Indian Journal of Animal Sciences **83** (8): 798–801, August 2013/Article

Effect of various levels of bromochloromethane and fibre level on *in vitro* methane production and fermentation pattern

S K SIROHI[1] and NAVNEET GOEL[2]

National Dairy Research Institute, Karnal, Haryana 13200 India

Received: 16 November 2012; Accepted: 19 March 2013

ABSTRACT

An *in vitro* study was conducted to investigate the effect of bromochloromethane (BCM) on methane production and fermentation characteristics in the 12 treatment combinations prepared by using the 3 levels of fibre (HF,MF,LF) and 4 levels of BCM (0, 4, 8 and 12 µM). BCM reduced total gas and methane production (>98% of control) in a dose-dependent manner. *In vitro* dry matter digestibility (IVDMD) in different treatment combinations were more or less not adversely affected by BCM but found increased in most of the cases with total mixed diet containing wheat straw and berseem as a roughage source. Acetate concentration was also reduced while, percentage of propionate and butyrate was found increased. Acetate : propionate (A : P) ratio was reduced by BCM addition in all treatment combinations. On the basis of present study 4 mM dose of BCM seems to be effective in methane reduction without affecting other rumen fermentation parameters adversely.

Key words: Bromochloromethane, Methane inhibition, Fermentation, Wheat straw

Methane production in the rumen is metabolically essential but nutritionally wasteful process represented 2 to 15% feed energy loss (Moss 1993, Johnson *et al*. 1991). Livestock methane emission affects the atmosphere adversely as its global warming potential is 21 times more than CO_2 (IPPC 1996) and half life less than 12 years which makes this right candidate for mitigation. Halogenated methane analogues such as bromochloromethane (BCM) are believed to inhibit methane production by reacting with reduced vitamin B_{12} which inhibits cobamide-dependent methanogenesis (Wood *et al*. 1968, Chalupa 1977).

In one study conducted in Brahman *(Bos indicus)* crossbred steers, the supplementation of BCM decreased methanogen populations by 1.72 fold and methane emission by 30% without affecting dry matter (DM) intake and live weight gain (Denman *et al.* 2007). The present experiment has been designed to understand the effect of BCM on methanogenesis and rumen fermentation in diet containing wheat straw containing different level of fibre by *in vitro* gas production test.

MATERIALS AND METHODS

Treatment and diets

Three diets viz. high fiber (80R: 20C), medium fiber (50R: 50C) and low fiber (20R: 80C) were prepared to evaluate the effect of different doses of BCM (0, 4, 8 and 12 µM) on *in vitro* methane production and fermentation pattern. Wheat straw and berseem form the roughage part were mixed together in the ratio of 70: 30 while the concentrate part composed of maize (33%), groundnut cake (21%), mustard cake (12%), wheat bran (20%), deoiled rice bran (11%), mineral mixture (2%) and salt (1%) respectively. The treatments were organized in factorial randomized block design (FRBD) with 3 replications for each treatment combination. Rumen fistulated adult male Murrah buffalo fed on a standard diet (60 parts roughage: 40 parts concentrate) was used as a donor of rumen liquor. A stock solution (0·1: 0·9, v/v) of BCM was prepared in ethanol. The stock solution was further diluted with distilled water to different concentrations and fresh diluted solution was used in the experiment; 200±10 mg substrate along with 30 ml incubation medium (comprising buffer and rumen liquor in 2: 1 ratio) was incubated in 100 glass ml syringe at 39±0.5°C for 24 h as per Menke and Steingass (1988).

Gas sample was collected from the syringe using gas tight syringes and methane proportion in total gas was determined by gas chromatograph fitted with flame ionization detector (FID) and stainless steel column packed with Porapak-Q. Volume of methane produced was calculated as follows:

$$\text{Methane production (ml)} = \text{Total gas produced (ml)} \times \text{\% methane in sample}$$

Present address: [1]Principal Scientist (sirohisk@gmail.com), [2]Research Associate, Dairy Cattle Nutrition Division (navneetgyl40@gmail.com).

In vitro *dry matter digestibility:* To estimate true DM degradability of feed sample of each syringe containing residues after incubation was estimated as per the prescribed method (Goering and Van Soest 1970).

Volatile fatty acids and ammonia nitrogen: The individual volatile fatty acid was estimated in 1.0 ml of fermented medium from each syringe using the standard protocol of Erwin *et al.* (1961) Thereafter, percent IVFA was estimated by gas chromatography (Sirohi *et al.* 2012).

