



Influence of monensin enriched UMMB feeding on *in vivo* methane emission in crossbred calves fed on wheat straw and concentrate based diet

DEBASIS DE¹, MADHU MOHINI² and G P SINGH³

National Dairy Research Institute, Karnal, Haryana 132 001 India

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Monensin, a biologically active compound produced by a strain of *Streptomyces cinnamonensis* (Haney and Hoehn 1968), is commonly used feed additive for feedlot cattle. It improves the efficiency of feed utilization of cattle by altering volatile fatty acid proportion towards more propionate (Dinius *et al.* 1976, Richardson *et al.* 1976, Goodrich *et al.* 1984, Andrae *et al.* 1995, Singh and Mohini 1999, Callaway *et al.* 2003, Packer *et al.* 2011) and thereby reduces methane production (Badawy *et al.* 1996; Mbanzamihigo *et al.* 1996). Monensin also depresses feed intake (Walker *et al.* 1980, Faulkner *et al.* 1985, Stock *et al.* 1995, Ramanzin *et al.* 1997). However, when ruminants were fed diets containing considerable -linked carbohydrate (roughage) ionophore did not depress intake (Bergen and Bares 1984). Supplementation of urea molasses mineral block increase the feed intake and utilization of straw (Garg and Gupta 1993) and reduce the methane production by decreasing methanogenic volatile fatty acids (Preston and Leng 1989, Singh *et al.* 1995). The methane production is not only loss of feed energy but it also plays a major role in global warming (Vangardingien 1991, Singh 1997, Martin *et al.* 2008). In the present study an attempt was done to enrich the UMMB with monensin for the mitigation of methane emission from ruminants.

Crossbred (Sahiwal × Holstein Friesian) male calves (4), ~ 1-year-old, body weight 249.25 to 258.5 kg, were used in a switchover design (Table 1) comprising 4 treatments, 4 animals and 4 periods. Each experimental period lasted for 23 days of which 15 days adaptation period followed by 3 days gas collection period and 5 days digestibility trial. The animals were kept in well ventilated byre, where there was provision for keeping UMMB licks separately. Before final collection of gas in canister, animals were trained with canister for 3 days during adaptation period to avoid sudden change in feed intake and to make them feel comfortable with canister and halter.

Present address : ¹Senior Scientist (debasiskrc@yahoo.com), Department of Animal Nutrition, KRC of CIBA, Kakdwip, South 24 Parganas, West Bengal 743 347 India.

^{2,3}Principal Scientist, Dairy Cattle Nutrition Division.

Table 1. Chemical composition of feed (%DM)

Parameter	Concentrate mixture	Wheat straw	UMMB
DM	87.89	90.50	82.55
OM	90.82	89.80	70.10
CP	19.10	3.38	38.38
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

Concentrate mixture: maize grain (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)

UMMB: (molasses 380 g kg⁻¹, urea 100 g kg⁻¹, common salt 50 g kg⁻¹, mineral mixture 60 g kg⁻¹, sodium bentonite 40 g kg⁻¹, lime powder 80 g kg⁻¹, deoiled rice bran 190 g kg⁻¹ and cotton seed cake 100 g kg⁻¹)

The animals of groups 1 and 2 were fed wheat straw *ad lib* and concentrate mixture as per requirement (Kearl 1982). Group 2 was also given monensin (30 mg/d/animal) along with concentrate mixture. Animals in group 3 were fed on wheat straw *ad lib*. plus 70% concentrate mixture of their requirement and the animals also had free access to UMMB. However, the animals under group 4 were fed on similar dietary plan as fed in group 3, except the enrichment of UMMB with monensin (100 ppm) for this group. Quantity of monensin (i.e. 30 mg/animal/d) for animals of group II was based on the consumption of monensin from UMMB in group 4 to keep the monensin level as close as possible in both the groups. Feed was offered once daily at 9.00 AM. Blocks in group 3 and 4 were placed after the consumption of concentrate mixture in plastic container in a slanting position to avoid biting of blocks by animals.

