Effect of Different Level of Monensin Supplemented with Cold Process Urea Molasses Mineral Block on *In vitro* Rumen Fermentation at Different Days of Adaptation with Monensin

Debasis De[1] and G. P. Singh*

Division of Dairy Cattle Nutrition, National Dairy Research Institute, Karnal: 132001 (Haryana), India

ABSTRACT : Effect of period of adaptation and levels of monensin were studied for microbial fermentation/ digestibility to find out the optimum period of adaptation of monensin in rumen and suitable level of monensin in wheat straw+concentrate and wheat straw+ UMMB diet. The mean digestibility of dry matter was decreased upto T-3 treatment (49.17%), however, digestibility of DM was affected upto period (P-2). NDF digestibility was affected due to treatment under P1 and P2 ($p<0.05$). Average digestibility of ADF was increased to 53.33% at T-3 level of monensin and P4 days of adaptation. TVFA (mmole/100 ml) were decreased from 9.49 in T-1 to 7.70 in T-7. Periods were not effectives except P2 (14 days of adaptation). Similarly, total gas was decrease with the increase of monensin levels in diet. Although acetate percentage in TVFA was not affected either due to level of monensin or period of adaptation but propionate was increased due to increase in monensin at 21 days of adaptation (P-3). Butyrate (%) was decreased significantly in T-2 to T-6 as compared to T-1 group. Total gas was significantly ($p<0.01$) higher in group T-1 (control) and it reduced significantly in T-5, however, differences in gas production between group T-3, T-5 and T-7 at P-1 was not significant. Methane production was reduced on P-3 and P-4 level of adaptation due to treatment. The overall result indicated that 21 days of adaptation with monensin was sufficient to mask the inhibiting effect of monensin to cell wall digestibility and 35 ppm monensin is optimum to reduce methane production and increase propionate productions. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 320-325)***

Key Words : Monensin, UMMB, Rumen Fermentation, *In vitro*

INTRODUCTION

During the last two decades, a number of active compounds have been discovered that when fed, can further improve the efficiency of production by increasing propionate production and decreasing methane production in ruminants. One such class of the compounds is Carboxylic poly ether antibiotics called ionophore. Monensin, one of the large scale uses of ionophores in farm animal, is produced by Streptomyces cinnamonensis (Honey and Hoehn, 1967; Bergen and Bates 1984). Feeding of monensin improved the rumen fermentation, daily gain and depressed filre digestibility in cattle (Singh 1997; Singh and Mohini, 1999; De and Singh, 2003; Owimer et al., 2003, Wang et al., 2003). However, the precise information on the dose of monensin and adaptation time in rumen are lacking on feeding of crop residue based ration. Therefore, an attempt was made to determine the adaptation period and the optimum level monensin for better rumen fermentation, when fed in cold processed urea molasses mineral block.

* Corresponding Author: G. P. Singh. National Research Centre on Camel, Jorbeer, P.B.No. 07, Bikaner: 334001 (Rajasthan), India. Tel: +91-151-2230187, Fax: +91-151-2230183, E-mail: gps @scientist.com

[1] ICAR Research Complex for NEH Region, Sikkim Centre, Tadong, Gangtok: 737102 (Sikkim), India.

Received February 16, 2004; Accepted July 22, 2004

MATERIALS AND METHODS

Effect of different levels of monensin on *in vitro* microbial fermentation was studied using concentrate mixture, UMMB and wheat straw as substrate (Table 1). Rumen liquor was collected from three fistulated steers on four periods, i.e. before supplementation of monensin (P I) and after 14 d (P II), 21 d (P III) and 35 d (P IV) of adaptation with monensin (50 mg/d). Steers were maintained with wheat straw and concentrate mixture (maize grain 320 g kg^{-1}, ground-nut cake 350 g kg^{-1}, wheat bran 300 g kg^{-1}, mineral mixture 25 g kg^{-1} and salt 5 g kg^{-1}) (60:40) to meet the maintenance requirement as per Kearl (1982). Rumen liquor was collected by plastic tube through permanent rumen fistula and brought through pre-gassed (CO_2) and autoclaved flat bottom flask. A cloth strainer was used during collection of rumen liquor for straining in case of bacterial count. The flask containing rumen liquor was kept in a thermostatic bucket containing water at 39±1°C. Rumen liquor of three steers were pooled and used for *in vitro* studies.

