Original Research

Effect of Herbs as Feed Additive on Rumen Fermentation Patterns and Haemato-Biochemical Parameters in Marwari Rams Fed Wheat Straw Based Complete Feed

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Abstract

A feeding trial was conducted to evaluate the effect of herbs as feed additive on rumen fermentation patterns and haemato-biochemical parameters in Marwari rams fed wheat straw based complete feed. Twenty Marwari rams of same breed age group (15-18 months) and of uniform conformation were randomly distributed into 5 groups of 4 animals in each subjected to five treatments. The dietary treatments were group-T1 fed complete feed without any herb (control), T2 fed complete feed with 4% Shatavari (Asparagus racemosus), T3 fed complete feed with 3% Brahmi (Bacopa monnieri), T4 fed complete feed with 3% Bhringraj (Eclipta alba) and T5 fed complete feed with 3% Jiwanti (Leptadenia reticulata). At the end of metabolic trial rumen liquor sample were collected from each animal at 0, 2, 4, 6, 8, 12 and 18 h post feeding for the estimation of pH, total volatile fatty acid, total protozoal count and various nitrogen fractions such as total nitrogen, ammonia nitrogen, TCA-precipitable nitrogen and non-protein nitrogen. Besides this, haemato-biochemical parameters were also estimated at the end of trial to study the physiological status of health of experimental rams. The results from this study showed highly significant ($P<0.01$) effect of period on pH, TVFA, total protozoal count, ammonia nitrogen, total nitrogen, TCA-precipitable nitrogen and non-protein nitrogen. Herb supplementation also significantly ($P<0.01$) improved the rumen fermentation resulting in increased TVFA, decreased total protozoal count and ammonia nitrogen, increased total nitrogen and TCA-precipitable nitrogen. Whereas, non-significant effect of treatment was observed on pH, NPN and various haemato-biochemical parameters. It was concluded that supplementation of Shatavari, Brahmi, Bhringraj and Jiwanti herbs as feed additives could be a viable proposition for better utilization of nutrients through improvement in rumen fermentation in the Marwari rams.

Key words: Complete Feed, Feed Additive, Herbs, Marwari Rams

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Introduction

Manipulation of rumen fermentation is possible to increase the nutrient utilization that subsequently improves the efficiency of production by farm animals (Kamra *et al.*, 2002; Wang and Wang, 2016). Uses of feed additives in diet of livestock for nutrient utilization as well as growth and production have immense importance. Feed additives that modify rumen fermentation such as organic acids, yeast, enzymes and ionophores are being used to optimize performance in animal production systems. Since the use of antibiotics as feed additives in animals is banned in many countries and is likely to be banned in others due to risk of antibiotic residues in milk and meat, the search for alternative feed additives has become the necessity of the day. With the demand of organic food, attention has recently been shifted to natural herbal feed additives. Traditionally, some herbs or their mixtures are used to promote feed intake due to their flavouring and medicinal properties for enhancing production of livestock.

The use of medicinal herbs and plants for human has been well known since the old civilization of ancient Egyptian. Many attempts were carried out to use natural materials such as medicinal herbs which are widely accepted as feed additives to improve the efficiency of feed utilization and productive performance of farm animal such as sheep, goat, buffaloes and cows (Allam *et al.*, 1999; Maged 2004; Shehata *et al.*, 2007 and Shwereb, 2012). In addition, using medicinal herbs in animal rations was the preventive solution to avoid the hazard of side effects using chemicals (EI-Kholany *et al.*, 2015). Therefore, this study was conducted to assess the effect of herbs viz. *Asparagus racemosus* (Shatavari), *Bacopa monnieri* (Brahmi), *Eclipta alba* (Bhringraj) and *Leptadenia reticulata* (Jiwanti) as feed additive on rumen fermentation patterns and haemato-biochemical parameters in Marwari rams fed wheat straw based complete feed.

Materials and Methods

The complete feed was prepared using wheat straw as basal roughage source and concentrate in the ratio of 60:40. The wheat straw based complete feed was fortified with optimum level of four herbs *viz.* Shatavari, Brahmi, Bhringraj and Jiwanti and designated as T_1 (complete feed without any herb), T_2 (complete feed + Shatavari herb at 4% level), T_3 (complete feed + Brahmi at 3% level), T_4 (complete feed + Bhringraj at 3% level) and T_5 (complete feed + Jiwanti at 3% level). Twenty Marwari rams were divided randomly into five groups of four each and allowed to acclimatize for a period of 10 days prior to experimental feeding. The animals in each group were offered respective complete feed every morning. At the end of metabolic trial rumen liquor sample were collected from each animal at 0, 2, 4, 6, 8, 12 and 18 h post feeding for the estimation of pH, total volatile fatty acid, total protozoal count and various nitrogen fractions such as total

