



Effect of fumaric acid supplementation on methanogenesis and rumen fermentation in Barbari goats

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ABSTRACT

Methane production in barbari goats was studied using Open Circuit Respiration chamber. A metabolism trial followed by respiration chamber trial was conducted in adult male Barbari goats (mean body weight of 15 ± 0.7 kg) which were divided into 3 groups (control, T₁ and T₂) of 2 each as per 2x3 switch over design. Animals were fed concentrate mixture and wheat straw in a ratio of 30:70 to meet out their nutrient requirements (NRC 2007). Animals of treatment 1 and 2 were fed on a diet supplemented with fumaric acid (FA) @ 2.5 and 5% of DM intake, respectively. The rumen liquor collection for rumen fermentation parameters and a respiration chamber study was carried out after metabolism trial. There was no significant difference in rumen fermentation parameters (ammonia nitrogen, total nitrogen, TCA-precipitable nitrogen, TVFA and protozoal counts) among 3 groups due to FA supplementation. However, linear increase in rumen pH with increasing levels of FA supplementation was highly significant ($P < 0.01$). Production of methane (L/d) did not differ significantly among the groups. But when expressed as L/kg DM intake, methane production was significantly ($P < 0.05$) reduced (18.45 and 14.97%) in FA supplemented group as compared to control group. The results showed that FA supplementation (@ of 2.5 and 5.0% of DMI) reduced substantially methane production (L/kg DMI) without altering rumen fermentation pattern in the Barbari goats.

Key words: Fumaric acid, Goat, Methane, Open Circuit Respiration Chamber, Rumen fermentation

Ruminants produce 97% of the methane generated in enteric fermentation (Mathison *et al.* 1998), but methanogenesis is an inescapable consequence of rumen fermentation as it is essential for utilization of reducing equivalents synthesized during carbohydrate fermentation in rumen; but being a carbon sink it lowers ruminal conversion efficiency. Energy lost as methane from cattle range from 2 to 12% of GE intake (Johnson and Johnson 1995) and the other major concern of methane emission is due to its greenhouse effect as it has 23 times more global warming potential as compared to carbon dioxide (Gowri and Danielle 2008). Antimicrobials are used in animal production but in many countries are either banned or seldom used due to microbial resistance and also concern for consumer health. This has necessitated the search of newer strategies to reduce methane production. One such possible way to impede methanogenesis is to promote alternative metabolic pathways to dispose of the reducing power, competing with methanogenesis for the H₂ uptake. Feed additives were used *in vitro* to reduce methane emissions, e.g. organic acids (Asanuma *et al.* 1999), malate (Martin 1998), fumarate

(Lopez *et al.* 1999), and acrylate (Ouda *et al.* 1999). Some reported variable reduction in methane production by supplementing fumaric acid (Bayaru *et al.* 2001, Newbold *et al.* 2002). Fumaric acid, a propionate precursor and key intermediate product in the succinate-propionate pathway (Russell and Wallace 1997), is reduced to succinic acid. In this reaction, reducing equivalents are needed and therefore, fumaric acid may provide an alternative electron sink for hydrogen. As a result, ruminal propionic acid formation was increased, thus fumaric acid has beneficial effects through decreased methane production and increased plasma glucose concentration resulting in net energy retention. Therefore, if precursors of propionate are added to the diet, they should reduce methane production by removing hydrogen produced during ruminal fermentation. The aim of the present study was to study the effect of supplemental fumaric acid on methanogenesis and rumen fermentation in goats.

MATERIALS AND METHODS

The experiment was carried out at Animal Nutrition Sheds of the institute

Feeding management: Animals were fed concentrate mixture and roughage (wheat straw) in a ratio of 30:70 at maintenance level (NRC 2007). The concentrate mixture was

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compounded by mixing crushed maize, wheat bran, soybean meal, mineral mixture and common salt in the ratio of 25, 22, 50, 2 and 1, respectively. All the goats were provided with 50 g available green daily to meet out their vitamin A requirement. Weighed amount of concentrate mixture was provided at 9.00 AM daily to meet out almost entire CP and greater part of TDN requirement. Wheat straw was provided *ad lib.* after complete consumption of concentrate mixture. Animals of treatments 1 and 2 were fed on diets supplemented with fumaric acid @ 2.5 and 5.0% of DM intake, respectively. Required quantity of fumaric acid was weighed and mixed in concentrate mixture daily and offered to the animals once in the morning.