In *in vitro* experiment (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 CO_2 (<2 ppm O_2) in to conical flask it was kept for incubation at 39°C in water bath having stirrer facility for

Table 1. Substrate for *in vitro* incubation

Treatment	Substrate
T1	Wheat straw+concentrate mixture (3:2)
T2	Wheat straw+UMMB (6:1)
T3	Wheat straw+monensin (35 ppm) enriched UMMB (6:1)
T4	Wheat straw+monensin (70 ppm) enriched UMMB (6:1)
T5	Wheat straw+monensin (100 ppm) enriched UMMB (6:1)
T6	Wheat straw+monensin (150 ppm) enriched UMMB (6:1)
T7	Wheal straw+monensin (200 ppm) enriched UMMB (6:1)

48 h. After 48 h of incubation 1.0 ml of 25% H_2SO_4 was added to arrest microbial fermentation.

Dry matter, cell wall digestibility and volatile fatty acid: *In vitro* DM, NDF and ADF digestibility was determined in the sample by measuring the difference of DM (AOAC, 1984), NDF and ADF content (Vansoest et al., 1991) before and after *in vitro* digestion of sample. Rumen fluid was analyzed for TVFA concentration (Bernett and Reid, 1957) and molar proportion of individual VFA (Erwin et al., 1961) after 48 h of incubation.

Total gas and methane

Total gas production in different samples (0.5 g) was measured by the gas tight plastic syringe of 100 ml capacity (Menke et al., 1979). Measurement of total gas production was done at 4, 8, 12, 18, 24, 30, 36, 42 and 48 h of incubation by observing the displacement of plunger of syringe. Proportion of methane in total gas was measured after 48 h of incubation 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%.

Table 2. Chemical composition of feeds (% DM)

	Concentrate mixture[2]	Wheat straw	UMMB[3]
Dry matter	89.62	87.15	84.91
Organic matter	92.93	90.15	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
Calcium	0.73	0.14	3.95
Phosphorus	0.59	0.09	1.62

[1] Values represent hexaplicate assays of each material.

[2] Composition of concentrate mixture: Maize 320 g kg^{-1}, ground nut cake 350 g kg^{-1}, wheat bran 300 g kg^{-1}, mineral mixture 25 g kg^{-1} and salt 5 g kg^{-1}.

[3] 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}, deoiled rice bran 190 g kg^{-1} and cotton seed cake 100 g kg^{-1}.

Statistical analysis

Data were analyzed statistically in two way analysis of variance (Snedecor and Cochran, 1986), using following model:

$$Y_{ij} = \mu+t_i+p_j+e_{ij}$$

Where Y_{ij} is the observation on the i^{th} treatment in j^{th} period

Table 3. Effect of different level of monensin on *in vitro* digestibility at different days of adaptation with monensin