For NH_3–N estimation, 5 ml supernatant was mixed with 1 N NaOH (2 ml) and steam passed on this using KEL PLUS-N analyzer and the NH_3 evolved was collected in boric acid solution having mixed indicator and titrated against N/100 H_2SO_4.

Protozoa counting: The protozoa in fermentation fluid were counted by haemocytometer as per (Dehority 1984).

Proximate analysis and Cell wall constituents: All the substrates were analyzed for proximate principles (AOAC 1995) and fibre fraction (Van Soest *et al.* 1991).

Statistical analysis: Data were analyzed in factorial arrangement in randomized design as per procedure of Snedecor and Cochran (1968). The effects of different diets and different levels of BCM (treatment combinations) were tested using OPSTAT statistical software (Sheoran 2010).

RESULTS AND DISCUSSION

The chemical composition of different diets used as substrate is given in Table 1. Chemical composition of different diets revealed a substantial variation particularly for CP, EE and CF in accordance to roughage and concentrate portion. The protein percentage increased as concentrate part increased and fibre part decreased as concentrate part increased or vice versa this is according to formulation of diets. The effects of different levels of BCM inclusion on *in vitro* rumen fermentation pattern and methane production of different diets presented in Table 2. Results indicated that on the addition of different levels of BCM, pH slightly decreased with maximum reduction in HFD at 8 μM level. The IVDMD values shown that there was not adverse effect of BCM addition, moreover IVDMD was found significantly higher ($P \leq 0.05$) in the treatment combinations having BCM at all levels. The highest increase, 8.87% in IVDMD was observed at 8 μM as compared to control in LFD. These results were more or less contradictory with previous studies. Non-significant affect of BCM on IVDMD were observed in crossbred Brahman cattle (Denman *et al.* 2007) and rams (Lalu *et al.* 2009). BCM addition significantly ($P \leq 0.05$) reduced *in vitro* methane production as compared to control. A reduction of 98–99% in methane production was observed in all the treatment combinations (Table 2). Our results indicated decrease in methane production corresponding to concentration of BCM. These findings are also in accordance to the inferences made by Denman *et al.* (2007), Goel *et al.*(2009) and Lalu *et al.* (2009). The methane reduction tendency of BCM was due to the reacting with vitamin B_{12} and inhibiting the cobamide-dependent methyl transferase step of methanogenesis (Wood *et al.* 1968 and Chalupa 1977). A significant ($P \leq 0.05$) reduction in protozoa number was also observed with increasing levels of BCM. The IVFAs concentration varied among the treatments. The propionic acid concentration increased with increasing dosage in all the 3 diets and highest (38.76%) increase was observed as compared to control at 8 mM level in LFD. Acetate concentration decreased in all cases and highest reduction (14.93%) was noticed as compared to control in LFD at 8 mM level. In present study, butyrate concentration was increased at all levels of BCM and diets. BCM treatments decreased molar proportion of acetate and increased that of propionate, so that the ratio of acetate and propionate (A/P) was also found decreased and highest reduction (38.46%) was found as compared to control in LFD at 8 mM level (Table 2). The similar results were observed during BCM supplementation in earlier findings (McCrabb *et al.* 1997, Goel *et al.* 2009). Similar observations of BCM on acetate, propionate concentration and their A/P ratio were also reported by Denman *et al.* (2007), when they fed diet added

Table 1. Physical and chemical composition of diets used as a substrate

Ingredient of roughage (g/kg on DM basis)							
Wheat straw: Berseem				700 : 300			
Ingredient of diets (g/kg on DM basis)							
		Diets		Roughage			Concentrate
		HFD		800			200
		MFD		500			500
		LFD		200			800
Chemical constituents of diets (g/kg on DM basis)							
Diets	OM	CP	EE	NDF	ADF	HC	TA
HFD (80R: 20C)	842.6	110.8	27.0	541.4	357.3	184.0	157.4
MFD (50R: 50C)	859.0	168.0	32.6	502.2	264.9	237.3	141.0
LFD (20R: 80C)	876.9	229.8	37.9	286.2	199.9	186.3	123.1

HFD, high fiber diet; MFD, medium fiber diet; LFD, low fiber diet; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; HC, hemicelluloses; TA, total ash.

