c}	42.99^{bc}	*
	±0.41	±2.01	±0.64	±1.50	±0.06	±3.76	±3.97	
Butyrate	8.70	7.98	7.44	8.84	7.72	5.37	8.59	NS
	±1.36	±0.53	±0.00	±0.05	±0.00	±0.68	±1.98	
Total gas	43.00	37.33	34.83	35.67	41.17	29.00	31.33	NS
	±5.67	±4.67	±5.36	±2.03	±4.60	±2.31	±2.40	
Methane	27.88^{a}	24.72^{d}	25.17^{c}	25.81^{b}	26.07^{b}	25.76^{b}	25.48^{c}	**
	±0.54	±1.16	±0.32	±0.28	±0.53	±0.14	±0.08	

TVFA, Total volatile fatty acid; NS nonsignificant; *P<0.05, **P<0.01; a,b,c, values bearing different superscripts in a row differ significantly.

inhibition of DM and cell wall digestibility in monensin added group when rumen microbes were suddenly exposed to monensin (Simpson 1978, 1980). However, rumen fluid obtained from animals adapted to monensin for 21 days i.e. period II shows similar DM and cell wall digestibility. After 21 days of monensin feeding rumen microbes might have been adapted sufficiently to mask any decrease in DM of fibre digestibility due to changes in microbial population.

Apparently lower TVFA production in all monensin treatments in period I might be due to sudden shock of monensin to rumen microbes but, no difference in TVFA production was observed when rumen microbes are already adapted (Davis *et al.* 1976, Potter *et al.* 1976, Richardson *et al.* 1976, Lemenager *et al.* 1978, Ricke *et al.* 1974, Bogaert *et al.* 1991, Rogers *et al.* 1991, Haimoud *et al.* 1995).

When rumen fluids were obtained from monensin adapted animal, propionate production was higher in monensin enriched UMMB treatments but acetate and butyrate proportion did not differ significantly. This increase in propionate proportion could be due to selection for succinate forming *Bacteriodes* and for *Selenomonas ruminatium*, a propionate producer that decarboxylates succinate to propionate, which could lead to an increase in propionate formation. Slight decrease in acetate proportion might be due to selection against H_2 and formate producer, *Ruminococcus albus*, *R. flavifaciens* and *Butyrivibrio fibrisolvens* which produces acetate and butyrate after fermentation of carbohydrate (Chen and Wolin 1979).

Significant reduction in gas production in all monensin treatments in period I could be due to same reason as discussed in case of TVFA production. Result indicated that in period II when animals were adapted to monensin there was no significant difference in gas production. Gas production can serve as an index of rumen microbial activity. These results thus indicated that rumen microbial activity was not adversely affected due to monensin treatments as no difference in gas production was observed after 21 days of adaptation with monensin. Methane production in period I though lower in all monensin treatments but differences were not statistically significant, this might be due to high standard error. Methane production in period II was negatively correlated with propionate production. This was because, monensin helps to increase propionate formation by utilizing hydrogen moleculer rather than diverting it for methane production. Monensin also helps in selection against hydrogen and formate producers which could lead to depress methane production (Chen and Wolin 1979).

So, it can be concluded from this study that adaptatin of donor animals to monensin for 21 days reduces the inhibitory effect of monensin to fibre degrading microbes but increases the propionate production by reducing the methane production. 35 ppm monensin level was sufficient to reduce methane production and diverting it for propionate production as there was no significant difference in propionate production and methane production with the increase level of monensin.

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