Period[1]	Treatments[2]							SEM
	T1	T2	T3	T4	T5	T6	T7	
DM digestibility (%)								
P 1**	66.00^{b}	65.33^{b}	52.00^{a}	50.67^{a}	50.67^{a}	51.33^{a}	53.33^{a}	2.16
P 2**	60.00^{e}	51.33^{cd}	48.67^{bc}	52.67^{d}	49.33^{bcd}	46.00^{ab}	44.00^{a}	1.17
P 3	46.00	46.00	44.67	48.67	44.00	44.67	49.33	2.62
P 4	51.33	52.00	51.33	50.67	46.67	45.33	48.67	1.65
Mean**	55.83^{c}	53.67^{c}	49.17^{ab}	50.67^{b}	47.67^{a}	46.833^{a}	48.83^{ab}	0.95
NDF digestibility (%)								
P 1**	56.33^{b}	57.01^{b}	52.90^{a}	44.90^{a}	44.68^{a}	43.79^{a}	41.34^{a}	1.75
P 2 *	53.84^{ab}	59.19^{c}	54.49^{abc}	51.67^{a}	53.55^{ab}	58.25^{bc}	56.52^{abc}	1.57
P 3	57.86	56.25	56.25	59.79	53.94	56.10	52.20	3.25
P 4	51.85	53.55	55.43	56.37	59.19	59.19	58.25	0.95
Mean	54.97	56.50	54.77	53.18	52.84	54.33	52.08	1.12
ADF digestibility (%)								
P 1**	49.21^{d}	51.04^{d}	41.39^{c}	40.35^{bc}	41.39^{c}	35.53^{ab}	30.69^{a}	1.90
P 2	49.12	52.83	51.22	48.53	48.29	54.14	52.64	1.67
P 3	52.62	51.07	50.79	54.20	51.94	52.33	48.77	2.17
P 4**	48.95^{a}	50.88^{b}	53.33^{c}	52.68^{c}	52.61^{c}	53.00^{c}	50.06^{ab}	0.51
Mean**	49.97^{bc}	51.45^{c}	49.18^{bc}	48.94^{b}	48.56^{b}	48.75^{b}	45.54^{a}	0.83

[a, b, c, d] Values bearing different superscripts in a row differ significantly (**p<0.01, *p<0.05).

[1] P 1: Rumen liquor taken from animal not adapted with monensin, P 2: Rumen liquor taken from animal adapted with monensin feeding for two weeks, P 3: Rumen liquor taken from animal adapted with monensin feeding for three weeks, P 4: Rumen liquor taken from animal adapted with monensin feeding for five weeks.

[2] See Table 1.

Table 4. Effect of different level of monensin on *in vitro* molar proportion of individual VFA at different days of adaptation with monensin

Period[1]	Treatments[2]							SEM
	T1	T2	T3	T4	T5	T6	T7	
Acetate (%)								
P 1	57.57	61.81	60.74	59.93	58.73	60.16	57.99	1.23
P 2	54.99	51.76	56.49	54.57	60.23	57.76	58.49	2.29
P 3	57.48	55.39	51.53	50.28	50.13	49.86	48.39	1.98
P 4	52.61	51.26	50.46	53.05	52.25	50.69	53.61	3.57
Mean	55.66	55.06	54.81	54.46	55.34	54.62	54.62	1.20
Propionate (%)								
P 1	35.06	31.39	34.00	33.63	34.82	34.21	35.34	1.16
P 2	38.97	40.78	39.45	40.73	36.55	37.49	33.32	2.48
P 3*	33.85^{a}	39.67^{ab}	41.03^{bc}	40.88^{bc}	42.15^{bc}	44.77^{c}	42.99^{bc}	2.08
P 4	41.68	43.35	46.44	43.90	43.51	43.59	41.93	3.50
Mean	37.39	38.05	40.23	39.79	39.26	40.01	38.40	1.23
Butyrate (%)								
P 1	7.37	6.80	5.27	6.44	6.44	5.64	6.67	0.70
P 2**	5.94^{bc}	7.46^{c}	4.06^{a}	4.70^{ab}	3.43^{a}	4.76^{ab}	8.16^{d}	0.56
P 3	8.70	7.98	7.44	8.84	7.72	5.37	8.59	1.04
P 4**	5.82^{b}	5.39^{b}	3.09^{a}	3.04^{a}	4.24^{ab}	5.72^{b}	4.46^{ab}	0.50
Mean**	6.93^{b}	6.91^{b}	4.96^{a}	5.76^{b}	5.46^{a}	5.37^{a}	6.97^{b}	0.36

[a, b, c, d] Values bearing different superscripts in a row differ significantly (**p<0.01. *p<0.05).

