

Effect of berseem fodder supplementation to wheat straw based diet on *in vitro* total gas and methane production and fermentation pattern

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ABSTRACT

A study was carried out to ascertain the effect of 4 levels of berseem fodder supplementation, viz. 0 (control T₀), 15 (T₁), 30 (T₂) and 45 (T₃)% to wheat straw and concentrate based diet on *in vitro* total gas, methane production, IVDMD, TVFA concentration and their molar proportion 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 T₃ followed by T₂ and T₁ and the lowest cumulative total gas production was recorded in T₀. Cumulative gas production in treatment T₃ was significantly higher than T₀ and T₁ treatments. Total gas production from slowly fermentable fraction 'b' ranged between 118.80 (T₀) to 150.20 (T₄) l/kg DM in different treatments. The reduction in methane production was 14 and 17% in treatment T₃ and T₄, respectively. Similarly, the cumulative methane production in T₁ was not differing significantly than that recorded in treatment T₀. The DMD in treatments T₂ and T₃ was significantly higher than that recorded in treatment T₀. Total volatile fatty acid (TVFA) recorded as 106.43 and 108.58 mM/l in treatments T₂ and T₃, was significantly higher than that recorded in control treatment (101.43 mM/l). The acetate proportion was significantly lower in treatment T₃ as compared to the control. The trend was reversed for the propionate production and significant increase over the control was recorded in treatment T₃. The proportion of butyrate in volatile fatty acid remained unchanged, irrespective of the diet. Protozoal numbers (log cfu/ml) decreased significantly by the supplementation of first cut berseem fodder at 45% level. It may be concluded from the study that the supplementation of berseem fodder up to 45% level to wheat straw and concentrate based diet, decreased methane production to the extent of 17% without affecting the fermentability of the diet and the saponins from berseem fodder up to the 0.75% level did not have any adverse effect on fermentation pattern.

Key words: Berseem, Methane, Total gas and TVFA

Various feeding strategies have been tried in India and abroad for the mitigation of methane emission from ruminants (Beauchemin *et al.* 2008 and Sirohi *et al.* 2009) but most of them could not succeed at the farmer's doorstep because of their own limitations like toxic to animal, toxic to the rumen micro organism, transient effect, high cost etc. Legumes, which are considered as a potent source of saponins and farmers grow them traditionally throughout the country, may be useful tools in the mitigation of methane emission. Saponins are well known for their anti-protozoal action and thereby indirect reduction in methane emission may be achieved by the feeding of saponins rich legume fodders like berseem, lucerne etc. Keeping these facts in mind, the study

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was planned to ascertain the effect of different levels of berseem fodder supplementation to wheat straw and concentrate based diet on total gas and methane production, kinetics of these gases and fermentability of different diets under *in vitro* conditions.

MATERIALS AND METHODS

Saponin content of berseem fodder (first cut) was estimated as per Yosioka (1974) by using methyl and n-butyl alcohol. The weight of saponin was expressed as per cent of dry weight of the sample taken as follows:

$$\text{Total saponin (\%)} = \frac{W_2 - W_1}{W} \times 100$$

where, W, dried weight of sample; W₁, dried weight of filter paper; W₂, dried weight of filter paper along with precipitates of saponin.

Four dietary treatments were prepared by using wheat straw, berseem fodder (first cut) and concentrate mixture. The roughage to concentrate ratio was kept similar as 60 : 40

with 12–13% CP in all the 4 treatments. Berseem fodder was supplemented at 15 (T₁), 30 (T₂) and 45 (T₃)% levels to the wheat straw and concentrate based ration, T₀ (control) treatment was not having berseem fodder and solely composed of wheat straw (60%) and concentrate mixture (40%). To minimise the variation, rumen liquor for the *in vitro* study was taken from the 3 crossbred male rumen fistulated cattle (bw 284.33 kg) fed on wheat straw and concentrate based diet (CP 20%, TDN 70%). 0.2 g substrate was taken in a small size nylon bag (4 × 6 cm, 50 µ) incubated with 20 ml Menke's buffer solution and 10 ml strained rumen liquor in a 60 ml well lubricated graduated plastic syringe. The piston of the syringe was adjusted to the surface of liquid to ensure the removal of air in the syringe. The 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 0, 8, 16, 24, 48, 72 and 96 h of incubation by recording the piston position. After recording the volume at each incubation period, gas was transferred and was analysed for methane using gas chromatograph fitted with flame ionization detector (FID) and a column (Porapak 'N') and the proportion was calculated as follows

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

Simultaneously, kinetics of total gas and methane with coefficients of determination (R²) was also derived by using the Sigma stat (2001) to fit the data in exponential model of Orskov and Mc Donald (1979) with the formula given below.

