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Effect of lucerne fodder (first cut) supplementation on *in vitro* methane production, fermentation pattern and protozoal counts

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ABSTRACT

The study was carried out to ascertain the effect of various levels of lucerne fodder (first cut) supplementation at 0 (T_0), 15 (T_1), 30 (T_2) and 45 (T_3) % to wheat straw and concentrate based diet on *in vitro* methane production, IVDMD, TVFA, molar proportion of VFA,s and protozoal counts. The roughage to concentrate ratio was maintained as 60: 40 irrespective of the diet. Highest total gas production was recorded in treatment T_3 followed by T_2 and T_1, however, the lowest cumulative total gas production was recorded in control treatment (T_0). Cumulative gas production in treatment T_3 was significantly higher than that recorded in $T_{0.}$ Total gas production from slowly fermentable fraction 'b' ranged between 118.80 (T_0) and 139.70 (T_3) litre/kg, DM in different treatments. The reduction in methane production was 15 and 18% in treatment T_2 and T_3**,** respectively. The DMD in treatments T_2 and T_3 was significantly higher than that recorded in treatment T_0. Total volatile fatty acid (TVFA) recorded as 105.58 and 107.93 Mm/litre in treatment T_2 and T_3, was significantly higher than that recorded in control treatment (101.43 Mm/litre). The acetate proportion was significantly lower in treatment T_3 as compared to the control and reverse trend was observed for the propionate production. Protozoal numbers (log cfu/ml) decreased significantly by the supplementation of first cut lucerne fodder at 30 and 45% level. Saponins containing feed may serve the purpose of methane mitigation from ruminants without giving any additional input as the farmers grow leguminous fodder traditionally. Thus, there is urgent need to explore the new feed resources containing saponin and fix the safe level of their inclusion in ration for achieving the goal.

Key words: Fermentability, Kinetics of gas, Lucerne, Methane production

Enteric fermentation in ruminants represents a loss of dietary energy (6–12%) in the form of methane (Johnson and Johnson 1995). This loss of energy is significant in situation where feeding is the costlier item and accounted almost 70% of total cost of production. For feeding 185, 97, 61 and 124 million of cattle, buffalo, sheep and goat in the country, there is an acute shortage of feed and fodders—estimated as 61 and 29% of green and dry fodder, respectively against the requirement (Faruqui and Kumar 2009). This situation gets more aggravated by the quality of feeds and fodders available for the feeding. Bulk of a ration is mainly composed of fibrous feed, which emits more methane on fermentation as compared to the green fodder and concentrate (Singhal *et al*. 2004). Besides the great energy loss, methane emission from ruminant contributes significantly to the global warming as it is 40–60 times more potent than CO_2 in terms of heat trapping (Khan *et al*. 2001). Livestock system alone on an average is adding 12Tg methane per year to the environment and 91% of it comes from the enteric fermentation only (Chhabra *et al*. 2009). Various feeding strategies were tried for the mitigation of methane emission but most of them could not succeed at the farmer's doorstep due to their own limitations. Saponin is well known for its anti-protozoal action (Hess *et al*. 2003) thereby indirectly responsible for methane reduction from ruminants. Legumes, which are considered as fair source of saponin (Hess *et al*. 2003) and farmers grow them traditionally throughout the country, may help mitigate methane emission from ruminants, therefore this study was planned to ascertain the effect of different levels of lucerne fodder (first cut) supplementation to wheat straw and concentrate based diet on total gas, methane production, kinetics of these gases, fermentability of diets and protozoal population under *in vitro* condition.

MATERIALS AND METHODS

Saponin content of lucerne fodder (first cut) was estimated as per Yosioka (1974) by using methyl and n-butyl alcohol. In the study lucerne fodder (I cut) was supplemented to the