[1] P 1: Rumen liquor taken from animal not adapted with monensin, P 2: Rumen liquor taken from animal adapted with monensin feeding for two weeks, P 3: Rumen liquor taken from animal adapted with monensin feeding for three weeks: P 4: Rumen liquor taken from animal adapted with monensin feeding for five weeks.

[2] See Table 1.

μ is the overall mean; t_i is the effect due to i^{th} treatment. p_j is the effect due to j^{th} period; e_{ij} is random error.

RESULTS

In vitro DM, NDF and ADF digestibility

In vitro DM digestibility (Table 3) were higher (p<0.01) in T1 and T2 than that of monensin enriched treatments (i.e. T3, T4, T5, T6 and T7) in P I, when rumen liquor was taken from animals not adapted to monensin. In P II, when donor animals were adapted to monensin for 14 d, DM digestibility of monensin (up to 100 ppm level) enriched UMMB treatments was at per with UMMB without monensin treatment (T2), and beyond 100 ppm level DM digestibility decreased. In P III and P IV, when donor animals were adapted to monensin for 21 d and 35 d, respectively, no significant (p>0.05) difference in DM digestibility was observed between treatments. However, when overall mean was considered OM digestibility was lower (p<0.01) in monensin enriched treatments than that of treatments without monensin.

Neutral detergent fibre digestibility (Table 3) of Tl and T2 was similar with T3 i.e. when 35 ppm monensin was added. But beyond 35 ppm level NDF digestibility was lower (p<0.01) in PI. In P II, NDF digestibility of UMMB without monensin treatment was higher (p<0.05) as compared to that of concentrate mixture without monensin. No significant difference was observed between all monensin enriched treatments (T3, T4, T5, T6 and T7) and T1. In P III and P IV, no significant (p>0.05) difference in NDF digestibility was observed between different treatments. No significant difference in NDF digestibility was also when overall mean of different treatments were considered. But average NDF digestibility of P I was lower (p<0.01) than that of other periods (i.e. P II, P III and P IV).

Acid detergent fibre digestibility Cfable 3) of monensin non-supplemented treatments (i.e. T1 and T2) was higher (p<0.01) than that of monensin enriched treatments in P I. But, it did not differ significantly between treatments in P II and P III. However, in P IV, NDF digestibility of monensin (up to 150 ppm) enriched treatments were higher (p<0.01) than that of monensin non-supplemented treatments. When overall mean of different treatments were considered it was found that up to 35 ppm monensin level ADF digestibility was similar with monensin non-supplemented treatments. However, average ADF digestibility in P I was lower (p<0.01) than that of other periods.

Total volatile fatty acids production and molar proportion of individual VFA

In P I, P II and P III, no significant difference in TVFA production (Table 5) among treatments was observed. In P IV, TVFA production was higher (p<0.01) in T1 and T2 as compared to that of T5, T6 and T 7, but similar to T3 and T4. However, when mean of four periods were considered, it was found that TVFA production was similar in T1 and

Table 5. Effect of different level of monensin on *in vitro* TVFA, total gas and methane production at different days of adaptation with monensin

