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Effect of ration balancing on methane emission, faecal archaeol concentration and its relation to enteric methane in crossbred cows

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ABSTRACT

A field study was conducted to evaluate the effect of ration balancing on methane emission, faecal archaeol and its relation with enteric methane in crossbred cows. Crossbred cows (25) in their early stage of lactation (60–80 d postpartum) were identified under field conditions. After recording their average daily feed intake and milk yield for 1 week, methane emission was measured by SF_6 tracer technique. A balanced ration was worked out for individual cows and fed to them for 30 d. After feeding a balanced ration, feed intake and methane emission was measured again. On feeding a balanced ration, methane emission (g/d) was reduced by 10.1%. Archaeol concentration in faeces being considered as an indicator of methane emission, its concentration in faeces was measured before and after feeding a balanced ration, which reduced significantly. There existed a significant correlation (r=0.12) between methane emission measured by SF_6 tracer technique (g/d) and archaeol concentration (mg/kg DM of faeces). However, more data need to be generated in this regard.

Key words: Crossbred cows, Faecal archaeol, Methane emission, Ration balancing

Greenhouse gases are well known for their contribution to climate change and global warming through absorption of infrared radiation in the atmosphere. Methane (CH_4) is the second largest anthropogenic greenhouse gas, behind carbon dioxide especially due to its 25 times higher global warming potential (Forster *et al.* 2007). Ruminant livestock contribute up to 50% of the total methane emission in India (INCCA 2010). Over a wide range of diets, enteric methane accounts for 2 to 12% loss of dietary gross energy intake and such losses are higher when the diet of ruminants is imbalanced in terms of energy, protein, minerals and vitamins (Garg *et al.* 2012). Therefore, manipulating diet composition to induce changes in rumen fermentation characteristics remains the most feasible approach to achieve reduction in methane emission (Bayat and Shingfield 2012). Measurement of methane emission from ruminants by sulphur hexafluoride (SF_6) tracer technique is energy intensive and time consuming. Alternatively, methane emission from ruminants can also be estimated by using concentration of archaeol derivatives in faeces (Gill *et al.* 2011). In the present study, a relationship between methane emission from ruminants using SF_6 technique and concentration of archaeol in faeces was studied to see whether or not archaeol concentration in faeces could be used as an indicator of methane emission in field animals and the results of the study are reported in this communication.

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MATERIALS AND METHODS

Experimental design: Early lactating (60–80 d postpartum) crossbred cows (25) belonging to 22 farmers were identified from 3 villages of Banaskantha district in Gujarat. The selected cows were in their third lactation, with an average milk yield 13.3 kg/d. The feed intake of individual animal was measured and representative sample was taken for analysis of proximate principles. Thereafter, the ration of all animals was balanced for crude protein (CP), metabolisable energy (ME), calcium and phosphorus using the ration balancing (RB) software developed by National Dairy Development Board (NDDB), which is based on National Research Council (1989) standards for cattle. The balanced diet was fed to all the cows for 30 days.

Methane measurement: Methane emission from the cows was measured using SF_6 tracer technique (Johnson *et al.* 1994). About 5 days prior to collection of respiratory gases, all cows randomly received a previously calibrated SF_6 permeation tube in the form of oral bolus, releasing an average 2.1±0.10 mg of SF_6/d (range 1.34 to 3.16 mg/d). The breath samples of all experimental cows were collected daily for four consecutive days in canisters for analysis of methane and SF_6 at the start of study. After feeding the balanced ration for 30 d, the breath samples were collected again in a similar manner. Methane and sulfur hexafluoride

concentrations were analysed in triplicate by gas chromatography technique. Methane emission rate was calculated as under.

$$Q\ CH_4 = Q\ SF_6 \times (CH_4)/(SF_6)$$

where: Q CH_4, methane emission rate (g/min); Q SF_6, known release rate of SF_6 from permeation tube (g/min); CH_4, methane concentration of collected sample in canister ($\mu g/m^3$); SF_6, SF_6 concentration of collected sample in canister ($\mu g/m^3$).

Faecal analysis: Analysis of faecal archaeol was conducted as per Gill *et al*. (2011). Fresh faecal samples were collected once daily over 4 days during the week, after methane measurement. Approximately, 200 g faecal samples were obtained by rectal grab, stored at –20°C, thawed, composited and dried at 60°C for 48 h. Total lipid extract (TLE) was obtained from samples (0.5–1.0 g) of dried, ground (1 mm screen) faeces through solvent extraction in 200 ml of dichloromethane (DCM): acetone (9:1 v/v) for 24 h using Soxhlet apparatus. Internal standard (100 µg of preg-5-en-3β-ol) was added to the TLE. An aliquote of TLE was subjected to saponification to separate 'neutral' and 'acid' fractions (Bull *et al*. 2003). Neutral fraction was further subjected to column chromatography to obtain 'alcohol' fraction (Bull *et al*. 1999). Analytes in the alcohol fraction were derivatised and thereafter analyzed by gas chromatography (system equipped with a non-polar fused silica capillary column, CPSil–5CB, 50 m × 0.32 mm × 0.12 µm). The temperature programme used was: initial temperature 70°C, rising to 130°C at 20°C/min, then to 300°C at 4°C/min, with a final hold at 300°C for 25 min. Archaeol was identified and quantified by comparison to the internal standard.