Table 2. Effect of different levels of bromochloromethane on digestibility and methane inhibition in berseem based diets

Diets	Parameters									
	pH	IVDMD%	CH_4 (mM/gDM)	M/TDS (A)	Protozoa ($\times 10^5$/ml)	Acetate (mM/100 ml)	Propionate (mM/100 ml)	Butyrate (mM/100 ml)	A: P ratio	NH_3-N (mg/100 ml)
HFD (0)	7.04	57.25	1.600	0.0276	2.00	68.88	25.99	5.13	2.65	6.62
HFD (4)	7.01	58.93	0.030	0.0005	1.67	63.46	28.85	7.69	2.20	9.15
HFD (8)	6.94	58.95	0.023	0.0004	1.33	66.62	25.46	7.29	2.62	9.24
HFD (12)	6.95	58.01	0.020	0.0004	1.67	65.92	26.69	7.39	2.47	9.71
MFD (0)	6.99	60.95	1.880	0.0302	1.67	68.78	25.37	5.85	2.71	12.88
MFD (4)	6.92	61.15	0.020	0.0003	1.50	61.16	30.55	8.09	1.99	9.65
MFD (8)	6.97	62.69	0.020	0.0003	1.00	60.20	30.70	9.29	1.97	10.17
MFD (12)	6.96	64.63	0.017	0.0002	1.67	62.07	28.52	9.41	2.18	10.27
LFD (0)	6.97	67.20	2.75	0.0407	1.83	69.77	23.30	6.93	2.99	7.28
LFD (4)	6.89	71.58	0.018	0.0003	1.33	62.37	28.68	8.95	2.18	7.65
LFD (8)	6.91	73.17	0.017	0.0002	1.33	59.35	32.33	8.32	1.84	9.43
LFD (12)	6.92	71.16	0.010	0.0002	1.17	60.98	30.39	8.63	2.01	10.36
Least significant difference (LSD)										
Diet	0.010	0.61	0.039	0.0007	0.11	0.92	0.80	0.33	0.10	1.09
Treatment	0.011	0.70	0.046	0.0008	0.13	1.06	0.91	0.38	0.12	1.26
D*T	0.019	N.S	0.080	0.0014	0.22	1.84	1.60	0.66	0.20	2.18

IVDMD, *In vitro* dry matter digestibility; CH_4, methane; A: P, acetate to propionate ratio; NH_3-N, ammonia nitrogen.

with BCM to steers, resulting in a methane reduction of 59%. A significant ($P \leq 0.05$) effect of BCM on ammonia-N concentration was also observed in all treatment combinations.The concentration of ammonia nitrogen increased with increasing concentrations of BCM in HFD and LFD while, decreased in MFD. The maximum increase (46.68%) was found in HFD at 12 mM level. This may be due to some effect on ammonia utilizating bacteria which may be affected due to addition of BCM.

It may be concluded from the present results that BCM addition in diets containing wheat straw and berseem as roughage source significantly reduced methane production in all HFD, MFD and LFD without affecting the fermentability of diet. The BCM level 4 mM emerged out as potent concentration for significant methane reduction without affecting other rumen fermentation parameters adversely. However, more detailed studies are warranted to validate the results under *in vivo* conditions.

ACKNOWLEDGEMENTS

Authors acknowledge the ICAR, Ministry of Agriculture, New Delhi 100012 for providing the grant under National Fund for Basic and Strategic Research in Agriculture (NFBSRA) to carry out research work.

REFERENCES

AOAC. 1995. Association of Official Analytical Chemists. *Official Methods of Analysis*. 16th edn, chapter 4, Pp: 13.Arlington, VA.

Busquet M, Calsamiglia S, Ferret A and Kamel C. 2005. Screening for effects of plant extracts and active compounds of plants on dairy cattle rumen microbial fermentation in a continuous culture system. *Animal Feed Science Technology* **123–124**: 597–613.

Chalupa W. 1977. Manipulating rumen fermentation. *Journal of Animal Science* **46**: 585–99.

Choi N J, Lee S Y, Sung H G, Lee S C and Jong K Ha. 2004. Effects of halogenated compounds, organic acids and unsaturated fatty acids on *in vitro* methane production. *Asian-Australasian Journal Animal Science* **17**(9): 1255–59.

Dehority B A. 1984. Evaluation of sub-sampling and fixation procedures used for counting rumen protozoa. *Applied Environmental Microbiology* **48**(1): 182–85.

Denman S E, Tomkins N W and McSweeney C S. 2007. Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. *FEMS Microbiology and Ecolology* **62**(3): 313–22.

