Indian Journal of Animal Sciences **78** (3): 293–297, March 2008

Influence of lucerne fodder supplementation on enteric methane emission in crossbred calves

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Received: 10 March 2007; Accepted: 5 October 2007

ABSTRACT

Crossbred calves (6) were fed on wheat straw based total mixed ration (TMR–1) consisting R: C 60:40 and than shifted on isonitrogenous TMR–2 in which 50% wheat straw of TMR–1 was replaced with lucerne fodder (saponins 2%) on DM basis. DMI, nutrient utilization and methane emission were studied on both feeding regimes. DM intake (kg/d) in crossbred calves was higher on TMR–2 than on TMR 1. DMI/100 kg BW was higher on feeding TMR–2 than on TMR. Similarly, nutrient digestibility and nitrogen intake on feeding TMR–2 was higher than that on TMR–1, which, resulted in higher nitrogen excretion through faeces and urine. Methane emission (g/ kg DMI) on feeding TMR–1 and TMR–2 was 17.38±1.49 and 12.20±0.99, respectively, showing reduction of methane emission by adding 30% lucerne fodder (second cut) in TMR–2 as compared to TMR–1, which was attributed to the dietary saponins through lucerne fodder (total intake 21.78g). Gross energy loss in the form of methane was more on feeding TMR–1 (5.44%) than that on TMR–2 (3.72%), showing that the incorporation of lucerne fodder to the wheat straw based total mixed ration diet at 30% level can increase DM intake and nutrient utilization and reduce methane emission. Therefore, the incorporation of saponins in the form of lucerne in total mixed ration at the level of 0.6%/kg DMI resulted in reduction in methane emission.

Key words: Calves, Methane emission, Lucerne fodder, Nutrient digestibility

India possess about one-fifth of the world's total animal population, which thrive mainly on crop residues based ration. Hence, India is being held responsible for their large contribution to the methane emission due to enteric fermentation of low quality roughages, which are responsible for higher methane emission than that from good quality green fodders. Methane is the second largest contributor to global warming after CO_2 and 40–60 times more potent than CO_2 in heat trapping (Khan *et al.* 2001). Annually methane emission in India is around 9–10 Tg (Singhal and Mohini 2002) in which cattle emit approximately 5.50 Tg (Singh 2001). Apart from the global warming, methane emission from the ruminants also represents a loss of dietary energy and this loss may be up to 6–12% of energy intake (Johnson and Johnson 1995). This loss is significant in the animal production system being practiced in our country because feed cost accounts 60–70% of total cost of animal production. Reduction in enteric methane emission through dietary supplementation of halogenated methane analogues, dietary lipid and ionophores had been attempted but these have their own limitations therefore, not being adopted at farmers' level.

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Rumen protozoa provide hydrogen to the attached methanogens for the reduction of CO_2 to methane (Hess *et al.* 2003). Therefore, reduction in rumen protozoa numbers may serve the purpose to mitigate enteric methane emission. Saponins adversely affect rumen protozoa and thereby reduce methane emission; as various plant species having saponins like *Sapindus saponaria* (Diaz *et al.* 1993), *Sapindus rarak* (Thalib *et al.* 1995) and *Yucca schidigera* (Wang *et al.* 2000), also decreased enteric methane emission through the reduction in rumen protozoa numbers. Lucerne fodder of second cut was selected for the supplementation to the crossbred calves as it was having highest saponin content among the cuts hence, the study was planned to investigate the effect of saponins rich lucerne fodder (second cut) supplementation to the wheat straw based total mixed ration on nutrient utilization and enteric methane emission in crossbred calves.

MATERIALS AND METHODS

Six male crossbred calves (Karan Fries, BW 105.08±2.40) selected from the institute herd, were initially fed on wheat straw and concentrate based total mixed ration (TMR–1, R:C 60:40) for 21 days to acclimatize the ruminal microflora to the diet. Metabolism trial of 7 days duration after preliminary

Table 1. Composition of total mixed ration used for the feeding of control and experimental crossbred calves

Ingredients	Composition (% on DM basis)						
	DM	OM	CP	EE	ADF	NDF	Ash
Wheat straw	90.04	93.18	3.00	1.12	51.80	76.20	6.19
Lucerne (II cut)	23.18	89.42	16.70	3.20	35.83	43.64	10.58
Concentrate (TMR–1)	94.90	92.30	25.70	3.92	14.23	39.59	7.70
Concentrate (TMR–2)	95.16	93.29	15.75	3.68	13.97	34.68	6.71