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wheat straw and concentrate based diet at the level of 0 (T_0), 15 (T_1), 30 (T_2) and 45 (T_3) % on DM basis. Roughage to concentrate ratio for all the dietary treatments was maintained similar as 60: 40 with 12–13% CP. Rumen liquor for the study was taken from the 3 crossbred male rumen fistulated animals (average body weight 284.33 kg) fed on wheat straw and concentrate based diet (CP 20%, TDN 70%) in the ratio of 60: 40 to meet their maintenance requirement. Each animal was offered 2 kg lucerne fodder to fulfil their vitamin A requirement. Temperature and anaerobic conditions were ensured during the collection and handling of rumen liquor. 0.2 g dry sample was taken in small nylon bag (4 cm×6 cm, 50 µ) and incubated with 20 ml buffer and 10 ml strained rumen liquor in a 60 ml well lubricated graduated plastic syringe for measuring the gas production as per Menke *et al*. (1979). Outlet of the syringe was connected with a silicon rubber tube of 4–5 cm length through 3-way valve by a plastic clip and then the syringes were kept in incubator at 39±0.5°C. The total gas production was recorded at 8, 16, 24, 48, 72 and 96 h of incubation by recording the position of piston at particular time period thereafter gas was transferred to another graduated plastic syringe with the help of 3–way valve. Gas sample was then immediately taken to the Environmental Laboratory of the Dairy Cattle Nutrition Division of the Institute for the analysis of methane by using gas chromatograph fitted with flame ionization detector (FID) and Porapak Q column. The proportion of methane in gas sample was calculated as follows

$$\text{Methane (ppm)} = \frac{\text{(Area covered by gas sample} \times \text{concentration of std.)}}{\text{Area covered by standard}}$$

Kinetics of total gas and methane with their coefficients of determination (R^2) was also derived by using the Sigma stat (2001) software to fit the data in exponential model of Mc Donald (1981) with the formula given below.

$$Y = b\ (1\text{-}e^{-c\ (t\text{-}1)})$$

where, Y is cumulative gas volume, b is slowly fermentable fraction, c is the fermentation rate, t, time i.e. 96 h, and l, discrete time lag phase.

At the end of incubation period, nylon bags were retrieved and washed with clean tap water until water became clear. The bags were then kept in hot air oven for drying at 80°C, thereafter dry weight of samples was recorded and dry matter digestibility was calculated by difference in the initial and final weight. Total volatile fatty acid (TVFA) and molar proportion of individual fatty acids were estimated as per Barnett and Reid (1957) and Erwin *et al.* (1961), respectively. Protozoal population was enumerated by using microscopic method by staining them with brilliant green dye and then taking the sample into haemocytometer. The data thus obtained were statistically analysed as per Snedecor and Cochran (1986).

RESULTS AND DISCUSSION

Chemical composition: Composition of different feed ingredients along with saponin content of lucerne fodder (first cut) and the proportion in which they used for diet formulation are given in Table 1.

Total gas production and its kinetics: Cumulative total gas production increased from 106.90 to 128.70 (l/kg DM) with the increasing levels of lucerne fodder from 0 (T_o) to 45 (T_3) % (Table 2). The highest total gas production was recorded in treatment T_3 followed by T_2 and T_1 however, the lowest cumulative total gas production was recorded in the control treatment (T_0). Results of the study revealed significantly (P<0.05) higher cumulative gas production in treatment T_3 as compared to treatment T_0 and T_1. Similarly, gas production in treatment T_2 was also significantly (P<0.05) higher than that recorded in treatment T_0 and T_1 however, no significant variation was found between T_0 and T_1. Significant (P<0.05) higher gas production on lucerne fodder supplementation in treatment T_2 and T_3 may be attributed to the high level of lucerne fodder supplementation and/or its saponin content, which might have lead the shift in the metabolism of carbohydrates resulting more gas production and moreover to the associative effect of palatable lucerne

Table 1. Chemical composition of feed ingredients and composition of concentrate mixture

Ingredients	DM (%)	Composition (% DM basis)					
		OM	CP	EE	CF	Ash	Saponin%
Wheat straw	89.37	93.05	3.00	1.09	38.16	6.95	
Lucerne fodder	18.51	87.70	20.80	3.40	18.44	12.30	1.17

Composition of concentrate mixture

Treatment	Concentrate ingredients						
	Maize grain	Groundnut cake	Mustard cake	Wheat bran	Deoiled rice bran	Mineral mixture	Salt
T_0	14	18	37	28		2	1
T_1	28	11	24	34		2	1
T_2	42	35	20			2	1
T_3	30				67	2	1

Table 2. Effect of lucerne fodder supplementation on cumulative total gas, methane production and their kinetics