Period[1]	Treatments[2]							SEM value
	T1	T2	T3	T4	T5	T6	T7	
TVFA (mmole/100 ml)								
P 1	9.35	9.80	8.25	8.51	8.50	8.03	7.63	0.51
P 2**	9.05^{b}	8.68^{b}	8.55^{b}	8.68^{b}	7.47^{a}	6.98^{a}	6.80^{a}	0.34
P 3	10.82	10.33	10.33	9.82	9.22	8.77	9.67	0.69
P 4	8.73	8.42	8.27	6.93	7.53	8.37	6.70	0.59
Mean**	9.49^{c}	9.31^{c}	8.85^{bc}	8.49^{ab}	8.18^{ab}	8.04^{a}	7.70^{a}	0.28
Total gas (ml/0.5 g substrate)								
P 1**	49.50^{d}	45.00^{cd}	34.00^{ab}	39.67^{bc}	29.33^{a}	39.67^{bc}	33.50^{ab}	2.30
P 2	44.00	39.33	39.00	38.67	31.67	36.33	32.67	3.48
P 3	43.00	37.33	34.83	35.67	40.17	29.00	31.33	4.36
P 4	42.00	33.83	35.00	45.00	39.33	35.17	29.33	4.62
Mean**	44.63^{c}	38.88^{b}	35.71^{ab}	39.75^{bc}	35.13^{ab}	35.04^{ab}	31.71^{a}	1.83
Methane (%)								
P 1	25.84	24.70	19.81	24.16	22.42	21.02	21.06	1.53
P 2	25.59	25.23	24.51	25.89	26.31	26.24	26.67	0.61
P 3**	27.88^{c}	26.72^{b}	25.17^{a}	25.81^{ab}	26.07^{ab}	25.76^{ab}	25.48^{a}	0.36
P 4**	26.67^{d}	25.34^{cd}	22.92^{a}	24.74^{bc}	25.19^{bc}	24.34^{bc}	24.86^{b}	0.34
Mean**	26.49^{c}	25.50^{bc}	23.10^{a}	25.15^{b}	24.99^{b}	24.34^{ab}	24.52^{b}	0.44

[a,b,c,d] values bearing different superscripts in a row differ significantly (**p<0.01, *p<0.05).

[1] P 1: rumen liquor taken from animal not adapted with monensin, P 2: rumen liquor taken from animal adapted with monensin feeding for two weeks, P 3: rumen liquor taken from animal adapted with monensin feeding for three weeks, P 4: rumen liquor taken from animal adapted with monensin feeding for five weeks.

[2] See Table 1.

T2. Even incorporation of 35 ppm monensin (T3) in UMMB did not affect TVFA production. But when monensin dose was 70 ppm (T4) and 100 ppm (T5), TVFA production was lower ($p<0.01$) than TI and T2 and similar with T3. However, monensin level beyond 100 ppm, i.e., at 150 ppm and 200 ppm, TVF A production was lower ($p<0.01$) than that of other treatments. Average TVFA production in different periods though differed ($p<0.01$) but did not show any definite trend.

Acetate molar percent (Table 4) was not affected due to treatment in different periods. When overall mean was considered, no significant differences were also observed among treatments. However, significant ($p<0.01$) and definite declining trend in acetate molar percent was observed as the days of adaptation with monensin increased. Acetate molar percent was higher ($p<0.01$) in P I as compared to P II, which was again higher ($p<0.01$) than that of P III and P IV. However, no significant difference in acetate molar percent was observed between P III and P IV.

Molar percent of propionate (Table 4) did not differ significantly among the treatments in P I, P II and P IV. However, in P III, propionate percent was higher in the UMMB groups, which was further increased when UMMB was treated with monensin. But, propionate molar percent in all monensin treatments was similar ($p>0.05$). Propionate molar percent was apparently higher ($p>0.05$) in all monensin treatments though not significant when overall mean was considered. There was increasing trend in propionate molar percent with the increase of days of adaptation with monensin.

No significant difference in butyrate molar percent (Table 4) among treatments was observed in P I and P III. However, it differed significantly among treatments in P II and P IV. When overall mean of butyrate percent of different treatment was considered it was found that butyrate molar percent was lower ($p<0.01$) in all monensin enriched UMMB treatments except 200 ppm monensin level. There was a decreasing trend in butyrate percent except in P III as days of adaptation with monensin increasing. A/P ratio in T1 to T7 was 1.49, 1.45, 1.36, 1.37, 1.41, 1.37 and 1.42, respectively. It was less in all monensin enriched treatments and least in 35 ppm monensin enriched UMMB treatment.