Laboratory analysis: Feeds and fodder samples were analyzed for proximate composition as per AOAC (2005). Gross energy of feed and fodder samples was calculated as per the conversion factors of Jarrige (1989). Energy content of methane was taken as 13.34 kcal/g (Brouwer 1965).

Statistical analysis: The statistical analysis of the data was by Student's 't' test as per Snedecor and Cochran (1994) with the SPSS package (1999).

The statistical model was:

$$t = \frac{\bar{X}_1 - \bar{X}_2 - \Delta}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

where: $\bar{X}_1$ and $\bar{X}_2$, mean of two samples; Δ, hypothesized difference between the population means, S_1 and S_2, standard deviation of 2 samples; n_1 and n_2, size of 2 samples and n–1, degrees of freedom.

RESULTS AND DISCUSSION

Plane of nutrition: Feeding practices followed by the farmers were found imbalanced in terms of nutrient requirements of animals as evident from the overfeeding of CP, ME and Ca up to the extent of 18.1, 8.0 and 19.5%, respectively, and underfeeding of P up to the extent of 9.0%.

Table 1. Effect of ration balancing (RB) on plane of nutrition of cows

Parameter	Before RB	After RB	SEM
Concentrate: roughage ratio	42: 58	45: 55	-
DM intake (kg/d)	14.5	13.7	0.37
DM intake (kg/100 kg body wt)	3.8	3.5	0.10
OM intake (kg/d)	13.0	12.2	0.33
CP intake (g/d)	1780.4	1729.2	71.52
ME intake (Mcal/d)	28.8	27.1	0.22
GE intake (Mcal/d)	31.9	30.6	0.98

SEM, standard error of difference between means.

Slightly lower (P>0.05) intakes of DM, CP and ME in present study (Table 1) was mainly due to restriction on intakes of nutrients after RB which otherwise fed excess by the farmers and is in accordance to Erickson *et al*. (1998).

Methane emission and faecal archaeol: In present study, feeding of balanced ration to cows reduced enteric methane emissions by 10.1% (P<0.01). Similarly, energy loss as methane was also reduced (P<0.01) from its initial level of 2.8 Mcal/d to 2.5 Mcal/d (Table 2) similar to Mohini and Singh (2010) and Kannan *et al.* (2010). In present study, lower methane emissions on higher concentrate diet after RB might be due to several factors, like higher ruminal passage rates, an increase in the flow of reducing equivalents to propionate and direct inhibition of methanogens at low pH similar to earlier reports (Johnson and Johnson 1995, Van Kessel and Russell 1996). Methane emission relative to DM and OM intake was unaffected after ration balancing (P>0.05, Table 2) mainly due to similar DM intake of animals (Table 1). Changing feeding pattern of cows from imbalanced to balanced ration reduced (P<0.01) GE loss as methane (Table 2) similar to Mohini and Singh (2010) where GE loss as methane was higher (9.1 vs. 7.0%) in underfed cows compared to cows fed as per requirements.

The occurrence of archaeol in faeces of cows was detected chromatographically (Fig. 1) and quantified in comparison to the internal standard. It was observed that after RB, excretion of faecal archaeol reduced (P<0.01) by 23.1% (158.3 vs. 121.6 mg/kg faecal DM, Fig. 2) with concomitant reduction in methane emission. In present study, after RB the proportion of concentrate in the diet of animals increased which might have resulted in lower

Table 2. Effect of ration balancing (RB) on methane emission from lactating cows

Parameter	Before RB	After RB	SEM
Methane emission (g/d)	211.4[a]	190.1[b]	5.70
Methane emission (g/kg DM intake)	14.5	13.8	0.62
Methane emission (g/kg OM intake)	17.1	15.8	0.70
Energy loss as methane (Mcal/d)	2.8[a]	2.5[b]	0.07
Energy loss as methane (% of GE)	8.8	8.2	0.48

Means with different superscripts in a row differ significantly ([a,b]P<0.01), SEM, standard error of difference between means.

