Original Research

Evaluation of *In vitro* Gas Production and Digestibility of Wheat Straw Based Complete Feed Supplemented with Different Levels of Herbs in Rumen Liquor of Marwari Sheep

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Abstract

In order to assess the effect of herbal feed additive at different levels in wheat straw based complete feed on rumen fermentation, in vitro trials were conducted by taking these herbs viz. Shatavari (Asparagus racemosus), Brahmi (Bacopa monnieri), Bhringraj (Eclipta alba) and Jiwanti (Leptadenia reticulata) in complete feed. The different herbs were incorporated in the complete feed @ 0, 1, 2, 3, 4 and 5% level of the feed and designated as T_0, T_1, T_2, T_3, T_4 and T_5 treatment groups respectively. The data of in vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) were recorded at 48 h and 72 h as well as total gas production (IVTGP) at 24 h for each group, respectively. The results indicated a linear increase ($P < 0.01$) in IVDMD and IVOMD at 48 h and 72 h incubation with the increase in the level of supplementation of Shatavari herb up to 4% level (T_4) and 3% level (T3) for Bhringraj, Brahmi and Jiwanti herb, respectively. The total gas production was higher in the treatments group incorporated with herb at different levels than control (T_0). The supplementation of Shatavari herb as feed additive beyond 4% level and Brahmi, Bhringraj and Jiwanti herb each beyond 3% level of incorporation did not have any beneficial effect on total gas production. The total gas production was significantly higher in treatment groups at 4% level in Shatavari and 3% level in Brahmi, Bhringraj and Jiwanti supplemented feeds, respectively. The results suggested that supplementation of Shatavari herb as feed additive at 4% level and Brahmi, Bhringraj and Jiwanti herb at 3% level of incorporation have beneficial effect on IVDMD, IVOMD and IVTGP.

Key words: Complete Feed, Feed Additives, Herbs, IVDMD, IVOMD, Total Gas Production

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Introduction

The demand of feed and fodder for livestock is much higher than availability. Various hormones, antibiotics and feed additive have been used to increase the animal productivity (Chaturvedi *et al.*, 2015). Modification of ruminal fermentation using feed additives, such as antibiotics, has proved to be a useful strategy to improve production efficiency in dairy cattle. The use of antibiotics as feed additives has proved to be a useful tool to reduce energy and nitrogen losses from the diet (McGuffey *et al*., 2001). Since the use of antibiotics as feed additives in animals is banned due to risk of antibiotic residues in milk and meat. The increasing public concern about the use of antibiotics in the animal feed industry made scientists look for alternative to manipulate gastrointestinal microflora (Syadati *et al*., 2012). The use of herbal preparations appears as one of the most natural alternatives to the antibiotic use in animal nutrition.

The *in vitro* dry matter digestibility, *in vitro* organic matter digestibility, and *in vitro* gas production is a good indicator of the nutritive quality diet (Murrilo *et al*., 2011). The determination of the *in vitro* gas production is of value to nutritionist because it provides information on fermentation kinetics of forage consumed by ruminants, which is dependent on the rate of passage and the degradation rate (Mould *et al*., 2005). The rate and extent of dry matter fermentation in the rumen are crucial determinants utilized by ruminants. Therefore, this *In vitro* study was conducted to identify the optimum level of herbs in complete feed to increase the nutrient utilization from the *In vitro* rumen fermentation and total gas production by using sheep rumen liquor.

Materials and Methods

This study was carried out using four herbs viz. Shatavari, Brahmi, Bhringraj and Jiwanti. The complete feed was prepared by taking wheat straw as roughage source and concentrate in ratio of 60: 40 and was used as substrate for *In vitro* experiments. Concentrate mixture was composed of barley, deoiled rice bran, groundnut cake, guar korma, common salt and mineral mixture. The feed ingredients and four herbal feed additives were dried in hot air oven at 70^0C temperature for 24 h. After drying the herbs were grinded in the laboratory Willey Mill using 1.5 mm sieve. Each herbs were used at 1, 2, 3, 4 and 5% level of substrate i.e. wheat straw based complete feed to assess nutrient utilization by *In vitro* experiments. Four adult Marwari rams of same age and uniform conformation were selected as donor of rumen inoculum for *In vitro* study. The dietary requirements of the donor sheep were met by feeding the same complete feed as per ICAR (1985) which was used as substrate for *In vitro* studies.