Total gas production

In P I, total gas production was higher ($p<0.01$) in T1 as compared to that of mmonensin enriched UMMB treatments (Table 5). In P II, P III and P IV, total gas production was not affected ($p>0.05$) due to treatments.

In P I, total gas production was relatively slower in first 24 h than next 24 h in all the treatments. Gas production in P II, P III and P IV was higher in first 24 h than the next 24 h. However, in all UMMB treatments, either with or without mmonensin, gas production was less in first 24 h as compared to the next 24 h. When average gas production of different treatments of all the four period was considered, it was observed that gas production of T1 was higher ($p<0.01$) than that of all UMMB treatments except at 70 ppm

monensin level. No difference ($p>0.05$) in total gas production was observed due to period effect.

Methane production

Methane production (%) did not differ ($p>0.05$) among treatments in P I and P II. But in P III, methane production was lower ($p<0.01$) in all UMMB treatments (T2 to T7) either with or without monensin as compared to that of concentrate mixture treatment i.e., T1. Again, among UMMB treatments, methane production was lower ($p<0.01$) in all monensin enriched treatments as compared to that of UMMB without monensin treatment (Table 5). In P IV, methane production in T1 was higher ($p<0.01$) than that of all monensin enriched UMMB treatments (i.e. T3 to T7) but similar to T2 i.e. UMMB without monensin treatment. Among monensin enriched UMMB treatments, methane production was lower ($p<0.01$) at 35 ppm monensin level. However, no significant difference in methane production was observed among 70, 100, 150 and 200 ppm monensin level. When overall means of four periods were considered, it was found that methane production was lowest ($p<0.01$) at 35 ppm monensin enriched UMMB treatment and it was higher ($p<0.01$) in concentrate supplemented wheat straw treatment as compared to that of all monensin enriched UMMB supplemented wheat straw treatments. Average methane production (%) of P I was lower ($p<0.01$) as compared to that of P II, P III and P IV. Methane production in P II and P III, and P II and P IV did not differ significantly.

Discussion

In vitro studies indicated that when UMMB was supplemented with wheat straw, DM, NDF and ADF digestibility were similar to that of concentrate mixture supplemented wheat straw. *In vitro* study using rumen fluid from animals not previously exposed to monensin shows that the marked inhibition of DM and cell wall digestibility occurred when rumen microbes are suddenly exposed to monensin (Simpson, 1978, 1980). However, *in vitro* study using rumen fluid from animals adapted to monensin for 14 days (i.e. P II) shows that DM digestibility was higher ($p<0.01$) in T1. NDF digestibility was higher ($p<0.05$) in T2 as compared to other treatments, but no difference ($p>0.05$) in ADF digestibility was observed among treatments. In P III and P IV, similar DM and cell wall digestibility were observed except for ADF digestibility in P IV, where ADF digestibility was higher ($p<0.01$) in monensin enriched UMMB treatments as compared to that of treatments without monensin supplementation. Similar DM and cell wall digestibility in all treatments in P III and P IV might be due to the fact that after 14 d of monensin feeding rumen microbes might have been adapted sufficiently to mask any decrease in DM or fibre digestibility caused due to sudden exposure to monensin. The increase in ADF digestibility in P IV could be due to increase in ionophore resistant cellulose digesting bacteria (e.g. *Fibrobacter succinogens)* when animals were adapted to monensin. *In vitro* results indicated that up to 35 ppm monensin level there was no reduction in TVFA production as compared to treatments without monensin. But beyond 35 ppm monensin level TVFA production reduced. This reduction in TVFA production might be due to inhibition of microbial activity. Acetate molar percent did not differ significantly. Average molar percent of propionate was apparently higher ($p>0.05$) in all monensin enriched treatments. It was 5.73 and 7.60 percent higher in 35 ppm monensin enriched UMMB treatment when compared with UMMB without monensin and concentrate mixture without monensin treatment, respectively. Similar result was reported by Zinn et al. (1994), who reported 9.40 percent increase in propionate percent due to monensin supplementation. Average butyrate molar percent was lower ($p<0.01$) in monensin enriched treatments except at 200 ppm monensin level. Decreased butyrate molar percent due to monensin was also reported in earlier works (Potter et al., 1976; Richardson et al., 1976; Boling, 1977; Dinius et al., 1978; Goodrich et al., 1984; Beever et al., 1987). The slight increase in propionate proportion and significant decrease in butyrate proportion could be due to selection for succinate forming *Fibrobacter succinomonas* and for *Selenomonas ruminatium*, a propionate producer that decarboxylates succinate to propionate which could lead to an increase in rumen propionate formation and selection against hydrogen and formate producer. *Ruminococcus albus*, *R. flavifaciens* and *Butyrivibrio fibrisolvens*, which produce acetate and butyrate after fermentation of carbohydrate (Chen and Wolin, 1979).