Experimental protocol: A 2×3 switch over design was used for this experiment. Each period of experiment consisted of 30 days of feeding trial followed by 3 days for methane estimation in Open Circuit Respiration Chamber. During this period daily feed (DM) intake was recorded. After each period, animals were kept on control feeding for 7 days to minimize carry over effect. The animals were weighed (before feeding and watering) in the beginning and at the end of each period to record live weight changes during that period.

Measurement of methane production: After 3 days of adjustment period during which total faeces and urine was collected and sampled. The gas exchange of individual animal was measured for 24h. Hastings mass flow meter was used to record the flow rate and total volume of air coming out of respiration chamber. Dry and wet bulb temperatures were recorded. Atmospheric pressure was recorded electronically. Oxygen consumption, carbon dioxide and methane production were determined by measuring the total air flow in the system and the difference in concentration of respective gases between out flow and incoming air. Sub-samples of chamber out flow air were collected in Douglass bags. The samples of outflow and incoming air collected were analyzed for oxygen by a dual type pragmatic oxygen analyzer with a 100 ml burette and methane by infra Red (IR) gas analyzer.

Analysis of rumen liquor: The representative rumen liquor samples at 0 h feeding were collected at the end of each respiration chamber study. About 50 ml of rumen liquor was collected at a time and strained through the muslin cloth and was taken to laboratory immediately in cold condition and pH was recorded. Strained rumen liquor (SRL) sample (25 ml) from each animal was poured into a bottle having 1:4 H₂SO₄ (to preserve SRL) for estimation of N fractions and for estimation of TVFA. The pH of SRL was determined by using a digital pH meter immediately after collection. For counting of rumen protozoa equal amount of rumen liquor and formal methyl green were mixed and kept overnight for proper staining. Glycerol (30%) was used for further dilution. The number of protozoa was counted under the microscope (10×) in a known volume of sample and the total number was calculated (Kamra *et al.* 1991). For the analysis of

different nitrogen fractions in the rumen liquor (SRL) were pipetted and total-N by Micro-Kjeldhal procedure (AOAC 1995). TCA pricipitable-N (Lowry *et al.* 1951) and ammonia-N (Weatherburn 1967) were analyzed. Total volatile fatty acids were analyzed by Markham apparatus method.

Statistical analysis: The data generated was subjected to general linear model univariate (SPSS 2002).

RESULTS AND DISCUSSION

Animals were fed concentrate mixture and wheat straw in a ratio of 30:70 to meet entire CP and greater part of TDN requirement (NRC 2007). Chemical composition of concentrate mixture and wheat straw is given in Table 1.

Table 1. Chemical composition (% DM basis) of concentrate mixture and wheat straw

Attributes	Concentrate mixture	Wheat straw
Organic matter	90.01	91.68
Crude protein	24.97	3.27
Ether extract	2.23	1.09
Neutral detergent fibre	33.54	85.81
Acid detergent fibre	17.03	63.86
Hemicellulose	16.51	21.94
Total carbohydrates	55.77	87.33
Calcium	1.11	0.78
Phosphorus	0.23	1.06