TMR–1consisted of wheat straw 60 parts and concentrate mix. 38 parts, urea 0.80 parts, mineral mixture 0.80 parts, and salt 0.40 parts. TMR–2 consisted of wheat straw 30 parts, lucerne fodder 30 parts, concentrate mixture 28 parts, wheat bran 10.8 parts, mineral mixture.80 parts, and salt 40 parts.

feeding was conducted and than 4 successful collection of gas sample released due to enteric fermentation, from each animal was taken to analyze the methane in the sample using sulphur hexafluoride gas technique (Johanson *et al.* 1994). After completing the studies on nutrient utilization and methane emission, these calves were shifted on experimental diet (TMR–2) in which lucerne fodder (second cut) was supplemented at 30% level maintaining the roughage and concentrate ratio similar to TMR–1. The saponin content of lucerne fodder was 1.17±0.04, 2.00±0.05 and 0.92±0.05% on DM basis at their first, second and third cut, respectively. second cut lucerne fodder was selected for the study in view of its higher saponin content among the 3 cuts of fodder in our *in vitro* study. Chemical composition of TMR–1 and TMR–2 is given in Table 1. Animals were kept in well ventilated shed having an arrangement for individual feeding. To avoid the external and internal parasites infection/ infestation, butox 0.5% (v/v) and albandazole (0.5 mg/kg bw) were applied. Control ration (TMR–1) was prepared by mixing wheat straw and concentrate mixture in the ratio of 60:40. Concentrate mixture consisted of maize grain 33%, groundnut cake 20.2%, mustard cake 12%, wheat bran 20%, deoiled rice bran 11%, urea 0.8%, mineral mixture 2% and common salt 1%. Experimental ration (TMR–2) was prepared by mixing wheat straw, lucerne fodder, concentrate mixture, which consisted of maize grain 24%, groundnut cake 15%, mustard cake 8%, wheat bran 42%, deoiled rice bran 8%, mineral mixture 2% and common salt 1% in the ratio of 30:30:40. Variation in composition of concentrate mixture used in TMR–2 was introduced to make both the TMR isonitogenous (CP 12.0%). Both the total mixed rations were prepared in such a manner that roughage to concentrate ratio remained at 60:40. Feeding of the animals was done as per NRC (2001). Feed, feed residues and faeces samples were analyzed for their proximate principals (AOAC 1995) as well as fibre fractions (Goering and Van Soest 1970) i.e. NDF and ADF and chemical composition of the different dietary ingredients has been given in Table 2. Urine samples were analyzed for their N content as per AOAC (1995). Analysis of the gas samples was done in the Environmental Laboratory of the Institute using gas chromatograph. The methane emission rate (QCH_4) was calculated from the ratio of CH_4 to SF_6 (Johnson *et al.* 1994) in collected gas and the known release rate of SF_6 (QSF_6). Background methane [$\{CH_4\}$ b] was subtracted from methane concentration in the PVC canister [$\{CH_4\}$ Y].

$$QCH_4 = \frac{QSF_6[\{CH_4\}Y - \{CH_4\}b]}{[SF_6]}$$

Table 2. DMI, nutrient digestibility and nitrogen utilization in crossbred calves as influenced by lucerne fodder supplementation in total mixed ration

Attributes	Crossbred calves	
	TMR–1	TMR–2
Body weight (kg)	105.08±2.40	130.83±2.48
DMI (kg/d)	2.85ᵃ±0.40	3.63ᵇ±0.13
DMI (kg/100 kg BW)	2.70±0.20	2.94±0.34
Digestibility (%)		
DM	53.93ᵃ±1.63	59.73ᵇ±1.03
OM	61.29ᵃ±1.72	68.44ᵇ±1.84
EE	64.25ᵃ±2.14	72.39ᵇ±0.620
CP	58.46ᵃ±1.03	65.15ᵇ±0.86
ADF	54.39ᵃ±1.37	61.44ᵇ±1.78
NDF	61.46ᵃ±0.66	68.34ᵇ±1.48
N utilization		
N intake (g/d)	55.02ᵃ±1.78	70.91ᵇ±0.58
N excreted (g/d)		
Faeces	20.46±1.08	24.72±0.73
Urine	22.92±0.72	30.74±1.21
Total	43.38	55.46
Urinary N as% total N intake	41.65	43.35
Faecal N as% total N intake	37.18	34.86
N absorbed (g/d)	34.55ᵃ±1.47	46.19ᵇ±0.73
N retained (g/d)	11.63ᵃ±0.87	15.44ᵇ±0.77
N retained as% of total N intake	21.13	21.77
Faecal N/ kg DM consumed (g)	71.17	6.80

Values bearing a,b superscripts in a row differ significantly ($P<0.05$).