Methane emission was measured *in vivo* using SF₆ tracer technique (Singh 1996). Using gas chromatograph fitted with an electron capture detector (350°C) to determine SF₆, and a flame ionization detector (250°C) to determine CH₄, the ratio

of CH_4 and SF_6 in the sample was determined. Gas chromatograph was fitted with a 3.3 m molecular sieve column. The column and injector temperature were both 85°C . Nitrogen gas was used as carrier gas at a flow rate of 40 ml/min. The methane emission rate (QCH_4) was calculated from the ratio of CH_4 and SF_6 in collected gas and the known release rate of SF_6 (QSF_6). Background methane level $[(\text{CH}_4)_b]$ was subtracted from methane concentration in the yoke $[(\text{CH}_4)_y]$.

$$\text{QCH}_4 = \text{QSF}_6 \times [(\text{CH}_4)_y - (\text{CH}_4)_b] / \text{SF}_6$$

After 3 successful gas collection, a digestibility trial of 5 days duration was conducted to determine DM and OM digestibility. Wheat straw, UMMB and concentrate mixture were analyzed for proximate principles (Table 2) and faeces was analyzed only for DM and OM content (AOAC 1984). Data obtained were analyzed statistically according to Snedecor and Cochran (1986).

DM, OM, digestible DM (DDM) and digestible OM (DOM) intake did not differ significantly between different treatment groups. DM and OM digestibility also did not differ significantly among the groups (Table 2). Methane production (l/d) in different groups were significantly ($P<0.01$) different to each other. Methane production was highest in group 1 followed by group 3, 2 and 4. Methane production l/kg DDM intake and l/kg DOM intake were significantly ($P<0.01$) higher in groups 1 and 3 as compared to that of group 2 which was again significantly ($P<0.01$) higher than that of group 4. However, no significant difference was observed between group 1 and group 3.

Results of the study indicated that when monensin (30mg/d/animal) was supplemented with concentrate mixture (group 2), methane emission (l/d) was reduced ($P<0.01$) by 21.57% as compare to that of animal fed concentrate mixture without monensin (group 1). However, a reduction ($P<0.01$) of 12.38% in methane emission was found when the animals were fed on UMMB based diet (group 3) when compared to those fed on concentrate mixture without monensin (group 1). Enrichment of UMMB with monensin, reduced ($P<0.01$) methane emission (l/d) by 40.49, 24.12 and 32.08% as compared to group 1, group 2 and group 3, respectively.

Reduction in methane emission in group 3 could be due to decrease in methanogenic bacterial population in UMMB fed animals caused by some ingredients (e.g. urea, molasses, calcium oxide, sodium bentonite and cotton seed cake) that were present in UMMB but not in concentrate mixture (De and Singh 2003). When methane emission was calculated on the basis of digestible DM and OM intake it was found that methane emission (l/kg DDMI) was reduced ($P<0.01$) by 20.72% as compared to that of animal fed concentrate mixture without monensin (group 1). Enrichment of UMMB with monensin, reduced ($P<0.01$) methane emission (l/kg DDMI) by 33.46, 16.08 and 31.09% as compare to group 1, group 2 and group 3, respectively. Methane emission (l/kg DOMI) was reduced ($P<0.01$) by 20.80% as compared to that of animal fed concentrate mixture without monensin (group 1). Enrichment of UMMB with monensin, reduced ($P<0.01$) methane emission (l/kg DDMI) by 32.88, 15.26 and 30.88% as compare to group 1, group 2 and group 3, respectively. When monensin was supplemented either with concentrate mixture or with UMMB, methane production was reduced. This reduction in methane emission due to monensin could be because of inhibition of hydrogen and formate producing bacteria and stimulation of succinate and propionate producers which could increase propionate formation by utilizing hydrogen rather than diverting it for methane emission (Chen and Wolin 1979). Reduction in methane emission in monensin treated groups might also be due to reduction in methanogenic bacterial and protozoal population (De and Singh 2003). Greater reduction in methane emission in monensin enriched UMMB fed animals (group 4) as compared to that of group II might be due to greater inhibitory effect of monensin enriched UMMB on methanogenic bacterial and total protozoal population (De and Singh 2003). Inhibitory effect of monensin on methane emission is more pronounced when used with UMMB as compare to when used with nonconcentrate because feeding of UMMB itself resulted reduction in methane emission due to its effect on methanogenic bacterial population (De and Singh 2003) and here action of monensin was additive in nature to further reduce methane emission. Reduction in