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nitrogen, ammonia nitrogen, TCA-precipitable nitrogen and non-protein nitrogen. Rumen fluid pH was determined immediately after collection using portable digital pH meter (pen type) at the site of collection. After pH determination one ml of saturated $HgCl_2$ solution was added to each collected sample to kill the microbes and to stop metabolic activity. Total volatile fatty acids were determined according to the method (Barnett and Reid, 1957) using Markham still distillation apparatus. Total protozoal count of the SRL was done by the method of Moir and Somers (1956) adopted by Purser and Moir (1959) using Sedgewick Rafter Cell (50×20×1 mm) in 10×10 magnification. Rumen ammonia nitrogen in SRL was estimated by Conway's micro diffusion method (1957) using Conway diffusion cell. Total nitrogen was estimated by Kjeldahl method. Haemato-biochemical parameters were also estimated at the end of trial to study the physiological status of health of experimental rams. Haemoglobin and PCV were estimated by Automatic Coult Counter. Blood serum parameters viz. total protein and blood glucose were estimated by Automatic Biochem Analyzer of Schiapparelli Biosystems, INC, using standard kits. Statistical analysis was done according to Snedecor and Cochran, (1994).

Results and Discussion

Rumen liquor samples were collected from the experimental animals at 0, 2, 4, 6, 8, 12 and 18 h post feeding to assess the effect of feeding complete feed containing Shatavari (4% level), Brahmi (3% level), Bhringraj (3% level) and Jiwanti (3% level) herb as feed additive on the rumen functioning.

Rumen pH

The results (Table 1) indicated that ruminal pH were non significantly affected by the dietary herb supplementation. However, the effects of time of sampling were found to be highly significant ($P<0.01$). The comparison of means revealed higher pH values at 18 h post feeding in all experimental groups. After feeding pH significantly decreased from 0 h to a minimum level at 4 h post feeding and after that increasing trend in pH was noticed and values at 18 h were close to the 0 h values. The results of present investigation are in consistent with the earlier reports of Hosoda *et al.*, 2006 and Amanullah *et al.*, 2009 reported non-significant effect on rumen pH values among the different treatment groups receiving dietary herb supplementation.

In the entire treatment group, a significant fall in pH was recorded at 4 h post feeding, possibly due to greater production of volatile fatty acids obtained at similar hour (Mc-Allan, 1991). While at 6 h post feeding pH tended to increase and could be explained on the basis of greater inflow of bicarbonate rich alkaline saliva buffering the ruminal contents (Turner and Hodgetts, 1955).

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Table 1: Rumen fermentation pattern in Marwari rams fed complete feed supplemented with herbal feed additives

Diet/ Hour	pH	Total protozoal count (×105per ml)	TVFA (meq/L)	NH_3-N (mg/dl)	Total N (mg/dl)	TCA-PN (mg/dl)	NPN (mg/dl)
Dietary Treatment							
T1	6.7	3.95^{c}	76.65^{a}	17.33^{d}	88.39^{a}	52.92^{a}	35.46
T2	6.72	3.35^{a}	80.01^{d}	15.86^{a}	92.02^{b}	55.66^{b}	36.36
T3	6.69	3.53^{b}	78.61^{c}	16.55^{bc}	91.50^{b}	55.93^{b}	35.57
T4	6.76	3.43^{a}	79.12^{c}	16.13^{ab}	92.28^{b}	57.09^{b}	35.19
T5	6.74	3.62^{b}	77.23^{b}	16.95^{cd}	89.43^{a}	53.37^{a}	36.05
	NS	**	**	**	**	**	NS
SEM	0.05	0.2	0.75	0.44	1.12	1.49	0.86
Time of Rumen Liquor Sampling (h)							
0 h	6.86^{d}	3.93^{c}	64.94^{a}	14.91^{a}	67.67^{a}	46.98^{a}	20.69^{a}
2 h	6.59^{b}	3.07^{a}	75.58^{d}	17.03^{c}	95.74^{d}	62.84^{c}	32.89^{c}
4 h	6.38^{a}	2.87^{a}	87.98^{f}	19.05^{e}	114.03^{f}	64.45^{d}	49.57^{f}
6 h	6.62^{b}	3.49^{b}	96.47^{g}	17.63^{d}	101.25^{e}	60.08^{c}	41.17^{e}
8 h	6.78^{c}	3.76^{c}	80.76^{e}	16.88^{c}	96.47^{d}	56.01^{b}	40.46^{e}
12 h	6.83^{d}	3.92^{c}	72.65^{c}	16.04^{b}	85.99^{c}	47.60^{a}	38.38^{d}
18 h	6.99^{e}	3.99^{c}	68.45^{b}	14.40^{a}	73.91^{b}	47.00^{a}	26.91^{b}
	**	**	**	**	**	**	**
SEM	0.04	0.16	0.61	0.36	0.91	1.21	0.7

*** (P<0.01), NS = Non-significant, N=Nitrogen, TVFA=Total Volatile Fatty Acids, PN= Perceptible Protein Nitrogen, NPN= Non Protein Nitrogen, SEM=Standard Error of the Mean*

