Effect of Ionophore Enriched Cold Processed Mineral Block Supplemented with Urea Molasses on Rumen Fermentation and Microbial Growth in Crossbred Cattle

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ABSTRACT : An experiment was conducted to study the effect of ionophore enriched cold processed mineral block supplemented with urea molasses on microbial growth and rumen fermentation. Twelve adult male crossbred cattle were divided into four groups on body weight basis. Animals were given wheat straw as a basal diet. The animals of group I and II were supplemented with concentrate mixture and animals of group III and IV were supplemented with cold processed urea molasses mineral block (UMMB). Thirty mg monensin/day/animal were supplemented to the animals of group II and 35 ppm monensin were incorporated in the UMMB supplemented to the animals of group IV. Dry matter (DM) intake did not differ significantly among groups. Mean rumen pH was higher in UMMB fed animals. Total volatile fatty acids (TVFA) concentration (mmole/L strained rumen liquor (SRL) in group III (113.19) was significantly ($p<0.05$) higher than those of group I (105.83) and II (108.74) but similar to group IV (109.34). TVFA production (mole/day) was similar in all the groups. The molar proportion of acetate was significantly ($p<0.01$) higher in the group I (59.56) than those of group II (51.73) and IV (55.91) but similar to group III (57.12). The molar proportion of propionate was significantly ($p<0.01$) higher in the monensin treated groups i.e. group II (38.38) and IV (36.26) than those of group I (27.78) and III (33.06). Butyrate molar percent was significantly ($p<0.01$) higher in group I (12.65) than those of group II (10.19), group III (9.83) and IV (7.84). The reduction of acetate and butyrate was due to UMMB and monensin resulted in lower A:P ratio. Average bacterial pool and bacterial production rate did not differ significantly among groups. Total N concentration (mg/100 ml SRL) was significantly ($p<0.01$) higher in the group I (55.30) and III (57.70) as compared to the group II (47.97) and IV (47.59). Ammonia-N concentration (mg/100 ml SRL) of group III (34.99) was significantly ($p<0.01$) higher than that of the group I (25.76) which was again significantly ($p<0.01$) higher than that of the group II (20.79) and IV (19.83) indicating slower release of ammonia due to monensin in diet. Total bacterial, cellulolytic, proteolytic bacterial and fungal count at 4 h post feeding did not differ significantly ($p<0.05$) among treatment groups. However, methanogenic bacterial count was significantly ($p<0.01$) higher in the group I (11.80) compared to the group II (8.43) which was significantly ($p<0.01$) higher than that of the group III (4.70) and IV (2.90). Average protozoal population was affected by both treatments. Thus feeding of UMMB and monensin in diet affected the rumen fermentation pattern towards propionate production, slower release of ammonia and reduction in methanogenic bacteria in the rumen. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 6 : 852-862)***

Key Words : Ionophore, UMMB, Rumen Fermentation

INTRODUCTION

Ruminant has unique ability to utilize efficiently cellulose and allied plant products in nature. But the crop residue based diets are not sufficient to support the body maintenance of the animals. Introduction of urea molasses mineral block (UMMB) overcomes the reluctance of farmers to use urea in the animal diet in India, which can meet the maintenance requirement of the animals when supplemented with crop residue (Garg, 1989). A long cherished dream of a ruminant nutritionist has been to manipulate and improve the efficiency of ruminal fermentation for the enhancement of overall productive efficiency of ruminants. Initial attempts to achieve this goal were by dietary manipulation but, during 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 antibiotic called ionophore. Monensin, one of the large scale use of ionophores in farm animals, is produced by *Streptomyces cinnamonensis*. Activity of the ionophore is lost due to action of heat. If the ionophore is incorporated in hot processed UMMB, its activity will be lost. But it will act in its own way or increase the urea utilization efficiency (Davis and Erhart, 1976) if it is incorporated with cold processed UMMB. The cold processed UMMB is prepared by using exothermic heat generated by calcium oxide and gelling and binding properties of sodium bentonite. In the present investigation two types of blocks, being a control UMMB and a UMMB enriched with monensin that were prepared through a cold process which does not involve use of external heat during preparation. The specific objective was to study the effect of monensin enriched UMMB on

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rumen fermentation and to compare it with the effect of monensin supplemented concentrate mixture in crossbred cattle.

MATERIALS AND METHODS

Twelve rumen fistulated male crossbred (Sahiwal× Holstein friesian) cattle (body weight 235 to 259 kg and age 2.5 to 3 years) were divided into four groups of three each on body weight basis and experiment was conducted in 2×2 factorial design. The four groups of the animals were allotted to different feeding regime as below:

Group I: Concentrate mixture (1.41 kg)+wheat straw *ad lib*
Group II: Concentrate mixture (1.41 kg)+monensin (30 mg/day)+wheat straw *ad lib*
Group III: Urea molasses mineral block (UMMB) at free choice+wheat straw *ad lib*
Group IV: UMMB enriched with 35 ppm monensin at free choice+wheat straw *ad lib*

The animals of group I and II were provided with concentrate mixture (maize 32 parts; groundnut cake 35 parts; wheat bran 30 parts; mineral mixture 2.5 parts; and salt 0.5 parts) to meet maintenance requirement (Kearl, 1982), while the animals in the group III and IV were given UMMB at free choice. However, UMMB offered to the animals of group IV contained 35 ppm monensin. Quantity of monensin (i.e. 30 mg/d) supplied to animals of the group II was based on the quantity consumed by the animals of the group IV. The daily consumption of wheat straw and UMMB were recorded. The animals of group I and II consumed all concentrate without any residue. The blocks were kept in a plastic milk crate in slanting position by the side of the feed manger so that the animals could have access to UMMB at their choice. The animals in all the groups were given vitamin supplement in drinking water once a week to meet their vitamin A and D requirements. The water was supplied *ad libitum* twice a day.