Reduction in average gas production of four periods in monensin enriched UMMB treatments was due to mark reduction in gas production of monensin enriched treatments in P I as microbes in the rumen liquor experienced monensin treatment for the first time. Another reason for comparatively lower gas production in all UMMB treatments (either with or without monensin) might be addition of 15 percent more straw as substrate in all UMMB treatments (T2 to T7) as compared to concentrate mixture treatment (T1), assuming that animal consume 15 percent more straw when UMMB was supplemented as compared to that of concentrate supplementation (Mohini, 1991). Results indicated that when animals were adapted to monensin since then there were no significant differences in gas production after 42 h of incubation. 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 treatment as no difference in gas production was observed after 14 d of adaptation with monensin.

Reduction in methane production in monensin enriched

treatments could be due to reason as described for increase in propionate and decrease in butyrate molar percent. Monensin helps in utilization of hydrogen molecule for propionate formation rather than diverting it for methane production. It also reduce hydrogen or formate production by depressing the bacteria responsible for it and ultimately reduce methane production.

So. it can be concluded that 21 d adaptation to the donor cattle with monensin can mask the inhibitory effect of monensin on *in vitro* DM and fibre digestibility and VFA production and 35 ppm monensin level is optimum when supplemented with UMMB to increase *in vitro* propionate production and reduce butyrate and methane production.

REFERENCE

Barnett, A. J. G. and R. L. Reid. 1957. Studies on the production of volatile fatty acid production from tiesh grass. J. Agric. Sci. Camb. 48:315.

Beever, D. E., H. R. Losada, D. L. Gala, M. C. Spooner and M. S. Dhanoa. 1987. The use of monensin or formaldehyde to control the digestion of the nitrogenous constituents of perennial rye grass and white clover in the rumen of cattle. Br. J. Nutr. 57:57-67.

Bergen, W. G. and D. B. Bates. 1984. Ionophores: Their effect on production efficiency and mode of action. J. Anim. Sci. 58:1465-1483.

Boling, J. A., N. W. Bradley and L. D. Campbell. 1977. Monensin levels for grazing and finishing steers. J. Anim. Sci. 44:867-871.

Chen, M. and M. J. Wolin. 1979. Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen Saccharolytic bacteria. Appl. Environ. Appl. Microbial. 38:72-77.

Debasis De and G. P. Singh. 2003. Effect of ionophore enriched cold processed mineral block supplemented with urea molasses on rumen fermentation and microbial growth in cross bred cattle. Asian-Aust. J. Anim. Sci. 16:852-862.

Dinius, D. A., H. K. Goering, R. R. Oltjen and H. R. Cross. 1978. Finishing beef steerson forage diets with additives and supplemental lipids. J. Anim. Sci. 46:761-768.

Dawson, K. A. and J. A. Boling. 1983. Monensin-resistant bacteria in the rumens of calves on monensin containing and unmedicated diets. Appl. Environ. Microbial. 46:160-164.

Erwin, E. S., G. A. Macro and E. M. Emery. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768-1775.

