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Research Paper

Effect of fenugreek leaf extract (*Trigonella foenum-graecum* L.) on *in vitro* methanogenesis and fermentation of wheat straw-based diet (*Triticum aestivum* L.) fed to buffaloes

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Abstract: The secondary metabolites present in some plants have the potential in modifying rumen fermentation due to their antimicrobial activity. The present study aimed investigating the effect of dosing extracts of fenugreek (*Trigonella foenum-graecum* L.) leaves on *in vitro* methanogenesis and fermentation of feed in buffalo (*Bubalus bubalis* Linnaeus, 1758). The leaves of fenugreek were dried at 45-50 °C and ground to pass through 1 mm sieve. Extracts (100 ml) were prepared with 10 g powdered sample using three solvents namely, ethanol, methanol and water, and stored at 4 °C for subsequent use. The extracts were tested at three levels (0, 1.0 and 2.0 ml). A 60:40 mixture (200 ± 5 mg) of wheat (*Triticum aestivum* L.) straw and concentrate feed was used as substrate and incubated with 30 ml buffered rumen fluid in 100 ml calibrated glass syringes at 39 °C for 24 hrs following the standard IVGP protocol. Gas production was recorded by displacement of piston in the syringe. Methane in the gas phase and volatile fatty acids (VFA) in the fermentation medium were estimated by GLC. The total gas and methane production was reduced significantly ($p<0.001$) with the increase in microbial biomass production by inclusion of methanol extract of fenugreek leaves. Acetate production was reduced with increase of propionate, which reduced the acetate to propionate ratio ($p<0.05$). The truly degradable dry matter content was increased by the addition of methanol extract. Results of this study suggested the potential use of methanol extract of fenugreek leaves as an anti-methanogenic feed additive to improve rumen fermentation in buffaloes.

Keywords: Buffalo, fenugreek leaves, feed degradability, methanogenesis, rumen fermentation

Introduction

Livestock emits 37% of the global anthropogenic methane, mostly from enteric fermentation by ruminants (Steinfeld *et al.,* 2006). Methane produced from the ruminant livestock also represents a loss of energy intake by the animals. Therefore, reducing methane emission from ruminants has implications not only for global environmental protection but also for effective animal production.The secondary metabolites of plants have the potential to modify fermentation by their antimicrobial activity (Gershenzon and Croteau, 1991) on specific group of organisms in rumen. Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume grown widely as a spice, medicinal plant and forage. Similar to most medicinal plants,

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fenugreek foliage also contains secondary metabolites *viz*. saponins, flavanoids, alkaloids and tannins (Taylor *et al.,* 1997), which may modify the rumen fermentation leading to reduced methane production and thus, improved feed utilization. Therefore, the aim of the present study was to investigate the effect of dosing extracts of fenugreek leaves on *in vitro* methanogenesis and fermentation of feed in buffalo.

Materials and Methods

Fenugreek (*Trigonella foenum-graecum* L.) foliage at vegetative stage was collected fresh from market. The leaves were separated manually and dried in electric oven at 45 – 50 °C for 24 hrs. The dried material was ground to pass through 1 mm sieve. Extracts (100 ml) were prepared with 10 g of powdered sample using three solvents namely, ethanol, methanol and water. Aliquots of 200 ml of ethanol or methanol were used in a Soxhlet apparatus for extraction, and the extracts were subjected to vacuum evaporation under low temperature. In the case of water extraction, the powdered leaves were taken in a conical flask and boiled for 5 min on a low flame. The flask was stoppered and incubated at 39 °C on a rotary shaker for 24 hrs and filtered through Whatman No 1 filter paper. The final volume of all three extracts were made up to 100 ml with distilled water and stored at 4 °C for further use.

The extracts were tested at three levels (0, 1.0 and 2.0 ml) in three replicates for each treatment. Specific blanks (containing the additive of interest, inoculum and medium without substrate) were used to correct gas production (Araujo *et al.,* 2011). A 60:40 mixture of wheat (*Triticum aestivum* L.) straw and concentrate feed consisting of maize grain (32 %), groundnut cake (15 %), mustard cake (12 %), wheat bran (38 %), mineral mixture (2 %) and common salt (1 %) was used as the substrate. Three fistulated buffaloes fed on a similar diet were used to collect rumen liquor just before feeding, in an insulated flask under strict anaerobic condition, pooled in equal proportions, and used as source of inoculum.

The substrate was milled to pass through 1 mm sieve and 200 ± 5 mg was weighed in glass syringes of 100 ml capacity. The incubation medium (Menke and Steingass, 1988) and inoculum was dispensed (30 ml) anaerobically in each syringe and kept at 39 °C for 24 hrs. Gas production was recorded by displacement of piston in the syringe. Methane in the gas phase was estimated by injecting 200 µl of head space gas into Nucon-5700 gas chromatograph equipped with thermal conductivity detector (TCD) and Porapak-Q stainless steel column. For estimation of volatile fatty acids (VFA), 1 µl of processed supernatant was injected in gas chromatograph equipped with flame ionization detector (FID) and chromosorb glass column as described by Cottyn and Boucque (1968). The *in vitro* true degradability of dry matter (TDDM) was estimated and microbial biomass production (MBP, mg/g DDM) was calculated (Blümmel *et al.,* 1997). Data were analyzed using the General Linear Model procedure of SPSS (1996) as a randomized complete block design and means were compared using Tukey's test.

Results and Discussion

The total gas production (ml/g DM) was reduced significantly ($p<0.001$) with the addition of methanol extract (M-2) of fenugreek leaves (Table 1). However, it was either increased or remained similar with both doses of ethanol and aqueous extracts and at lower dose of the methanol extract. Methane production (ml/g DDM) followed a similar trend (Figure 1).