Chemical analysis

Wheat straw, concentrate mixture and UMMB were analyzed for proximate principles (AOAC, 1984). Drymatter (DM), organic matter (OM), ether extract (EE), crude fibre (CF) and nitrogen free extract (NFE) content of UMMB were lower than those of concentrate mixture. However, nitrogen (N), calcium (Ca) and phosphorus content of block were higher than those of concentrate mixture (table 1).

Table 1. Chemical compositions of feed

Parameter	Concentrate mixture	Wheat straw	UMMB
DM	87.89	90.50	82.55
OM	90.82	89.80	70.10
N	3.06	0.54	6.38
EE	4.42	0.78	0.53
CF	9.47	38.37	6.76
NFE	57.83	47.27	56.43
Total ash	9.18	10.20	29.90
Ca*	0.68	0.16	4.04
P	0.58	0.11	1.72

* Ca content of water 0.0019 g/100 ml.

Rumen fermentation studies

After an adaptation period of one month, rumen liquor samples were collected on three consecutive days at predetermined intervals depending on parameter under study. Rumen liquor samples were collected through cannula with help of stainless steel probe kept at four different sites in the rumen to get a representative sample of rumen liquor. Immediately after collection, microbial activity was stopped by adding 0.2 ml of 10 N H_2SO_4 except in sample for pH measurement. Immediately after each collection, pH was measured with digital pH meter. Rumen fluid volume and flow rate of liquid digesta was estimated using poly ethylene glycol (PEG) (Smith, 1959). Protozoa were enumerated by method of Langer et al. (1968).

Total volatile fatty acid concentration, production rate using ^{14}C sodium acetate and molar proportion of individual volatile fatty acids

Single dose isotope dilution technique was followed to estimate total volatile fatty acid (TVFA) production rate as described by Gray et al. (1960). Water was offered one hour before infusion of isotopes. 1,2-^{14}C sodium acetate was infused at the dose of 180 μci in the rumen and rumen contents were thoroughly mixed manually by inserting arm covered with gloves. Samples of rumen liquor were collected at 120, 150, 180, 210, 240, 270, 360 and 480 min interval after infusion. One ml of strained rumen liquor (SRL) was taken in a Markham distillation apparatus. One ml Scaris brick buffer was added. Hundred ml of distillate was collected in volumetric flask. An aliquot of distillate was taken in a test tube and its contents were made alkaline by using 0.01 N NaOH. The remaining distillate was titrated against standard 0.01 N NaOH using phenolphthalein as indicator to find out the concentration of TVFA. Out of the aliquot of the distillate which was made alkaline, 2.0 ml solution was taken in scintillation vial to which 5.0 ml of Bray's scintillation fluid (Bray, 1960) was added. The samples were counted in liquid scintillation counter. The rate of decline in specific radioactivity as a function of time was determined statistically. The pool size

of TVFA (mmoles) was calculated using following formula

Pool size (mmoles) = [Dose infused (dpm)/(Specific radio-activity at zero h/mmole of TVFA)]

Production rate (mmole/min) was calculated as

Production rate (mmole/min) = P×m

where, P = pool size (mmoles) and m = rate constant of decline in radioactivity per minut. Molar proportion of individual volatile fatty acids (VFA) were estimated according to method of Erwin et al. (1961).

Total nitrogen

Two ml of SRL was digested with 5 ml of concentrated H_2SO_4 and pinch of digestion mixture (containing Potassium sulphate and Copper sulphate) in Kjeltec digestion unit. Digested contents were transferred to micro-kjeldahl unit for distillation. Sixty ml of distillate was collected in a conical flask containing 10 ml of 2% boric acid having mixed indicator (0.1% methyl red and 0.1% bromocresol green in the ratio of 2:1). Distillate was titrated against 0.01 N H_2SO_4. Total N was calculated as follows

Total N (mg/100 ml) = [(Volume of acid used×strength of acid×0.014×10^5)/Volume of sample taken]

Trichloro acetic acid (TCA) precipitable N

Five ml of SRL was precipitated with 5 ml of 30% TCA. After 4 h, the contents were centrifuged at 3,000 rpm for 15 min. Two ml of supernatant was digested and distilled in micro-kjeldahl unit. The distillate was titrated against 0.01 N H_2SO_4 and non protein nitrogen (NPN) was calculated as

NPN (mg/100 ml SRL) = [(Volume of acid used×strength of acid×0.014×10^5)/Volume of SRL taken]

TCA precipitable N was calculated as

TCA precipitable N (mg/100 ml) = [Total N (mg/100 ml) -NPN (mg/100 ml)]

Ammonia N

Micro diffusion technique of Conway (1957) was followed to determine the ammonia -N concentration. For this, 1.0 ml of SRL was taken in the outer compartment of Conway micro diffusion cell and 1.0 ml of 2% boric acid with mixed indicator was placed in the inner compartment. One ml of saturated sodium carbonate solution was put in outer chamber. The cover of Conway dish was put and contents of outer chamber were mixed thoroughly by tilting and gently rotating the Conway dish. Dish was placed in incubator at 39±1°C for 4 h. After incubation, the content of inner chamber was titrated against 0.01 N H_2SO_4 using a micro burette titrater.