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

where, Y, cumulative gas volume; b, slowly fermentable fraction; c, fermentation rate; t, time, i.e. 96 h; 1, discrete time lag phase.

At the end of incubation period (96 h), nylon bags were retrieved and washed with clean tap water until washing water became clear. The bags were kept in hot air oven for drying at 80°C and weight of the samples along with bag was recorded. The difference in the initial and final weight of the samples was considered as *in vitro* digestibility of sample. Total volatile fatty acid (TVFA) in incubation medium was estimated as per Barnett and Reid (1957) however, molar proportion of individual fatty acids was estimated as per Erwin *et al.* (1961) by using gas chromatograph. Protozoal population was counted at the end of incubation by staining with brilliant green dye and microscopic counting (Santra *et al.* 1998). The data obtained were subjected for the statistical analysis as per Snedecor and Cochran (1986).

RESULTS AND DISCUSSION

Chemical composition

Composition of different feed ingredients and the proportion of different items used for the preparation of concentrate mixture for different treatment are given in Table 1.

Total gas and its kinetics

Cumulative total gas production (l/kg, DM) as influenced by different levels of berseem fodder supplementation has been given in Table 2. Data showed that the cumulative total gas production increased from 106.90 to 138.20 (l/kg DM) with increasing the levels of berseem fodder from 0 (T₀) to 45 (T₃)%. The highest total gas production was recorded in treatment T₃ followed by T₂ and T₁ treatments and lowest total gas production was recorded in the control treatment (T₀). Our results revealed significantly (P<0.05) higher cumulative gas production in treatment T₃ as compared to treatments T₀ and T₁. Similarly, total gas production in treatment T₂ was also significantly (P<0.05) higher as

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

Ingredients	DM (%)	Proximate composition (% DM basis)				
		OM	CP	EE	CF	Ash
Wheat straw	89.4	93.0	3.0	1.09	38.2	6.95
Berseem fodder*	12.2	88.6	17.8	3.68	18.3	11.36

Proportion of concentrate ingredients.

Treatment	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	27	12	24	34	—	2	1
T ₂	40	35	22	—	—	2	1
T ₃	30	—	7	—	60	2	1

*Saponin content of berseem fodder (first cut) was 0.74% (DM basis) with ranging between 0.73 to 0.76%.

Table 2. Effect of supplementation of graded levels of berseem fodder on cumulative total gas (TG) and methane (CH₄) production and its 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 (27.74)	3.98	14.75 (22.24)	3.28	17.60 (21.31)	3.75	18.75 (18.72)	3.51
16	37.60 (17.77)	6.68	40.25 (15.13)	6.09	42.70 (18.36)	7.84	49.50 (16.42)	8.13
24	61.45 (21.97)	13.50	71.50 (18.53)	13.25	88.70 (13.84)	12.28	103.10 (12.67)	13.06
48	93.05 (21.11)	19.64	100.85 (18.16)	18.31	117.55 (14.29)	16.80	133.35 (12.28)	16.37
72	102.80 (20.43)	21.00	110.20 (17.53)	19.32	124.90 (14.30)	17.86	136.70 (12.72)	17.39
96	106.90 ^a (19.92)	21.29 ^b	113.20 ^{ab} (17.31)	19.59 ^{bc}	126.50 ^{bc} (14.45)	18.28 ^{ac}	138.20 ^c (12.78)	17.66 ^{ac}
b	118.8	23.56	124.35	21.58	138.4	19.32	150.2	18.48
c	0.137	0.0302	0.149	0.0303	0.162	0.0368	0.175	0.0409
R ²	0.98	0.97	0.99	0.96	0.96	0.99	0.95	0.98

Figure in parenthesis is the proportion (%) of methane; mean values bearing different superscripts in a row differ significantly ($P < 0.05$); CD value at 5% = 13.47 (TG), CD value at 5% = 2.09 (CH₄).