Goodrich, R. D., J. E. Garrett, D. R. Gast, M. A. Krick, D. A. Larson and J. C. Meiske. 1984. Influence of monensin on the performance of cattle. J. Anim. Sci. 58:1484.

Haney, M. E. Jr. and Hoehn. 1968. Monensin, a new biologically active compound. I. Discovery and isolation. Antimicrob. Agents Chemother, 1967. pp. 349-352.

Joblin, K. N. 1981. Isolation, enumeration and maintenance of rumen anaerobic fungi in rolled tubes. Appl. Environ Microbiol. 42:1119-1122.

Kearl, C. L. 1982. Nutrients requirement of ruminants in developing countries. International Feedstuffs Institute. Utah Agricultural Experiment Station, Utah State University, Logan Utah.

Mohini, M. 1991. Effect of urea molasses mineral block supplementation to straw based diets on fibre digestibility, rumen fermentation pattern and nutrient utilization and growth in buffalo calves, Ph.D Thesis, NDRI, Kamal.

Mc Dougall, E. I. 1948. The composition and output of sheep's saliva. Biochem. J. 43:99.

Menke, K. H., L. Raap, A., Salawsky, H. Steingaso, D. Fritz and W. Scheneider. 1979. The estimation of the digestibility and metabolisable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. J. Agric. Sci. Camb. 93:217-223.

Nagaraja, T. G. and M. B. Taylor. 1987. Susceptibility and *resistance* of ruminal bacteria to antimicrobial feed additives. Appl. Environ. Microbiol. 53:1620-1625.

Owaimer, A. N., M. S. Kraidees, M. Al. Saiady, S. Zahran and M. A. Abouheif. 2003. Effect of feeding monensin in combination with zeranol implants on performance, carcass trait and nutrient digestively of growing lambs. Asian-Aust. J. Anim. Sci. 16:1274-1279.

Potter, E. L., A. P. Raun, C. O. Cooley, R. P. Rathmacher and L. F. Richardson. 1976. Effect of monensin on carcass characteristics, carcass composition and efficiency of converting feed to carcass. J. Anim. Sci. 43:678-683.

Richardson, L. F., A. P. Raun, E. L. Potter, C. O. Cooley and R. P. Rathmacher. 1976. Effect of monensin on rumen fermentation *in vitro* and *in vivo*. J. Anim. Sci. 43:657-664.

Simpson, M. E. 1978. Effects of certain antibiotics on *in vitro* cellulose digestibility and volatile fatty acid production by ruminal organisms. J. Anim. Sci. 47(suppl.1):439.

Simpson, M. E. 1980. Effect of added antibiotics on *in vitro* rate and extent of digestion of a wheat straw cell wall. J. Anim. Sci. (suppl. 1):394.

Singh, G. P. and M. Mohini. 1999. Effect of different levels of rumensin in diet on rumen fermentation, nutrient digestibility and methane production in cattle. Asian-Aust. J. Anim. Sci. 12:1215-1221.

Snedecor, G. W. and W. G. Cochran. 1986. Statistical Methods. Oxford and IBH Publishing Co., Calcutta, India.

Tilley, J. M. A. and R. A. Terry. 1963. A two stage technique for the *in vitro* digestion of forage crops. J. Br. Grassland Soc. 18:104-112.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Method of dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3582-3597.

Wang, Y., T. A. Mcallister, J. Baah, R. Wilde, K. A. Beauchemin, L. M. Rode, J. A. Shelford, G. M. Kamande and K. J. Cheng. 2003. Effects of Tween 80 on *In vitro* fermentation of silages and interactive effects of tween 80, monensin and exogenous fibrolytic enzymes on growth performance by feedlot cattle. Asian-Aust. J. Anim. Sci. 16(7):986-978.

Zinn, R. A., A. Plascencia and R. Barajas. 1994. Interaction of forage level and monensin in diets for feed lot cattle on growth performance and digestive function. J. Anim. Sci. 72(9):2209-2215.