Ammonia-N = [(Volume of acid used×strength of acid×0.014×10^5)/Volume of SRL taken]

Rumen microbial count

Sampling time: Total anaerobic bacteria, cellulolytic, proteolytic and methanogenic bacterial and fungal counts were made from rumen liquor collected at 4 h post-feeding.

Sampling procedure: Rumen liquor was collected by plastic tube through permanent rumen fistula and brought 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.

Clarified rumen fluid (CRF) for the medium: Freshly collected rumen liquor was strained through double layer of muslin cloth and clarified through centrifugation at 12000 rpm for 30 min and kept at -5°C. Before adding to the medium, the rumen liquor was thawed and again centrifuged to remove any sedimented material. The CRF was used to provide unidentified growth factor to the rumen microbes.

Enumeration of bacteria: Half ml of freshly collected SRL of desired dilution was mixed with 2 ml of molten media (specific for each category of bacteria table 2 and table 3) in a roll tube and then tube was rotated on platform of crushed ice manually. Before adding SRL in media, cystein hydrochloride and vitamin solution was added. After the agar had set, purified CO_2 was introduced into the roll tube and the original rubber stopper was replaced by butyl rubber stopper. The roll tube was incubated in an upright position at 39±0.5°C for four weeks. Bacterial colonies developed on the agar film of the roll tubes were enumerated at the end of the incubation.

Enumeration of fungi : Freshly collected rumen liquor was used without straining as a source of inoculums and stirred for 5 to 7 min. on a magnetic stirrer. The stirring was performed with a continuous flow of CO_2 in front of the flame. Then stirred rumen liquor was diluted with anaerobic diluents to get requisite dilution. The inoculum (0.5 ml) of desired dilution was added in roll tubes containing molten media (table 2) and antibiotic solution (0.25 ml each of penicillin and streptomycin sulphate stock solution). The tubes were then rolled over platform of crushed ice to solidify the media uniformly as a thin film on the inner wall of the roll tube. The tubes were kept in incubator at a

Table 2. Anaerobic media for enumerating total anaerobic bacteria, cellulolytic, proteolytic and methanogenic bacteria and anaerobic fungus

Ingredients	Total anaerobic bacteria (Hungate, 1957)	Cellulolytic bacteria (Hungate, 1957)	Proteolytic bacteria (Abou-Akkada and Blackburn, 1962)	Fungi (Joblin, 1981)
Mineral solution I	3.75 m	15.0 ml	15.0 ml	17.0 ml
Mineral solution II	3.75 ml	15.0 ml	15.0 ml	17.0 ml
CRF	30.0 ml	20.0 ml	10.0 ml	15.0 ml
Yeast extract	0.05 g	-	-	0.05 g
Starch	0.05 g	-	-	-
Glucose	0.025 g	-	-	-
$NaHCO_3$	0.5 g	0.05 g	0.40 g	0.25 g
Cystein HCl	0.025 g	0.025 g	0.05 g	0.10 g
Na_2S, $9H_2O$	0.025 g	-	-	-
Resazurin (0.1%)	0.10 ml	0.10 ml	0.10 ml	0.30 ml
Bacto-casitone	-	0.25 g	-	-
Cellobiose	-	0.025 g	-	0.20 g
Cellulose	-	0.80 g	-	
Tryptose	-	-	0.30 g	0.10 g
Casein	-	-	0.50 g	-
Hemin solution (0.05%)	-	-	-	0.2 ml
Agar	2.0 g	2.0 g	2.0 g	2.0 g
Distilled water	62.5 ml	50.0 ml	60.0 ml	43.6 ml

temperature of 39±1°C on inverted position for four weeks. During incubation, the roll tubes were regularly inspected for appearance of colonies visible through naked eyes. The number of colonies were then counted.

Bacterial Protein synthesis rate : Bacterial protein synthesis rate was estimated by purine estimation of microbial protein following rapid assay technique (Zinn and Owens, 1986). Purine N content in the sample was calculated considering N content of purine 48.95 percent (Lehninger et al., 1993). Bacterial concentration in rumen liquor collected at different hours was calculated considering purine N content (Qi et al., 1993). Taking mean of bacterial concentration at different hours, average bacterial concentration in rumen liquor was determined.