compared to the treatment T₀. The significant ($P < 0.05$) higher gas production in different treatments due to berseem fodder supplementation may be attributed to the associative effect of berseem fodder, which increases the fermentability of wheat straw based diet by colonizing the fibre for microbial action. Data of the study also suggested that maximum fermentability of the feed occurs within 48 h of incubation, irrespective of the treatments (Table 2) and the feed ferment rapidly and exhaustively in those treatments having high level of berseem fodder (Table 2). Cumulative total gas production from slowly fermentable fraction 'b' ranged between 118.80 (T₀) and 150.20 (T₄) l/kg, DM in different treatments, however, the rate of gas production (c) was recorded as 0.137 to 0.175 l/h in treatment T₀ and T₃, respectively (Table 2). Since gas production on incubation of feeds in buffered rumen fluid is associated with feed fermentation (Getachew *et al.* 2004), therefore higher rate of gas production was reported in berseem supplemented treatments than the control. Getachew *et al.* (2004) and Bueno *et al.* (2005) reported more gas production on legume supplementation. Our results are in consonance with the findings of Monforte - Briceno *et al.* (2005) and Das and Singh (1998), who reported higher total gas production on supplementation of leguminous fodder to straw based diet. The results of the study are fairly supported by the findings of Bonsi *et al.* (1995) who reported lower total gas volume from slowly degradable substrate like Veronica than from highly degradable substrate like Sesbania. Highly degradable substrates got degraded in a short period, and availability of degraded products to the microbial digestion increased the gas production (Blummel and Orskov 1993).

In vitro methane production and its kinetics

As the level of berseem fodder (first cut saponin content 0.74% DM basis) increased from 0 to 45%, *in vitro* methane production decreased accordingly and among the 4 dietary

treatments, minimum production was recorded in treatment T₄ (17.66 l/kg DM). The highest methane production was recorded in treatment T₀ (21.0 l/kg DM) composed of wheat straw and concentrate however, *in vitro* methane production for rest of the 2 treatments was in between the T₀ and T₄ treatments. Data revealed that the supplementation of berseem fodder at 45% level decreased the methane production significantly ($P < 0.05$) as compared to the control however, no significant variation was noticed between T₃ and T₄ treatments. Similarly, the cumulative methane production in T₁ did not differ significantly than that recorded for treatment T₀. The reduction in methane production due to the berseem fodder supplementation at 30 and 45% levels was 14 and 17% in treatment T₃ and T₄, respectively. The production of methane in treatments T₃ and T₄ during first 24 h of incubation was more because of high fermentability of the diet due to berseem fodder (Table 3). The decrease in methane production on the supplementation of berseem fodder may be due to a decrease in protozoa numbers (Table 3), which are responsible for the hydrogen transfer to the methanogenic bacteria (Krumholz *et al.* 1983). Similarly, Reddy and Krishna (2009) also quoted that feeding forage legumes like lucerne or red clover tended to decrease methane losses compared to grass. Kinetics of methane production showed that slowly fermentable fraction 'b' of diet produced maximum methane (23.56 l/kg DM) in treatment T₀, which decreased gradually by the increasing levels of berseem fodder supplementation. The lowest methane production in treatment T₄ (18.48 l/kg DM) may be attributed to the shift in the fermentation pattern towards propionate production at the cost of acetate (Table 3). Similarly, the rate of methane production (l/h) was lowest in treatments having high level of berseem fodder as compared to the treatments having low level or no berseem fodder (Table 2). The findings of Benchaar *et al.* (2001) strengthen the results of study who reported a reduction in methane production by 15 to 21% on

Table 3. Effect of different levels and cuts of berseem or lucerne fodder supplementation on IVDMD, TVFA, molar proportion of VFA and protozoal counts