Table 2. Effect of monensin enriched UMMB on intake digestibility and *in vivo* methane production

Parameter	Group 1	Group 2	Group 3	Group 4	CD
DM intake (kg/d)	5.35±0.59	5.43±0.35	4.62±0.13	4.60±0.38	NS
DM digestibility (%)	58.87±2.32	56.54±3.16	60.39±4.45	60.00±3.95	NS
Digestible DM intake (kg/d)	3.15±0.31	3.07±0.19	2.79±0.06	2.76±0.14	NS
OM intake (kg/d)	4.88±0.53	4.96±0.31	4.17±0.11	4.15±0.35	NS
OM digestibility (%)	60.87±3.98	58.47±2.86	62.83±2.90	62.17±3.17	NS
Digestible OM intake (kg/d)	2.97±0.29	2.90±0.18	2.62±0.06	2.58±0.14	NS
Methane production (l/d)	150.07 ^d ±6.47	117.70 ^b ±4.32	131.49 ^c ±6.23	89.31 ^a ±2.63	10.98**
Methane production (l/kg DDMI)	48.80 ^c ±4.50	38.69 ^b ±2.25	47.12 ^c ±1.50	32.47 ^a ±0.82	5.39**
Methane production (l/kg DOMI)	51.73 ^c ±4.74	40.97 ^b ±2.38	50.23 ^c ±1.49	34.72 ^a ±0.87	6.05**

** $P<0.01$; NS- Nonsignificant; a, b, c and d values bearing different superscripts in a row differ significantly.

methane production might be due to removal of protozoa (Kreuzer *et al.* 1986) as ciliate protozoa are symbiotic with methanogenus (Stumn and Zwart 1986, Finlay *et al.* 1994). When concentrate mixture was fed to animal, the increase in methane production might be due to an increase in hydrogen transfer between this microorganisms (Krumholz *et al.* 1983). When UMMB was fed, due to partial defaunating properties of UMMB (Garg 1989) acetate and butyrate production was less (De and Singh 2003) and hydrogen might have been utilized towards more propionate production and methane emission was reduced as compare to when animals were fed with concentrate mixture. The principal fermentation products of protozoa are acetate and butyrate (Gutierrez 1955), therefore, the removal of these microorganisms from rumen might shift towards propionate and decrease the formation of methane (Itabashi *et al.* 1984). Results revealed that the enrichment of UMMB with monensin is more effective to inhibit methane emission as compared to that of when monensin was supplemented with concentrate mixture. So, from this study it can be concluded that addition of monensin either with urea molasses mineral block or with concentrate mixture can reduce methane production by 10.11 to 16.33 litre/kg DDMI or 10.76 to 17.01 litre/kg DOMI or 32 to 60 litre per day.

SUMMARY

Crossbred (4; Sahiwal × Holstein Friesian) male calves (~1 yr, avg. body weight 249.25 to 258.5 kg) were used in a switchover design to study the effect of monensin addition either in concentrate or enrichment of urea molasses mineral block (UMMB) with monensin on methane emission. In group 1, animals were fed concentrate mixture and wheat straw *ad lib*, group 2 were supplemented with 30 mg monensin/day, group 3 and 4, a deduction of 30% in concentrate was done, which was fulfilled through UMMB. In group 4, UMMB was enriched with monensin (100 ppm). Methane emission (l/d) was affected significantly ($P < 0.01$) due to monensin. It was significantly ($P < 0.01$) lower in group 4 (89.31 l/d) followed by group 2 (117.70 l/d), group 3 (131.49 l/d) and group 1 (150.07 l/d). Methane emission (l/kg DDMI and l/kg DOMI) was significantly ($P < 0.01$) lower in groups 2 (38.69, 40.07) and 4 (32.47, 34.72) as compared to groups 1 (48.80, 51.73) and 3 (47.12, 50.23). However, no significant difference was observed between groups 1 and 3 and also between groups 2 and 4. So, monensin enriched UMMB can reduce the methane production significantly.

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