Pool size was calculated as follows:

Table 3. Media for Methanogenic bacteria (Ranade and Gadre, 1988)

Ingredients	Quantity (for 100 ml)
KH_2PO_4	0.03 g
NH_4Cl	0.10 g
NaCl	0.06 g
$MgCl_2 \cdot 6H_2O$	0.01 g
$CaCl_2$	0.008 g
Yeast extract	0.05 g
Trypticase	0.05 g
Trace mineral solution	0.1 ml
Trace vitamin solution	0.1 ml
Sodium acetate	0.68 g
Resazurin (0.1%)	0.1 ml
Cystein hydrochloride (2% w/v)	0.65 ml
Sodium sulphide	0.60 ml
Agar	2.0 g
N_2:CO_2	80:20

Pool size = Rumen fluid volume × Average bacterial concentration (g/litre)

Bacterial production rate was calculated as follows:

Bacterial production rate (g/h) = Pool size × b
(b = the rate of change of bacterial concentration per hour)
Bacterial production (g/d)= Pool size × b × 24

Statistical analysis

Differences between trearment effects were tested using analysis of variance in a 2×2 factorial design (Snedecor and Cochran, 1986).

RESULTS

Dry matter intake

Wheat straw intake (kg/d) in monensin treated groups i.e. in the group II (1.80) and the group IV (2.54) were lower (41.37 and 17.53 percent, respectively) compared to their respective control groups, i.e. group I (3.07) and group IV (3.08). Dry matter (kg/d, kg/100 kg body weight and g/kg metabolic body size) did not differ significantly to each other (table 4).

Ruminal pH

The mean ph of the group I (6.52) was significantly ($p<0.01$) lower (table 5) than that of group II (6.84) which was again significantly ($p<0.01$) lower than that of the group III and IV. However, pH of group III (7.15) and IV (7.10) did not differ significantly to each other. In all the

Table 4. Dry matter intake in cattle fed on concentrate and urea molasses mineral block based diet with or without monensin

Parameter	G-I	G-II	G-III	G-IV
Body weight (kg)	246.33	248.67	250.33	245.00
Wheat straw intake (kg/d)	3.07	1.80	3.08	2.54
Conc. mix. intake (kg/d)	1.41	1.41	-	-
UMMB intake (kg/d)	-	-	0.79	0.68
TDMI (kg/d)	4.48	3.21	3.87	3.22
TDMI (kg/100 BW)	1.81	1.29	1.54	1.32
DM intake (g/kg $W^{0.75}$)	71.82	51.24	61.23	52.32

Treatments: G-I Wheat straw *ad lib.*+Concentrate mixture, G-II Wheat straw *ad lib.*+Concentrate mixture+monensin (30 mg/d), G-III Wheat straw *ad lib.*+UMMB at free choice, G-IV Wheat straw *ad lib.*+monensin (35 ppm) enriched UMMB.
SEM values: Body weight-5.43; Wheat straw intake-0.53; Total DM intake (TDMI)-0.56; TDMI (kg/100 kg BW)-0.22 and DMI (g/kg $W^{0.75}$)-8.53.

groups, lowest pH was observed at 4 h post feeding (figure 1).

TVFA concentration

TVFA concentration (mmole/L SRL) in the group III (113.19) was significantly ($p<0.05$) higher than those in the group I (105.83) and II (108.74) but similar to the group IV (109.34). However, there was no significant differences between groups I and II (table 5). TVFA concentration was lowest at zero hour, i.e. before feeding and increased at 4 h post feeding; afterwards it started declining (figure 2).

TVFA production rate

TVFA production rate (mmole/min and mole/d) was similar in all the groups. Considering that 1 mole TVFA provides 2.8 MJ ME (Leng, 1982), the metabolizable energy was calculated from TVFA production in different groups which did not differ significantly (table 5).

Individual VFA molar proportion: The molar proportion of acetate was significantly ($p<0.01$) higher in the group I (59.56) than those in the group II (51.73) and IV (55.91) but similar to the group III (57.12). Acetate percent in the group III and IV did not differ but it was significantly ($p<0.01$) higher than those in the group II (table 6).

The molar proportion of propionate was significantly ($p<0.01$) higher in the monensin treated groups i.e. group II (38.08) and IV (36.26) than those in the group I (27.78) and III (33.06). Propionate percent in the group III was significantly ($p<0.01$) higher than that of the group I. However, no significant difference was observed between the group II and IV.

Butyrate molar percent was significantly ($p<0.01$) higher in the group I (12.65) than those in the group II (10.19) and III (9.83), which were also significantly higher than that of group IV (7.84).

A:P ratio was significantly ($p<0.01$) higher in the group I (2.20) than those in the group II (1.38), III (1.78) and IV (1.58). However, no significant difference in A:P ratio was observed between the group II and IV and between the group III and IV.

Bacterial production rate

Average bacterial pool (g), bacterial production rate (g/h or g/d) did not differ significantly among treatment groups (table 7). When bacterial production rate per kg digestible OM (calculated from digestibility data of the particular group in nonfistulated condition) was calculated, it was observed that although bacterial production (g/kg DOMI) was higher in the monensin treated groups (group II, 156.31 and IV, 192.56) than those in their respective control (group I, 120.86 and III, 150.64) but there were no statistical

Table 5. Total volatile fatty acids production rate in cattle fed on concentrate and urea molasses mineral block based diet with or without monensin

Parameter	G-I	G-II	G-III	G-IV
PH	6.52^{a}	6.84^{b}	7.15^{c}	7.10^{c}
TVFA (m mole/l SRL)	105.83^{a}	108.74^{a}	113.19^{b}	109.34^{ab}
Pool size (m mole)	2618.72	2794.90	2941.90	2762.78
m value ($\times 10^{-4}$)	35.03	35.52	35.79	36.22
Production of TVFA				
m mole/min.	9.17	9.81	10.53	10.01
Moles/d	13.21	14.13	15.17	14.41
ME (MJ)	36.98	39.56	42.47	40.36