Rumen liquor of animals was collected at 2 h post feeding. For *in vitro* studies 10 ml of strained rumen liquor along with 40 ml of fresh Mc Dougall buffer was added to the Erlenmeyer flask (Fig.1) containing substrate as per Tilley and Terry method (1963). The flask containing substrate along with rumen liquor and buffer was incubated at 39 ± 1^0C with periodic shaking for 48h of incubation in BOD incubator. After

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incubation, pH of *In vitro* rumen liquor was measured immediately using electronic pH meter calibrated against standard buffer solution. The contents were refluxed for 1h and filtered through pre-weighed Gooch crucibles. For *In vitro* studies at 72 h incubation of samples was done same as mentioned above for 48 h incubation studies. After 48 h of incubation the reaction was stopped by adding 2 ml of 6N hydrochloride acid and 0.1 g pepsin powder (1:3000) to each flask. Flasks were further incubated for another 24 h and contents of each flask were filtered through pre weighed Gooch crucible, dried and weighed. The dry matter content of the residue was weighed and *in vitro* dry matter digestibility of feed was calculated as follows-

$$In\ vitro\ \text{DM digestibility (\%)} = \frac{(\text{DM of feed taken for incubation} - \text{residue})\ \text{X100}}{(\text{DM of feed taken for incubation})}$$

The crucibles containing residue were kept for ashing in muffle furnace at 500^{0C} and organic matter present in residue was calculated. The organic matter digestibility was calculated as follows-

$$In\ vitro\ \text{OM digestibility (\%)} = \frac{(\text{OM of feed taken for incubation} - \text{OM in residue})\ \text{X100}}{(\text{OM of feed taken for incubation})}$$

An another flask containing 0.2g feed was incubated at 39 ± 1^{0}C for 24h with 30 ml medium in 100 ml capacity Hohenheim gas syringe (Fig. 2) (Menke and Steingass, 1988) for estimation of *In vitro* total gas production (IVTGP) in ml/200mg. Total gas production was calculated as displacement of piston in 24hr. The data obtained in the experiment were analyzed using statistical procedures as suggested by Snedecor and Cochran (1994). Significance of means differences were tested by Duncan's New Multiple Range Test (DNMRT).

Fig. 1: Erlenmeyer flask

Fig. 2: Hohenheim gas syringe

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Results and Discussion

The results indicated a linear increase (P<0.01) in IVDMD and IVOMD at 48 h and 72 h incubation with the increase in the level of supplementation of Shatavari herb up to 4% level (T4). Inclusion of Shatavari herb beyond 4% level of substrate did not have any additional improvement on IVDMD and IVOMD (Table 1 and 2). Similarly, IVDMD and IVOMD at 48 h incubation and 72 h incubation were highest (P<0.01) at 3% level of Brahmi, Bhringraj and Jiwanti supplementation in the complete feed, respectively but beyond 3% levels, IVDMD and IVOMD were reduced significantly (P<0.01).

Table 1: *In vitro* DM digestibility of complete feed containing different levels of herb as feed additive at 48 & 72 h incubation (% DM basis)

Herbs	Treatment Groups						SEM
	T_0	T_1	T_2	T_3	T_4	T_5	
48 h							
Shatavari	49.23^{a}	54.74^{b}	55.37^{b}	58.21^{c}	62.58^{d}	58.32^{c}	0.32
Brahmi	49.23^{a}	51.84^{b}	56.48^{d}	58.27^{e}	55.25^{c}	52.71^{b}	0.23
Bhringraj	49.23^{a}	55.71^{b}	58.35^{c}	62.14^{e}	60.28^{d}	59.95^{d}	0.28
Jiwanti	49.23^{a}	51.44^{bc}	51.92^{c}	56.38^{d}	52.01^{c}	50.91^{b}	0.21
72 h							
Shatavari	58.81^{a}	62.76^{b}	63.44^{b}	65.78^{c}	70.53^{d}	66.75^{c}	0.5
Brahmi	58.81^{a}	60.34^{b}	64.78^{b}	69.45^{e}	65.66^{d}	61.54^{c}	0.26
Bhringraj	58.81^{a}	64.65^{b}	66.87^{c}	70.69^{e}	68.35^{d}	65.43^{b}	0.26
Jiwanti	58.81^{a}	60.87^{b}	61.54^{bc}	65.78^{d}	62.56^{c}	59.77^{a}	0.27

Mean with different superscripts in a row differs significantly.