Methane production: Methane production data on respiration chamber studies are presented in Table 2. However, methane production (L/kg) per unit of dry matter digested and organic matter digested did not differ significantly. Similarly total dry matter (DM) intake and digested DMI (g/d) and organic matter digested (g/d) did not differ significantly between the groups. Methane production per kg DM intake differed significantly ($P<0.05$) and the values were 28.15 ± 1.86 , 23.21 ± 1.63 and 24.19 ± 3.01 in 3 respective groups. This showed significant ($P<0.05$) decrease (14.97 to 18.45%) in methane emission owing to FA supplementation (@ 2.5 and 5.0% DMI, respectively). This is in agreement with the results of Wood *et al.* (2009) who supplemented 100 g/kg of FA and encapsulated FA in growing lambs. Bayaru *et al.* (2001) reported that FA supplementation (20 g/kg diet DM) reduced CH₄ production by 23.0% in steers. Similarly Newbold *et al.* (2002) and Wallace *et al.* (2005) also found large reductions in methane emissions. In contrast McGinn *et al.* (2004) who supplemented 80 g/d FA in growing beef cattle found FA had no effect on CH₄ emission, and Beauchamin and McGinn (2005) also found no measurable effect on CH₄ emission by supplementing FA (175 g/d with sodium bicarbonate 75 g/d) in Angus heifers. German *et al.* (2008) also found similar results in wether lambs.

Rumen fermentation: Effect of supplementation of fumaric acid on rumen fermentation parameter, viz. mean values of

Table 2. Methane production in different groups during respiration studies

Attributes	Control	T ₁	T ₂	SEM
CH ₄ (L/d)	10.08±1.80	10.08±2.09	7.82±1.03	1.117
CH ₄ (L/kg DMI)*	28.45 ^a ±1.86	23.2 ^b ±1.63	24.19 ^b ±3.01	1.556
CH ₄ (L/kg DMD)	53.88±4.04	45.09±4.24	47.17±8.25	3.646
CH ₄ (L/kg OMD)	54.50±4.34	45.84±4.15	47.85±8.22	3.833
DMI (g/d)	346.80±45.31	423.82±65.44	322.78±7.14	53.742
DM digested (g/d)	182.76±22.35	216.82±26.45	170.45±10.62	30.285
OM digested (g/d)	180.38±21.06	213.55±27.62	167.53±9.55	29.608

^{abc} Means bearing different superscripts in a row differ significantly; * (P<0.05).

Table 3. Rumen fermentation pattern in different groups

Attributes	Control	T ₁	T ₂	SEM
Total nitrogen (mg/dl)	127.92±2.92	125.83±3.27	127.50±3.10	4.310
Ammonia nitrogen (mg/dl)	9.72±0.66	9.34±0.29	9.25±0.22	0.606
TCA-ppt. N (mg/dl)	47.94±5.35	51.35±4.37	51.50±5.14	4.377
pH**	6.60 ^b ±0.18	6.70 ^b ±0.20	6.83 ^a ±0.21	0.042
Total VFA (mmol/dl)	7.02±0.34	6.72±0.23	6.67±0.35	0.417
Protozoa (10 ⁶ /ml)	1.27±0.05	1.21±0.04	1.22±0.04	0.072

^{abc} Means bearing different superscripts in a row differ significantly; ** (P<0.01).

nitrogen fraction, total volatile fatty acid, protozoal count and pH in rumen liquor are presented in Table 3. There was no significant difference among treatments in any of parameters except rumen pH. This is in agreement with Lopez *et al.* (1999) and Yuangklang *et al.* (2005). Conversely, Abdl-Rahman *et al.* (2010) reported a decremental effect on ammonia nitrogen concentration. The significant linear increase (P<0.01) in rumen pH with increasing level FA supplementation in the present study corroborated well with the findings of German *et al.* (2008), but Asanuma *et al.* (1999) suggested that the quantity of the free acid fed has to be restricted as it causes drop in ruminal pH and digestive problems (Castillo *et al.* 2004). Wood *et al.* (2009) also reported when free FA is used the pH of the digesta might be compromised. However, Bayaru *et al.* (2001) and Beauchemin and McGinn (2005) reported no effect on pH by FA supplementation. In the present study the linear increase in rumen pH with increasing FA supplementation levels was unexpected.

It is deduced that supplementation of fumaric acid at 2.5 and 5.0% in the diets of goats reduced methane production by 18.45 and 14.97%, respectively, when expressed as L/kg DMI without altering rumen fermentation.

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