[a,b] Values bearing different superscripts in a row differ significantly ($p<0.05$).
m- Rate of decline in radio activity from rumen with passage of time.
Treatments: G-I Wheat straw *ad lib.*+concentrate mixture, G-II Wheat straw *ad lib.*+concentrate mixture+monensin (30 mg/d), G-III Wheat straw *ad lib.*+UMMB at free choice, G-IV wheat straw *ad lib.* + monensin (35 ppm) enriched UMMB.
SEM values: pH-0.29; TVFA (m mole/l SRL)-2.98; Pool size-68.44; m value-0.44; TVFA production (m mole/min)-0.37; TVFA production (mole/d)-0.53 and ME (MJ)-1.49.

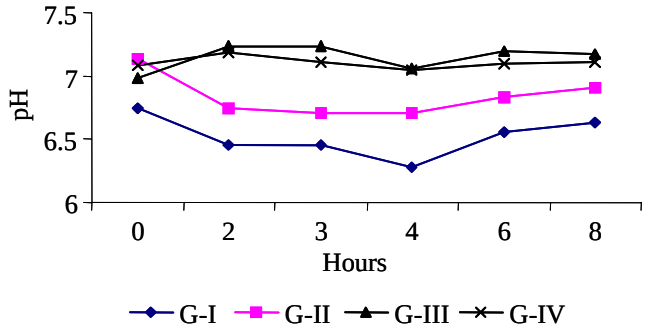

Figure 1. Rumen pH of different groups of animals at different hours

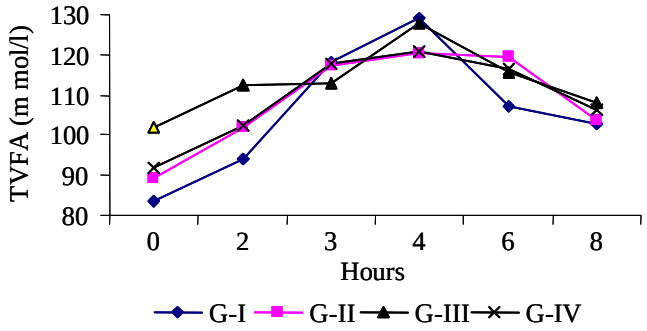

Figure 2. TVFA concentration of different groups of animals at different hours

difference between the groups because of higher variation within group resulting in higher standard error.

Concentration of various nitrogenous constituents in rumen liquor

The mean concentration of total N (mg/100 ml SRL) was significantly ($p<0.01$) higher in the group I (55.30) and III (57.70) as compared to the monensin treated groups i.e. group II (47.97) and IV (47.59). However, difference between groups I and III and between groups II and IV was not significant (table 8).

The mean concentration of TCA precipitable N (mg/100 ml SRL) was significantly ($p<0.01$) higher in the groups I (29.14), III (30.28) and IV (28.24) than those in the group II (24.55). However, no significant difference was observed between the groups I, III and IV.

The mean ammonia N concentration (mg/100 ml SRL) of the group III (34.99) was significantly ($p<0.01$) higher than that of the group I (25.76), which was again significantly ($p<0.01$) higher than that of the group II (20.79) and IV (19.83). However, no significant difference was observed between the groups II and IV.

Rumen fluid volume and flow rate of liquid digesta

Effect of different treatments on rumen fluid volume and flow rate along with other essential data of body weight, water intake and DM intake are presented in table 9. DM intake (kg/d) in the group II (3.69) and III (3.91) was 19.78 and 15.00 percent lower respectively than that of group I (4.60) and DM intake in group IV (3.16) was 19.18 percent lower than that of group II (3.91). However, the difference was not significant. The mean water intake in all the groups was in the range of 30 to 31 (l/d) and was similar in all the groups. The rumen fluid volume in the groups I to IV were 25.50, 26.17, 26.02, 25.71 litre, respectively, which did not differ significantly to each other. Flow rate also did not differ significantly among the groups. Rumen fluid volume, calculated as percent of body weight in all the groups were in the range of 10.33 to 10.55 percent ($p>0.05$).

Table 6. Molar proportion of individual volatile fatty acids in cattle fed on concentrate and urea molasses mineral block based diet with or without monensin

Molar percent	G-I	G-II	G-III	G-IV
Acetate	59.56^{c}	51.73^{a}	57.12^{bc}	55.91^{b}
Propionate	27.28^{a}	38.08^{c}	33.06^{b}	36.26^{c}
Butyrate	12.65^{c}	10.19^{b}	9.83^{b}	7.84^{a}
A/P ratio	2.20^{c}	1.38^{a}	1.78^{b}	1.58^{ab}

^{a,b,c} Values bearing different superscripts in a row differ significantly ($p<0.01$).

A/P-Acetate/Propionate.

Treatments: G-I Wheat straw *ad lib.*+Concentrate mixture, G-II Wheat straw *ad lib.*+Concentrate mixture+monensin (30 mg/d), G-III Wheat straw *ad lib.*+UMMB at free choice, G-IV Wheat straw *ad lib.*+monensin (35 ppm) enriched UMMB.