Table 2: *In vitro* OM digestibility of complete feed containing different levels of herb as feed additive at 48 & 72 h incubation (% DM basis)

Herbs	Treatment groups						SEM
	T_0	T_1	T_2	T_3	T_4	T_5	
48 h							
Shatavari	56.81^{a}	59.92^{b}	61.03^{b}	62.77^{b}	69.54^{c}	62.04^{b}	0.77
Brahmi	56.81^{a}	57.34^{a}	61.23^{c}	63.78^{d}	59.65^{b}	57.14^{a}	0.27
Bhringraj	56.81^{a}	61.54^{b}	64.43^{c}	66.92^{d}	63.60^{c}	64.38^{c}	0.31
Jiwanti	56.81^{a}	59.06^{b}	58.56^{b}	62.65^{c}	59.09^{b}	58.82^{b}	0.41
72 h							
Shatavari	62.90^{a}	69.46^{b}	70.94^{c}	72.02^{c}	78.57^{e}	74.75^{d}	0.33
Brahmi	62.90^{a}	64.74^{b}	68.56^{d}	75.22^{f}	70.44^{e}	66.35^{c}	0.49
Bhringraj	62.90^{a}	69.84^{b}	72.55^{d}	76.36^{f}	73.14^{e}	70.39^{c}	0.37
Jiwanti	62.90^{a}	64.62^{c}	65.36^{cd}	70.16^{e}	66.12^{d}	63.84^{b}	0.21

Mean with different superscripts in a row differs significantly.

These results also suggested that inclusion of Shatavari herb beyond 4% level and Brahmi, Bhringraj and Jiwanti herb each beyond 3% level of substrate did not have any significant improvement on *In vitro* nutrient utilization. The improvement in dry matter digestibility of complete feed might be due to enhanced

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microbial activity in rumen liquor due to presence of herbs. These herbs might have also enhanced the activity of microbes adhered to complete feed. The finding in the present study corroborated with that of Bakshi *et al*. (2004) who reported that the *In vitro* digestibility of nutrients were improved with herbal feed additives. The findings were also in line with observations of Sardar *et al*. (1998) who found that a mixture of herbs supplementation (50 mg/g) improved the *in vitro* dry matter digestibility (IVDMD) of concentrate mixture, oat fodder and concentrate mixture + oat fodder. Kumar *et al*. (2006) also reported significant ($P<0.05$) improvement in IVDMD from the substrate when mixed with *Asparagus racemosus*, *Cryptolepis buchanani*, *Eurphorbia hirta* and *Urtica dioica*.

The statistical analysis of data revealed significant ($P<0.01$) effect of herbs as feed additive on IVTGP (Table 3). *In vitro* total gas production at 24 h incubation were highest ($P<0.01$) at 4% level of Shatavari and 3% level of Brahmi, Bhringraj and Jiwanti supplementation in the complete feed.

Table 3: *In vitro* total gas production (ml/200mg) in complete feed containing different levels of herb at 24h incubation

Herbs	Treatment Groups						SEM
	T_0	T_1	T_2	T_3	T_4	T_5	
Shatavari	20.20^{a}	21.50^{b}	22.80^{c}	29.10^{e}	32.00^{f}	26.90^{d}	0.32
Brahmi	20.20^{a}	23.50^{b}	27.40^{c}	32.20^{e}	28.80^{d}	28.60^{d}	0.37
Bhringraj	20.20^{a}	21.30^{b}	24.50^{c}	28.60^{e}	28.50^{e}	25.90^{d}	0.26
Jiwanti	20.20^{a}	20.10^{a}	22.30^{b}	26.50^{c}	23.40^{b}	22.40^{b}	0.27

Mean with different superscripts in a row differs significantly.

The increase in total gas production appeared to be associated with higher DM and OM degradability in herb supplemented groups due to increase in bacterial number and activity, rate of fermentation might have increased in herb supplemented groups and hence total gas production was increased in present study. These results corroborate well with earlier report, where higher production of total gas observed due to herbal feed additive supplementation (Bakshi *et al.*, 2004; Patra *et al.*, 2006; Wadhwa and Bakshi, 2006; Ganai *et al.*, 2011; Meel *et al.*, 2016 and Chaturvedi *et al.*, 2016).

Conclusion

The result of *in vitro* rumen fermentation revealed that Brahmi, Bhringraj and Jiwanti herb at 3% level and Shatavari herb at 4% level in the wheat straw based complete feed could be selected for *In vivo* efficiency of nutrient utilization in sheep.

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