Incubation period (h)	T_0		T_1		T_2		T_3	
	TG	CH_4	TG	CH_4	TG	CH_4	TG	CH_4
8	14.35	2.70	13.80	2.04	17.15	3.00	18.30	3.80
16	37.60	6.68	38.95	6.13	44.65	6.98	47.95	6.9
24	61.45	13.50	68.75	13.17	89.80	12.95	94.45	13.35
48	93.05	19.64	97.25	18.12	117.10	16.86	124.45	16.15
72	102.80	21.00	105.90	19.34	124.25	17.83	127.45	17.35
96	106.90[a]	21.29[x]	107.28[a]	19.56[xy]	125.95[b]	18.15[y]	128.75[b]	17.55[y]
b	118.8	23.88	116.35	21.85	136.95	19.45	139.7	18.50
c	0.137	0.028	0.156	0.0296	0.167	0.0355	0.177	0.0392
R^2	0.98	0.97	0.97	0.95	0.96	0.97	0.96	0.97

Mean values bearing different superscripts in a row differ significantly (P<0.05).

fodder resulted more colonization and fermentation of feed particles (Blumel and Orskov 1993). The increase in total gas production on the supplementation of lucerne fodder was due to the more production of propionate (Table 3) may be via succinate : propionate pathway in which CO_2 is released (Rejil 2005).

Cumulative total gas production from slowly fermentable fraction 'b' ranged between 118.80 (T_0) to 139.70 litre/kg, DM (T_3) in different treatments, however, the rate of gas production (c) was recorded between 0.137 and 0.177 l/h in treatment T_0 and T_3, respectively (Table 2). The gas production in different treatments was positively correlated with the fermentable fraction of the diet (0.96 to 0.98). Maximum gas production was recorded during 24–48 h of incubation in all treatments indicating more fermentation of diet during this period irrespective of the treatment. Correlation coefficients (R^2) varied in the range of 0.96 to 0.98, showing that gas production was highly correlated with different substrates and low fibre feed had higher cumulative gas production than those having high fibre. The higher rate of gas production in lucerne supplemented treatments showed the higher fermentability of wheat straw and concentrate based diet. Our results are in consonance of Monforte - Briceno *et al.* (2005), who reported higher gas production on supplementation of leguminous fodder to straw based diet.

In vitro methane and its kinetics: As the saponin (1.17% DM basis) containing lucerne fodder (first cut) level increased from 0 to 45% in wheat straw and concentrate based diet, *in vitro* methane production on the fermentation of diet, decreased accordingly and among the 4 dietary treatments, maximum reduction was recorded in treatment T_3 (17.55%).The highest methane production was recorded in control treatment (21.29 l/kg DM) composed of wheat straw and concentrate with zero lucerne fodder. Data revealed that the supplementation of lucerne fodder at 45% level decreased methane production significantly (P<0.05) as compared to control however, no significant variation was noticed between T_2 and T_3 treatments. Similarly, cumulative methane production in T_1 did not differ significantly from treatment T_0. The reduction in methane production on the supplementation of lucerne fodder was 8, 15 and 18%, respectively, in treatment T_1, T_2 and T_3, and this reduction may be attributed to the saponin of lucerne fodder (1.17% DM basis) or high soluble carbohydrates and better fibre quality of fodder, which may lead the shift in fermentation pattern from methanogenic volatile fatty acids to the non-metahnogenic volatile fatty acid (propionate), exert the anti protozoal action due to the saponin (Table 3) and thereby hampered the availability of hydrogen to methanogens (McMeniman *et al*. 1988). As far as kinetics of methane is concerned, slowly fermentable fraction 'b' in the control treatment produced highest methane (23.88 l/kg DM), whereas its production decreased gradually on lucerne fodder supplementation at varying level of 15 to 45% (Table 2). Similarly, the rate (l/h) of methane production 'c' was low in the treatments having lucerne fodder than that formulated without lucerne fodder. Our results are in good agreement with the findings of Benchaar *et al.* (2001), who reported a reduction of 15 to 21% on legumes supplementation.

Fermentation pattern

In vitro *dry matter digestibility (IVDMD) : In vitro* DMD significantly increased on the supplementation of lucerne fodder at 30 and 45% levels to the wheat straw and concentrate based diet, however, no significant difference was observed between T_2 and T_3 treatment (Table 3). The increase in DM digestibility on lucerne fodder supplementation in T_2 and T_3 was 12 and 18%, respectively, as compared to the control treatment, which may be attributed to the increased bacterial biomass due to the anti protozoal action of lucerne saponin or high soluble carbohydrate and better fibre quality of palatable nutritious lucerne fodder than that of wheat straw. It is also envisaged from the study that saponin from lucerne up to the level 1.17% of DM did not adversely affect digestibility and fermentability. The findings of the study are in consonance of Das (1996), who reported