SEM values: Acetate-2.31; Propionate-2.03; Butyrate-1.07 and A/P ratio-0.09.

Table 7. Bacterial production rate in cattle fed on concentrate and urea molasses mineral block based diet with or without monensin

Parameter	G-I	G-II	G-III	G-IV
Rumen fluid volume (1)	25.50	26.17	26.02	25.71
Bacterial pool size (g)	187.27	304.69	373.78	401.79
Bacterial production rate				
g/h	10.94	11.75	11.16	11.72
g/d	262.50	282.08	267.96	281.37
g/kg DOMI	120.86	156.31	150.64	192.56

Treatments: G-I Wheat straw *ad lib.*+concentrate mixture, G-II Wheat straw *ad lib.*+concentrate mixture+monensin (30 mg/d), G-III Wheat straw *ad lib.*+UMMB at free choice, G-IV Wheat straw *ad lib.*+monensin (35 ppm) enriched UMMB.

SEM values: Rumen fluid volume-3.08; Bacterial pool size- 93.09; Bacterial production rate (g/h)-0.91; Bacterial production rate (g/d)-21.93; Bacterial production rate (g/kg DOMI)-38.13.

Microbial population

Total bacterial count, cellulolytic bacterial count and proteolytic bacterial count at 4 h post feeding (table 10) in the groups I, II, III and IV did not differ significantly. However, methanogenic bacterial count ($\times 10^4$/ml SRL) was significantly ($p<0.01$) higher in the group I (11.80) than those in the group II (8.43), which was again significantly ($p<0.01$) higher than those in the group III (4.70) and IV

Table 8. Various rumen nitrogen metabolites in cattle fed on concentrate and urea molasses mineral block based diet with or without monensin

Treatment	Hour post feeding					Overall mean
	0 h	2 h	4 h	6 h	8 h	
			Total nitrogen (mg/100 ml SRL)			
G-I	38.09	37.80	42.18	93.98	64.46	55.30[b]
G-II	42.94	43.58	53.24	45.11	54.99	47.97[a]
G-III	54.69	35.35	70.27	75.17	53.03	57.70[b]
G-IV	34.92	36.06	50.75	68.08	48.13	47.59[a]
			TCA-Precipitable N (mg/100 ml SRL)			
G-I	11.73	15.05	14.70	65.28	38.94	29.14[b]
G-II	11.71	14.84	18.47	40.79	36.92	24.55[a]
G-III	33.86	16.89	34.22	40.08	26.34	30.18[b]
G-IV	22.66	18.03	28.35	40.60	31.55	28.24[a]
			Ammonia nitrogen (mg/100 ml SRL)			
G-I	21.84	24.57	31.52	24.90	25.95	25.76[b]
G-II	21.37	18.01	22.40	20.67	21.51	20.79[a]
G-III	33.86	16.89	34.22	40.08	26.34	34.99[c]
G-IV	15.78	18.16	19.14	23.06	22.99	19.83[a]

[a,b,c] Values bearing different superscripts in a column differ significantly ($p<0.01$)

Treatments: G-I Wheat straw *ad lib.*+concentrate mixture, G-II Wheat straw *ad lib.*+concentrate mixture+monensin (30 mg/d), G-III Wheat straw *ad lib.*+UMMB at free choice, G-IV wheat straw *ad lib.*+monensin (35 ppm) enriched UMMB, SEM values: Total N-1.99; TCA-precipitable N-1.59; Ammonia N-1.51.

Table 9. Rumen fluid volume and flow rate of liquid digesta in cattle fed on concentrate and urea molasses mineral block based diet with or without monensin

Parameter	G-I	G-II	G-III	G-IV
Body weight (kg)	246.33	248.67	250.33	245.00
Total DM intake (kg)	4.60	3.69	3.91	3.16
Water intake (l)	30.22	30.63	29.92	30.71
Rumen fluid volume (l)	25.50	26.17	26.02	25.71
Flow rate (ml/h)	873.15	885.95	863.92	820.82
Flow rate (l/d)	20.96	21.26	20.73	19.70
RFV as % of body weight	10.33	10.55	10.36	10.42

RFV-Rumen fluid volume.

Treatments: G-I Wheat straw *ad lib.*+concentrate mixture, G-II Wheat straw *ad lib.*+concentrate mixture+monensin (30 mg/d), G-III Wheat straw *ad lib.*+UMMB at free choice, G-IV wheat straw *ad lib.* + monensin (35 ppm) enriched UMMB.

SEM values: Body weight-5.43; Total DM intake-0.61; Water intake-2.81; Rumen fluid volume (l)-3.08; Flow rate (ml/h)- 64.51; Flow rate (l/d)-1.55 and RFV as % of body weight-0.85.

(2.90) (table 10). Though methanogenic bacterial count in the group IV was less than that of the group III but difference was not significant. Fungal population at 4 h post feeding (table 10) did not differ significantly among the four treatment groups.

Average protozoal population of group I (3.77) was significantly ($p<0.01$) higher than that of the group II (2.92) and IV (2.12) but similar to the group III (3.32). No significant difference was observed between the groups II and III. Again, protozoal population in the group II was significantly ($p<0.01$) higher than that of the group IV.