Dohme F, Machmuller A, Wasserfallen A and Kreuzer M. 2001. Ruminal methanogenesis as influenced by individual fatty acids supplemented to complete ruminant diets. *Letters of Applied Microbiology* **32**(1): 47–51.

Erwin E S, Macro G A and Emery E M. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatograph. *Journal of Dairy Science* **44**: 1768–71.

Goel G, Makkar H P and Becker K. 2009. Inhibition of methanogens by bromochloromethane: effects on microbial communities and rumen fermentation using batch and continuous fermentations. *British Journal Nutrition* **101(10)**: 1484–92.

Goering H K and Van Soest P J. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures and some Applications). *USDA Handbook* No. 379, Washington DC, USA.

Hironaka R, Mathison G W, Kerrigan B K and Vlach I. 1996. The effect of pelleting of alfalfa hay on methane production and digestibility by steers. *Science of the Total Environment* **180**: 221–27.

IPCC. 1996. Intergovernmental Panel on Climate Change.

Greenhouse Gas Inventories Revised Methodology. *Guidelines for National Greenhouse Gas Inventories*, Volume **3**. Blackwell.

Johnson D E, Wood A S, Stone J B and Moran E T. 1972. Some effects of methane inhibition in ruminants (steers). *Canadian Journal of Animal Science* **52**: 703–12.

Johnson K A and Johnson J E. 1995. Methane emissions from cattle. *Journal of Animal Science* **73**: 2483–92.

Johnson D E, Hill T M, Carmean B R, Lodman L W and Warm G W. 1991. New perspective on ruminant methane production. *Energy Metabolism of Farm Animal*. (Eds) Wenk C and Boessinger M. Zurich, Switzerland.

Lalu K, Bhar R, Das A and Mandal A B. 2009. Effect of Bromochloromethane supplementation and Dietary Energy Restriction on methane production and efficiency of energy utilization in Rams. *Indian Journal of Animal Nutrition* **26** (2): 97–102.

Martin S A. 1998. Manipulation of ruminal fermentation with organic acids: a review. *Journal of Animal Science* **76**(12): 3123–32.

McAllister T A, Okine E K, Mathison G W and Cheng K J. 1996. Dietary, environmental and microbiological aspects of methane production in ruminants. *Canadian Journal of Animal Science* **76**: 231–43.

McCrabb G J, Berger K T, Magner T, May C and R A Hunter. 1997. Inhibiting methane production in Brahman cattle by dietary supplementation with a novel compound and the effects of growth. *Australian Journal of Agricultural Research* **48**: 323–29.

Menke K H and Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Developement* **28**: 7–55.

Moss A R. 1993. *Methane: Global Warming and Production by Animals*. Chalcombe Publications, Kingston, UK.

Robertson J B and Van Soest P J. 1981. The detergent system of analysis and its application to human foods. Pp. 123. *Analysis of Dietary Fiber in Foods.* (Eds) James W P T and Theander O. Marcel Dekker, New York.

Sheoran O P. 2010. Online Statistical Analysis (OPSTAT) developed by CCS Haryana Agricultural University, Hissar, Haryana, India. http://hau.ernet.in/sheoranop/

Sirohi S K, Pandey P and Goel N. 2012. Response of Fumaric Acid Addition on ethanogenesis, Rumen Fermentation and Dry Matter Degradability in Diets Containing Wheat Straw and Sorghum or Berseem as Roughage Source. ISRN Veterinary Science. doi: 10.5402/2012/496801.

Snedecor G W and Cochran W G. 1968. *Statistical Methods*. 5th edn. Iowa State Univ. Press, Ames, I.A.

Tomkins N W and Hunter R A. 2003. Methane mitigation in beef cattle using a patented antimethanogen. *Proceedings of 2nd Joint Australia and New Zealand Forum on Non-CO_2 Greenhouse Gas Emission from Agriculture*. (Ed.) Eckard R. CRC for Greenhouse Accounting, Canberra.

Van Nevel C J and Demeyer D I. 1995. Feed additives and other interventions for decreasing methane emissions. *Biotechnology in Animal Feeds and Animal Feeding.* pp. 329–49. (Eds) Wallace R J and Chesson A. VCH, Weinheim, Germany.

Van Soest P J, Robertson J B and Lewis B A. 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–97.

Wood J M, Kennedy F S and Wolfe R S. 1968. The reaction of multihalogenated hydrocarbons with free and bound reduced vitamin B_{12}. *Biochemistry* **7**: 1707–13.