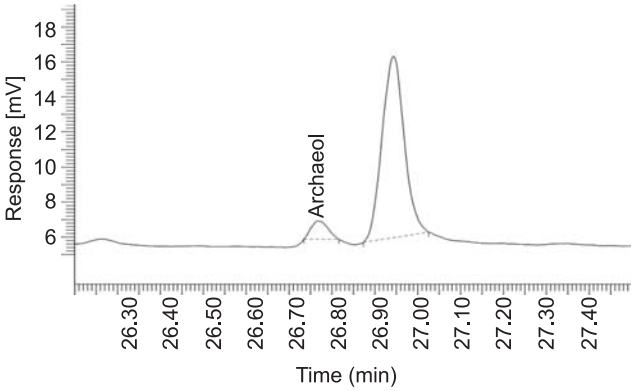

Fig. 1. Partial gas chromatogram showing occurrence of archaeol in the faeces of lactating cows.

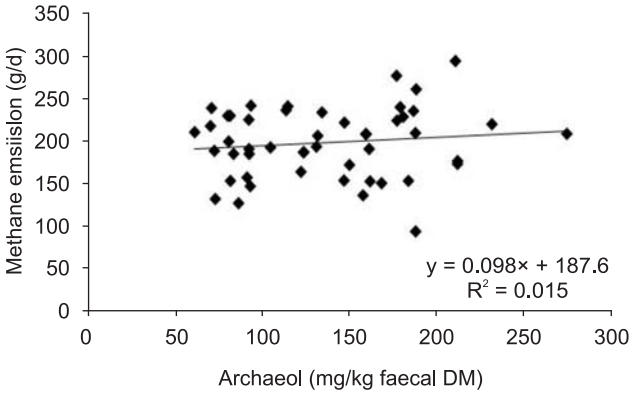

Fig. 3. Correlation between archaeol concentrations in bovine faeces with methane emission

emissions of methane and lower concentration of archaeol derivatives in the faeces. Similar to these findings, Gill *et al*. (2011) also reported lower methane emission (174 vs 341 g/d) and faecal archaeol concentration (5.1 vs. 30.6 mg/kg DM) on concentrate based diets compared to silage based diets. It is apparent that archaeol is generated during passage through digestive tract, as its absence in feed is already confirmed (Gill *et al.* 2011). Several reports also indicated that the rumen is the most probable source of archaeol (Janssen and Kirs 2008, Gill *et al*. 2010). Analysis of the 16S RNA gene also confirmed the absence of higher proportion of archaea in the hind gut compared to the rumen (Lin *et al*. 1997).

The methane emission data obtained by SF_6 technique and concentration of archaeol derivatives in faeces revealed that there existed a significant correlation between them (r=0.12, P<0.01, Fig. 3). These findings are corroborated with Gill *et al*. (2011) who indicated a relationship between faecal archaeol concentration and methane emission per unit DM intake (r=0.55, P<0.05). There are several unknown factors that may influence this relationship and includes selective retention of archaea in the rumen, differences in the species composition of the methanogen community contributing to differences in the archaeol concentration per cell and differences in the post-rumen digestibility of dialkyl glycerol ether i.e. archaeal lipids. In field conditions it requires large number of animals to be sampled for methane emission and faecal archaeol concentration to study the relationship between them and to our knowledge such reports have not been documented yet.

In conclusion, ration balancing under tropical field conditions can be used as a strategy to reduce enteric methane emission (g/d) up to 10%. Archaeol can be used as a biomarker for methanogens, but more data need to be generated to establish a relationship between archaeol derivatives in faeces and methane emission measured by SF_6 tracer technique. If the correlation between faecal archaeol derivatives and methane emission is confirmed, then there is a potential for faecal archaeol to be developed as a proxy for ruminant methanogenesis and it can be used as an alternative method to estimate methane emission from ruminants.

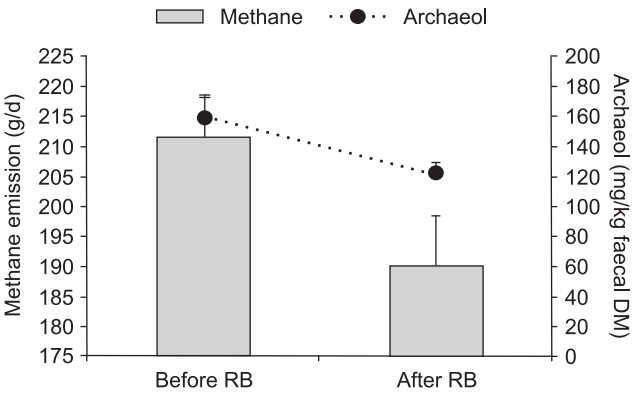

Fig. 2. Association between faecal archaeol concentration and methane emission in lactating cows.

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