DISCUSSION

Lower feed consumption in the present study could related to increased concentration of ruminal or blood propionic acid or some other chemostatic mechanism in the animal (Theurer et al., 1974). Decreased feed intake due to monensin treatment was reported by several workers (Davis and Erhat, 1976; Raun et al., 1976; Boling et al., 1977; Joyner et al., 1979). But Bergen and Bates (1984) reported no decrease in feed intake due to monensin when diet contained considerable β-linked carbohydrates. Potter et al. (1976) did also observe that monensin dosage of 200 mg/head/d and below had no effect upon DM consumption in cattle fed pasture or green crop. When UMMB or monensin enriched UMMB was supplemented to wheat straw diet, pH was significantly higher as compared to concentrate supplementation. Higher pH was due to consumption of urea present in block, which was degraded in rumen. No change in pH was observed when monensin was supplemented along with block. Similarly, no effect on pH due to ionophore feeding were reported several workers (Dyer et al., 1980; Zinn et al., 1991; Haimoud et al., 1995). On the contrary, in the present study pH was increased when monensin was supplemented with concentrate mixture. When concentrate mixture was offered to animals they consumed it very quickly, as a result lactic acid might be produced which helped to reduce the pH. When monensin was supplemented with the concentrate mixture, increase in pH could be due to inhibition of lactate producing rumen bacteria without interfering lactate utiliser (Dennis et al., 1981).

Table 10. Bacterial, protozoal and fungal biomass in the rumen fluid of cattle fed on concentrate and urea molasses mineral block based diet with or without monensin

Parameter	G-I	G-II	G-III	G-IV
Total bacterial count ($\times10^9$/ml SRL)	8.23	9.87	9.70	10.10
Cellulolytic bacteria ($\times10^7$/ml SRL)	5.33	8.33	7.00	8.67
Proteolytic bacteria ($\times10^5$/ml SRL)	2.77	3.33	3.47	4.57
Methanogenic bacteria ($\times10^4$/ml SRL)	11.80^c	8.43^b	4.70^a	2.90^a
Fungi ($\times10^3$/ml SRL)	1.61	1.55	1.72	1.61
Protozoa ($\times10^4$/ml SRL)	3.72^c	2.92^b	3.32^{bc}	2.12^a

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

Treatments: G-I Wheat straw *ad lib*.+concentrate mixture, G-II wheat straw *ad lib*.+concentrate mixture+monensin (30 mg/d), G-III wheat straw *ad lib*.+UMMB at free choice, G-IV wheat straw *ad lib*.+monensin (35 ppm) enriched UMMB.

SEM values: Total bacterial count-1.50; Cellulolytic bacteria-0.88; Proteolytic bacteria-0.61; Methanogenic bacteria-0.86; Fungi-0.14; and Protozoa-0.38.

Significant increase in TVFA concentration on supplementation of UMMB licks implies availability of more fermentable energy. However, Singh et al. (1995) reported similar concentration of TVFA on feeding of concentrate and UMMB. It is clear that monensin did not affect the production of TVFA and was also reported by several groups of workers (Boling et al., 1977; Haimoud et al., 1995; Mbanzamihigo et al., 1996).

Comparatively higher TVFA concentration and production rate than those observed by Srinivas and Gupta (1997) and Madhu Mohini (1991) imply an effective fermentation on supplementation of concentrate mixture or UMMB either with or without monensin. Which indicated that energy availability through TVFA for microbial protein synthesis was similar in the groups fed UMMB with or without monensin or concentrate mixture (at maintenance level) with or without monensin.

Addition of monensin in concentrate mixture and UMMB resulted in decreased acetate and butyrate and increased propionate. The effect of monensin was more pronounced when added in concentrate than those in UMMB because of consumption pattern of concentrate and UMMB. The increase in propionate on UMMB supplementation was obviously due to availability of more and highly soluble carbohydrate that increase the propionate production.

When proportion of individual VFA was compared between the groups fed concentrate (without monensin) and UMMB (without monensin), it was seen that acetate and butyrate percent decreased and propionate increased in the group fed UMMB. This increase in propionate and decreases in acetate and butyrate were further increased and Acetate/Propionate (A/P) ratio was further decreased when monensin was added in UMMB. Therefore, here action of monensin was additive in nature. Gram-positive bacteria are reported to be more sensitive to ionophore than gram-negative bacteria. *Ruminococcus albus* and *R. flavifaciens* have gram-positive anatomy, but *Butyrivibrio fibrisolvens*, although negative in gram-stain, has a gram-positive cell wall structure, are highly sensitive to monensin (Haney and Hoehn, 1968). Addition of monensin to the rumen tends to decrease the production of major fermentation products, acetate and butyrate, by inhibiting the growth of these carbohydrate fermenting species (Chen and Wolin, 1979). On the other hand, *Bacteroides* (succinate producer) are moderately sensitive and *Selenomonas ruminantium* (propionate producer) is insensitive to monensin due to their gram-negative cell wall structure (Costerton et al., 1974). *Selenomonas ruminantium* produces little or no formate and traces of hydrogen (Scheifinger and Wolin, 1973). Therefore, decrease in acetate and butyrate and increase in propionate proportion due to monensin treatment could be due to action against monensin sensitive *R. albus*, *R. flavifaciens* and *B. fibrisolvens* that produce acetate and butyrate and selection for succinate forming *Fibrobacter succinogens*, that uses cellulose, *Prevotella ruminicola* that uses starch and *S. ruminantium* that produces propionate by decarboxylation of succinate generated by other species (Chen and Wolin, 1979). Dennis et al. (1981) also reported that increase in propionate in cattle fed monensin might be due to selection for succinate producers and lactate fermenters.