The data thus obtained were subjected to the statistical analysis as per the procedure of Snedecor and Cochran (1986).

RESULTS AND DISCUSSION

DM intake

Higher ($P<0.05$) DM intake (kg/d) in crossbred calves fed on TMR–2 than on TMR–1 may be attributed to the replacement of wheat straw with highly palatable and nutritious lucerne fodder. Though, DMI/100 kg B.wt. was also higher on TMR–2 than that on TMR–1, but the variation between both the groups was not significant (Table 3). On the basis of saponins content of lucerne fodder (1.17±0.04, 2.00±0.05 and 0.92±0.05% on DM basis at first, second and third cut, respectively), lucerne fodder of second cut was selected for the supplementation at 30% level because this level of supplementation was found the most promising and effective in methane reduction in our *in vitro* study. The level of saponins in diet of animals received lucerne fodder based TMR was 0.60% of DM intake and total saponins intake was 21.78 g/day. Therefore, it can be inferred that saponin intake up to 22 g/day through lucerne fodder did not interfere the DM intake adversely. Similar to these observations, Said and Tolera (1993) reported higher DM intake following the supplementation of small amount of leguminous fodder however, saponins intake through fenugreek seed did not affect DM intake (El-Nor 1999).

Table 3. Effect of lucerne fodder supplementation on enteric methane emission in crossbred calves

Attributes	TMR–1	TMR- 2
GE intake (Mcal)	12.15	15.86
DE intake (Mcal)	7.22	9.82
ME intake (Mcal)	5.89	7.98
Methane emission		
g/day	49.55^{a}±2.61	44.29^{b}±1.92
g/kg DMI	17.38^{a}±1.49	12.20^{b}±0.99
g/kg DDM	11.31^{a}	5.62^{b}
Methane energy (Mcal)	0.66	0.59
Methane as% of GEI	5.44	3.72

Values bearing a, b superscripts in a row differ significantly ($P<0.05$).

Nutrient digestibility

DM digestibility of TMR–2 was higher ($P<0.05$) than that of TMR–1 in crossbred calves, which may be attributed to its higher soluble carbohydrates and better fibre quality of lucerne fodder than that of wheat straw as reported by Das (1996). Higher ($P<0.05$) organic matter digestibility of TMR–2 than of TMR–1 attributed to the association of rapid fermentation of ration and higher production of bacterial biomass as a result of lucerne fodder supplementation as was observed in our study (data not presented). Similar observations were recorded following the supplementation of trifolium hay to oat straw based diet (Mosi and Butterworth 1985). The EE digestibility in calves fed on TMR–2 was significantly higher ($P<0.05$) than that on TMR–1. The CP digestibility in calves fed on TMR–2 was higher ($P<0.05$) than those fed on TMR–1. These results are in agreement with the findings of Chauhan (1986) who supplemented trifolium hay to straw based diet in lactating buffaloes. ADF and NDF digestibility was significantly ($P<0.05$) higher in lucerne supplemented group (TMR–2) than on feeding TMR–1. The significant increases ($P<0.05$) in ADF and NDF digestibility of TMR–2 may be attributed to the higher degradability, more soluble carbohydrates and highly colonizable fibre of lucerne fodder than that of wheat straw however, Klita *et al.* (1996) reported no significant effect of alfalfa root saponins on NDF digestibility in sheep. These results revealed that fibre digestibility in TMR–2 due to supplementation was increased and the saponins of lucerne fodder did not affect it adversely. It appeared from these results that increase in nutrient digestibility following the supplementation of lucerne fodder was due to its higher carbohydrates and protein contents rather than its saponins content as substantiated by Allam *et al.* (1999) following the supplementation of saponins rich fenugreek seed (500 mg/kg) in Zaraibi goats.