Table 3. Effect of lucerne fodder supplementation on fermentation pattern and protozoal counts

Incubation period (h)	T_0	T_1	T_2	T_3
IVDMD (%)	$43.72^{a}\pm0.52$	$45.88^{ab}\pm0.68$	$48.76^{bc}\pm0.70$	$51.54^{bc}\pm0.67$
TVFA (Mm/litre)	$101.43^{a}\pm0.97$	$101.74^{a}\pm1.23$	$105.58^{b}\pm1.45$	$107.93^{b}\pm1.08$
Acetate (%)	$74.66^{b}\pm1.01$	$72.67^{ab}\pm0.90$	$70.67^{b}\pm0.85$	$68.23^{b}\pm1.26$
Propionate (%)	$16.34^{a}\pm0.51$	$17.55^{a}\pm0.46$	$20.99^{ab}\pm0.40$	$22.34^{b}\pm0.51$
Butyrate (%)	9.0 ± 0.43	9.78 ± 0.28	8.44 ± 0.35	9.43 ± 0.25
Protozoa (log cfu/ml)	$4.74^{a}\pm0.44$	$3.61^{ab}\pm0.38$	$3.47^{b}\pm0.21$	$2.99^{b}\pm0.16$

Mean values bearing different superscripts in a row differ significantly ($P<0.05$).

a significant increase in DM digestibility following the supplementation of leguminous fodders to straw based ration. Similarly, Navas-Camacho *et al.* (1993) did not find any adverse effect of *Sapindus saponaria* pericarp supplementation on *in sacco* DM degradability in sheep.

Volatile fatty acid: Total volatile fatty acid (TVFA) production was recorded as 105.58 and 107.93 Mm/litre in treatment T_2 and T_3, respectively, and the production of total volatile fatty acid in these treatments was significantly higher ($P<0.05$) than that recorded in treatment T_0 and T_1 (Table 3). High production of TVFA in treatment T_2 and T_3 on supplementation was due to the more fermentability of the diet as evidenced from the *in vitro* dry matter digestibility data (Table 2). The proportion of acetate on the supplementation of saponin containing lucerne fodder decreased significantly ($P<0.05$) in T_2 and T_3 as compared to T_0 and T_1. However, among the other treatments it did not vary significantly. Reverse trend was reported for the propionate production with the significantly ($P<0.05$) higher production in treatment T_3 (Table 3) however, no significant variation was reported between other treatments. Butyrate was the only volatile fatty acid which was not affected by the supplementation, and its production was somewhat constant irrespective of the treatment. The shift in the fermentation pattern from acetate to the propionate on the lucerne fodder supplementation may be attributed to the high soluble carbohydrate, better fibre quality and to the some extent to saponin of lucerne fodder. Das (1996) also reported a significant increase in TVFA concentration on berseem fodder supplementation to the wheat straw based diet. Findings of Abreu *et al.* (2004) also support the result of our study as they reported the significant decrease in acetate and increase in propionate on the supplementation of lucerne to rice straw based diet. Results from the study of Hristov *et al.* (1999) are in consonance of our findings as they reported the same trend in heifers fed on *Yucca schdigera* powder as a source of saponin.

Protozoal counts: Supplementation of lucerne fodder decreased the protozoal counts (log cfu/ml) significantly ($P<0.05$) in treatment T_2 and T_3 and the reduction in counts was 27 and 36%, respectively. The variation in protozoal numbers among the other treatments was not significant. The significant decrease ($P<0.05$) in protozoal numbers in treatment T_2 and T_3 (Table 3) was attributed to the saponin content in particular, which exerts a specific action against the protozoa due to the precipitation of medicagenic acid and sterols of the cell membranes beside the presence of cholesterol in protozoal cell membrane (Rejil 2005). The results are in good agreement with the findings of Hess *et al.* (2003). Protozoal number ($\times10^5$/ml) decreased significantly from 14.4 to 5.3 and 2.4 in rumen fluid of steers who received 0.5 and 1% of sarsaponin in control diet (Lila *et al*. 2005).

It may be envisaged from the study that first cut lucerne fodder supplementation (45% level) having saponins @1.17% of DM, decreased the methane production up to 18% from wheat straw and concentrate based diet, however, saponins from lucerne at this level did not have any adverse effect on the fermentability of diet. Thus, feeds or herbs having saponins up to the 1.1% level are safe to incorporate for the mitigation of methane production from livestock without any adverse effect of fermentation of the basal diet.

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