The microbial protein synthesis depends upon the amount of easily available energy and easily fermentable nitrogen source. In this experiment, optimum fermentable N and energy in both the groups fed concentrate and UMMB resulted in similar bacterial production rate in all the groups. No adverse effect of monensin on bacterial production rate was observed when monensin was added either with concentrate mixture or UMMB. In the present study, UMMB contained only 10 percent cotton seed cake (CSC) whereas concentrate mixture contained 35 percent ground nut cake (GNC) but there was no significant difference in bacterial production rate.

Higher bacterial production rate calculated in terms of g/kg DOMI, ($p>0.05$) in the monensin treated groups might be due to higher propionate in rumen that culminated to synthesize more protein per unit of DOMI. Present values of bacterial production (g/kg DOMI) were within the range

of 95 to 320 g bacterial protein per kg OM fermented as reported by Thomas (1973).

Total N and TCA precipitable N concentration in rumen liquor were similar in the groups fed concentrate and UMMB. However, addition of monensin with concentrate mixture resulted in a significantly ($p<0.01$) decreased total N and TCA precipitable N while only total N was decreased in the group fed monensin enriched UMMB. This decrease in total N and TCA precipitable N due to monensin treatment could be attributed to the reduction in protozoal number because of monensin (Badawy et al., 1996; Hino et al., 1994; Suda et al., 1995) as no reduction in bacterial production was observed due to monensin treatment.

Ammonia N concentration in the group fed UMMB (without monensin) was significantly ($p<0.01$) higher than the group fed concentrate (without monensin) but total N and TCA precipitable N did not differ significantly indicating that increased ammonia N was not utilised efficiently for the marked improvement in the TCA precipitable N, an indicator of better microbial protein synthesis (Gupta et al., 1970). When monensin was supplemented either with concentrate mixture or UMMB, ammonia N concentration was significantly ($p<0.01$) reduced. This reduction of ammonia N concentration due to monensin might be because of inhibition of urease, protease and deaminase activity by monensin (Van Nevel and Demeyer, 1977; Starness et al., 1984). So whatever might be the reason, feeding monensin would increase the quantity of dietary protein escaping ruminal degradation and would, therefore, be available for digestion and uptake in the small intestine. Monensin did not have any effect on rumen fluid volume and flow rate of liquid digesta.

So far as bacterial population is concerned, total count did not differ significantly due to treatment effect and monensin did not have any adverse effect on total bacterial count. However, 25 percent decrease in bacterial number due to *in vitro* was reported by suda et al. (1995). Significant ($p<0.01$) decrease in methanogenic bacterial count in the group fed UMMB compared to the groups fed concentrate might be due to some ingredients (e.g. urea, molasses, calcium oxide, sodium bentonite and cotton seed cake) that were present in UMMB but not in concentrate mixture. Result of present study also indicated that monensin reduced the methanogenic count as several methanogens are sensitive to ionophore (Hilpert et al., 1981) or could be due to suppression of hydrogen production from formate because of ionophore which ultimately inhibits methanogens (Dellinger and Ferry, 1984) or could be due to combination of both.

Fungal population was enumerated by counting the colonies which appeared in roll tube following incubation at 39°C. Actually on agar surface zoospores can not migrate, however, they mature to become vegetative sporangia. After bursting of sporangia, several zoospores are released which mature again and form visible colonies and these colonies are counted. Fungal population in monensin treated groups was some what less in number than those in their respective controls though differences were not significant. Cann et al. (1993) observed that the growth of mixed rumen fungi *in vitro* was suppressed by ionophore antibiotics (Salinomycin, Monensin, Portmycin).

It is clear from the study that protozoal number were similar in animal fed concentrate mixture (without monensin) and UMMB (without monensin). But when both feeds were supplemented with monensin, protozoal population was significantly ($p<0.01$) reduced as compared to their respective control groups. This reduction in protozoal population could be due to direct effect of monensin as anticoccidial agent (Fitzgerald and Mansfield, 1973; Poos et al., 1979). Protozoal population was significantly ($p<0.01$) lower in the group fed monensin enriched UMMB than those in the group fed monensin supplemented concentrate mixture. The lower protozoal population in cattle fed monensin enriched UMMB could be due to the combined effect of monensin and partial defaunating properties of UMMB (Garg, 1989).

Therefore, monensin supplementation either with concentrate or UMMB decreased molar proportion of acetate and butyrate, A/P ratio and increased molar proportion of propionate. Monensin decreased ammonia nitrogen and total nitrogen level of rumen liquor without affecting bacterial production rate. Both UMMB as well as monensin decreased methanogenic bacterial and protozoal population, however, cellulolytic, proteolytic bacterial population and fungal population were not affected by either dietary treatment or monensin supplementation.

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