Table 1. Effect of fenugreek leaf extracts on *in vitro* rumen fermentation of wheat straw-based diet

Treatments	TDDM* %	Gas ml/g DM	Methane ml/g DDM	MBP mg/100 mg DDM	TVFA mM/dl	A:P
Control	56.06^{b}	128.77^{b}	37.30^{b}	48.30^{c}	8.34^{b}	3.39^{b}
Ethanol extract						
E – 1**	53.45^{b}	158.99^{d}	62.56^{d}	33.03^{ab}	8.25^{b}	3.24^{b}
E - 2	48.10^{a}	152.84^{cd}	60.13^{d}	28.66^{a}	7.14^{a}	3.56^{b}
Methanol extract						
M – 1	54.55^{b}	141.11^{c}	41.87^{b}	33.57^{ab}	8.23^{b}	3.02^{ab}
M - 2	57.66^{b}	103.09^{a}	30.10^{a}	58.95^{d}	8.42^{b}	2.85^{a}
Aqueous extract						
A - 1	48.27^{a}	151.68^{cd}	45.39^{c}	28.88^{a}	7.32^{a}	3.42^{b}
A - 2	47.25^{a}	133.91^{bc}	41.49^{b}	36.11^{b}	7.30^{a}	3.34^{b}
SEM***	1.45	6.09	2.24	3.28	0.26	0.11

*TDDM = true degradable dry matter; MBP = microbial biomass production; TVFA = total volatile fatty acid; A:P = ratio of acetate to propionate; **E-1, E-2, M-1, M-2, and A-1 and A-2 are 1 ml and 2 ml doses of ethanol extract (E), methanol extract (M) and aqueous extract (A), respectively, per 30 ml buffered rumen fluid; ***SEM = standard error of the mean; Within each column means followed by the same letter are not significantly different ($p<0.001$) by the Tukey's test.

Fenugreek leaves are a rich source of saponins with minute amount of flavanoids and tannins (Abdouli *et al*., 2012). The primary effect of saponins in rumen fermentation appears to inhibit the protozoa (Wang *et al*., 2000). However, decreasing the activities and numbers of methanogens by saponins (Patra and Saxena, 2009) of fenugreek leaves may also be responsible for reducing methane production. Saponins may selectively affect specific rumen bacteria and fungi, which may alter the rumen metabolism depending upon the chemical structure and doses (Hess *et al*., 2004; Wallace *et al*., 1994). The increase in total dry matter degradability (TDDM) may be due to the higher numbers of major fibrolytic bacteria and reduction in protozoa count, which also significantly increased ($p<0.001$) the microbial protein production with the addition of methanol extract (M-2) of fenugreek leaves. However, at the corresponding dose the microbial protein production was reduced with aqueous and ethanol extracts.

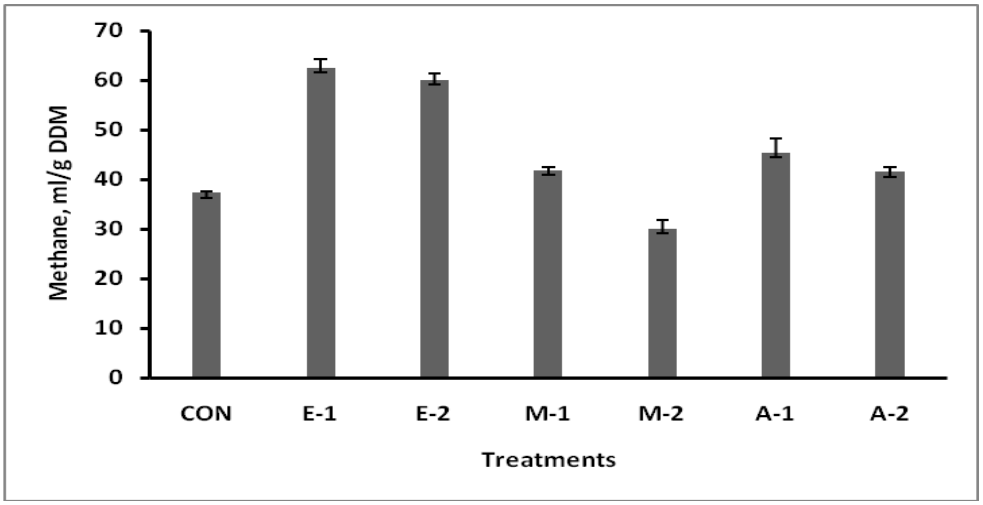

Figure 1. *In vitro* methanogenesis of wheat straw based diet dosed with extracts of fenugreek leaves (CON = Control; E-1, E-2, M-1, M-2, and A-1 and A-2 are 1 ml and 2 ml doses of ethanol extract (E), methanol extract (M) and aqueous extract (A), respectively, per 30 ml buffered rumen fluid). Vertical lines represent standard error of the mean.

The total volatile fatty acid (TVFA) production was not affected, however, the ratio of acetate to propionate (A:P) was reduced ($p<0.01$) with the inclusion of methanol extract. There was a reduction in TVFA production in treatments with aqueous and ethanol extracts. A decrease in protozoal numbers by saponins present in the methanol extract of fenugreek leaves may result in an increase in the proportions of propionate and reduce A:P ratio. Besides, saponins sometimes stimulate the growth of *Selenomonas ruminantium*, which is predominantly responsible for propionate production from succinate metabolism (Wolin *et al*., 1997).

Conclusion

Results of this study revealed that the methanol extract of fenugreek leaves would increase the feed degradability and microbial protein synthesis with reduction in methane production. Therefore, the methanol extract of fenugreek leaves could be used as an anti-methanogenic feed additive to improve the rumen fermentation in buffaloes.

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