Incubation period (h)	T ₀	T ₁	T ₂	T ₃
IVDMD (%)	43.72 ^a ±0.52	47.7 ^{ab} ±0.92	51.41 ^b ±0.84	54.41 ^b ±0.84
TVFA (Mm/l)	101.43 ^a ±0.97	102.46 ^a ±1.12	106.43 ^b ±1.34	108.58 ^b ±0.86
Acetate (%)	74.66 ^b ±1.01	72.68 ^{ab} ±0.74	70.69 ^{ab} ±0.58	68.78 ^a ±0.98
Propionate (%)	16.34 ^a ±0.51	18.54 ^{ab} ±0.46	19.88 ^{ab} ±0.62	21.36 ^b ±0.38
Butyrate (%)	9.0±0.43	8.78±0.37	9.43±0.44	9.86±0.50
Protozoa (log cfu/ml)	4.74 ^c ±0.44	3.73 ^{bc} ±0.33	3.56 ^{ab} ±0.28	3.09 ^a ±0.27

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

legumes supplementation. Singh and Mohini (1999) also reported that the methane production decreased significantly on complete replacement of wheat straw with leguminous fodder.

In vitro Dry matter digestibility (IVDMD)

Our results revealed an increase in *in vitro* DMD on increasing the levels of berseem fodder from 0 to 45% to the wheat straw and concentrate based diet. The DMD in treatments T₂ and T₃ was significantly higher (P<0.05) as compared to treatment T₀ (Table 3). The increase in dry matter digestibility on berseem fodder supplementation in T₂ and T₃ was around 17 and 24%, respectively, which may be attributed to the high soluble carbohydrates and better fibre quality of nutritious berseem fodder than that of wheat straw. The variation among the other treatments in DMD was not significant. The findings of the study are in line with the findings of Reddy *et al.* (1991) and Das (1996) who reported a significant increase in DM digestibility following the supplementation of leguminous fodders to straw based rations.

Volatile fatty acid

Total volatile fatty acid (TVFA) concentration was recorded as 106.43 and 108.58 mM/l in treatment T₂ and T₃, and the production of total volatile fatty acid in these treatments was significantly (P<0.05) higher than that recorded in control (101.43 mM/l). The higher production of TVFA in berseem supplemented treatments was due to the high fermentability of diets because of berseem fodder. The proportion of acetate in volatile fatty acids decreased significantly (P<0.05) in T₃ as compared to the control (Table 3), however, the acetate production in rest of the 2 treatments was somewhat lower than the control, but the variation was not significant. The trend was reverse for the propionate production and significant increase (P<0.05) in propionate production was noticed in treatment T₃ (Table 3) as compared to the control, however, the variation between other treatments was not significant. Butyrate was the only volatile fatty acid which was not affected by the supplementation of berseem and its proportion remained unchanged. It may be conferred from the study that the

supplementation of berseem fodder shifts the fermentation pattern of the diet from acetate to propionate production, which may be attributed to the high soluble carbohydrates and better fibre quality of berseem fodder. Das (1996) also reported a significant increase in TVFA concentration on the supplementation of berseem fodder to the wheat straw based diet. Findings of Wang *et al.* (2000) and Abreu *et al.* (2004) strengthen the results of the study who reported similar kind of the trend on legumes supplementation in rumen cannulated sheep, RUSITEC and African-type hair sheep, respectively.

Protozoal counts

The graded supplementation of berseem fodder decreased the protozoal numbers (log cfu/ml) significantly (P<0.05) and maximum reduction (35%) was noticed in treatment T₃ as compared to the control, however, the variation in protozoal numbers between the other treatments was not significant. The significant decrease (P<0.05) in protozoal numbers in treatment T₃ attributed to the comparatively high saponin content in T₃. Saponins exert a specific effect against the protozoa. The results are in good agreement with the findings of Diaz *et al.* (1993) and Hess *et al.* (2003). Klita *et al.* (1996) reported a significant reduction in ruminal protozoal numbers due to the supplementation of alfalfa root saponins in sheep diet. Protozoal number (×10⁵/ml) decreased significantly from 14.4 to 5.3 and 2.4 in rumen fluid of steers received 0.5 and 1% of sarsaponin to the control diet (Lila *et al.* 2005). Similarly, Makkar and Becker *et al.* (1997) also reported a decrease in number of protozoa on various legumes and saponin rich diet in sheep and cattle. It may be concluded from the study that the supplementation of berseem fodder up to 45% level to the wheat straw and concentrate based diet, decreased the methane production up to the extent of 17% without affecting the fermentability of the diet. Saponins up to the 0.75% on DM basis does not affect the fermentability thus, there is a urgent need to explore the saponins containing feed resources for the mitigation on methane production and to fix their safe level of incorporation in the ration of different animals.

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