Nitrogen balance

N intake (g/d) in crossbred calves fed on TMR–2 was higher ($P<0.05$) than those fed on TMR–1 and value was higher by 29%, which may be due to the higher intake of ration and better quality of protein of lucerne fodder. N intake/kg of digestible organic matter was almost similar in both groups showed that the supply of nitrogen was adequate for the optimum functioning of rumen. Lila *et al.* (2005) reported that the addition of 0.5 and 1% (DM basis) sarsaponin to the control diet did not affect the N intake (g/d) in Holstein steers. Faecal N excretion in crossbred calves fed on TMR–1 and TMR–2 was 20.46±1.08 and 24.72±0.73 g/d, respectively, and the N excretion through faeces was 21% higher on feeding TMR–2 in comparison to the feeding of TMR–1. Urinary N excretion (g/d) followed the pattern recorded for faecal N. Urinary N excretion as per cent of total N intake was higher on feeding TMR–2 than that on TMR–1, however reduced faecal N excretion as per cent of total N intake on feeding TMR–2 than on TMR–1 indicated better utilization of digested N because of higher microbial protein synthesis due to anti protozoal action of saponins of lucerne fodder. Faecal N/kg DM intake on feeding TMR- 1 and TMR–2 was 7.17 and 6.80, respectively, showed no significant variation between these 2 groups due to the saponins of lucerne fodder. Lower faecal N excretion/kg DM consumed in present study may be attributed to the difference in the quality and amount of dietary N in Indian and exotic dairy animals. N retention was higher ($P<0.05$) on feeding TMR–2 than on TMR–1. Contrary to present observations, Lila *et al.* (2005) reported increased N retention due to dietary sarsaponin as compared to control diet attributed to decreased urinary loss of nitrogen in sarsaponin supplemented group.

Methane emission

Methane emission (g/d) on feeding TMR–1 and TMR–2 was 49.55±2.61and 44.29±1.92 (Table 5) in crossbred calves, respectively. These values indicated about 12% lower ($P<0.05$) methane emission on feeding TMR–2 than on feeding TMR–1. Methane emission coefficient (g/kg DMI) on feeding TMR–1 and TMR–2 in crossbred calves was 17.38±1.49 and 12.20±0.99 (Table 5) and lower value on feeding TMR–2 may be due to the saponin content supplied by lucerne fodder (21.78g). Srivastava and Garg (2002) reported methane emission/ kg DMI in crossbred calves to the tune of 19.26 g on feeding paddy straw and fodder based diet, which was reduced to 18.40 following the replacement of fodder with concentrate mixture. Lower methane emission coefficient in present study, particularly on TMR–2, may be attributed to the anti-protozoal action of lucerne saponins, which might have resulted in the utilization of hydrogen for the production of propionate rather than its transfer to the methanogens for the production of methane. Higher gross energy (GE) content of TMR–2 (4.37 Mcal/kg) than that of TMR–1 (4.22 Mcal/kg) may be attributed to the replacement of wheat straw with lucerne fodder having comparitvely higher GE possibly due to its higher ether extract (Sen *et al.* 1978). Higher loss of dietary GE in the form of methane in calves fed on TMR–1 (5.44%) than those fed on TMR–2 (3.72%), thus, the loss of dietary energy was reduced by 30 % on dietary inclusion of saponins rich lucerne fodder in the total mixed ration. Lila *et al.* (2003) also reported significant decrease in energy loss following the supplementation of saponins rich lucerne fodder. Benchaar *et al.* (2001) also concluded that feeding of legumes reduced the methane production from ruminants by 15–21%. The reduction in methane emission could be due to the inhibition of hydrogen producing bacteria that produced pyruvate ferridoxin oxide reductase enzyme to metabolize pyruvate to acetyl CoA. Rejil (2005) also reported decreased ($P<0.01$) *in vitro* methane emission (litre/kg DM) following the dietary supplementation of saponins rich fenugreek seed.

These results revealed that the supplementation of lucerne fodder (second cut) to the wheat straw based diet at 30% level increased dry matter intake significantly and digestibility of nutrients than that based on wheat straw besides decreasing methane emission significantly due to the saponins of lucerne fodder. Hence, the traditional system of supplementing straw based ration of livestock with lucerne fodder is highly advantageous for the productivity as well as in mitigation of methane emission from ruminants.

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