Total Volatile Fatty Acids

Comparison of means revealed significantly higher values of total volatile fatty acid concentration in all the herbal supplemented groups compared to control group. The concentration of TVFA obtained at 0, 2, 4, 6, 8, 12 and 18 h post feeding initially showed a continuous rising trend with peat at 6 h post feeding. Thereafter, TVFA showed decline trend upto 18 h. The peak concentration of TVFA at 6 h post feeding in SRL have also been reported by Venkanna *et al.*, 1997, Tomar and Senger, 1999 and Gupta *et al.*, 2005. While assessing the effect of herb supplementation increased concentration of TVFA in herb supplemented group may be the result of stimulatory effect of herb on multiplication of microorganism and therefore, the concentration of TVFA increased. An increase in TVFA concentration on supplementation of herbs and herbal product has been reported by Sardar *et al.*, 1997, Manjunatha, 1998 and Pankaj *et al.*, 2008.

Total Protozoal Count

Statistical analysis of data (Table 1) revealed highly significant (P<0.01) effect of herb supplementation as well as time of sampling. The results suggested that addition of herbs as feed additive decreased rumen protozoa significantly at different hours of the day and numerically considerable reduction was observed at any hours of the day. The results of present investigation correspond well with the findings of Rejil *et al.*, 2008 and Amanullah *et al.*, 2009 observed decrease in rumen protozoal number in cattle on feeding

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Fenugreek seed and *Sapindus mukorrossi*, *Camelia sinensis* and *Acacia concinna* as feed additive. Across period, the protozoa number showed significantly decreasing trend across the period of sampling with least number at 4 h post feeding and an increase thereafter. The probable reason in the present study is that the pH of the rumen liquor was lowest at this hour of post feeding, since protozoal population is very sensitive to change in pH and may be inhibited or eliminated at low pH (Hungate, 1966).

Nitrogen Fractions

Rumen Ammonia Nitrogen

The overall mean values of ammonia nitrogen irrespective of period when subjected to DNMRT were found to be significantly lower for herb supplemented group and higher in control group. The ammonia nitrogen concentration was observed at peak level 4 h post feeding in all experimental groups. The peak concentration of ammonia at 4 h was possibly due to maximum proteolytic deaminase activity at this hour, while, decrease in concentration 6 h post feeding onwards may be due to simultaneous absorption or its utilization by the microbes in synthetic activity of rumen. A decreased ammonia nitrogen concentration was seen in steers fed peppermint as feed additive (Ando *et al.*, 2003), in swamp buffaloes fed eucalyptus leaf meal as feed additive (Thao *et al.*, 2015). The results of study in text corroborate well with the earlier reports where reduced ammonia nitrogen concentration due to higher incorporation of ammonia nitrogen into microbial protein was observed due to herb supplementation (Sardar *et al.*, 1997 Rejil *et al.*, 2008 and Ammanullah *et al.*, 2009). Similarly, Meel *et al.*, 2017 also reported that Ashwagandha, Reetha alone or in combination feeding to Rathi calves as feed supplement tended ($P<0.01$) to decrease the ammonia nitrogen concentration compared with those in the control group.

Total Nitrogen

The overall mean values of total rumen nitrogen irrespective of period when subjected to DNMRT were found to be significantly lower for control group and higher in herb supplemented group. However, the values obtained for Jiwanti supplemented group was comparable to the un-supplemented group. Across period, the total rumen nitrogen showed significantly increasing trend across the period of sampling with maximum concentration at 4 h post feeding and later decreased upto 18 h post feeding.

TCA-Precipitable Nitrogen

The comparison of means by DNMRT revealed significantly higher values in herbs supplemented groups as compared to control group. However, the values obtained in Jiwanti supplemented group were comparable to the control group. The TCA-precipitable nitrogen showed significantly increasing trend across the periods of sampling with maximum concentration at 4 h post feeding and then a decrease thereafter up to 18 h post feeding.

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Non Protein Nitrogen

Statistical analysis of data revealed non-significant effect due to inclusion of various herbs in complete feed. However, effect due to time of sampling was found to be highly significant (P<0.01). Across period, NPN concentration showed significantly increasing trend up to 4 h post feeding and later decrease up to 18 h post feeding. The peak concentration at 4 h post feeding might be due to more rumen microbial activity during this period. The peak concentration of total nitrogen, non-protein nitrogen, ammonia nitrogen in SRL has been reported during this period by Venkanna *et al.*, 1997 and Tomar and Sengar, 1999. The higher level of total nitrogen may be attributed to a significantly higher proteolytic activity of the rumen in herb supplemented group. Such higher proteolytic activity in rumen has been reported by Yoon and Stem (1996) and Moloney and Drennan (1994).

The higher concentration of TCA-precipitable nitrogen in herb supplemented group might be due to increased utilization of ammonia nitrogen by rumen microbes for microbial protein synthesis.

Haemato-Biochemical Studies

To ascertain the effect of different herbs supplementation in wheat straw based complete feed on physiological status of health, routine haemato-biochemical studies (haemoglobin, packed cell volume, blood glucose, blood urea nitrogen, total serum protein and serum creatinine) were made in different treatment groups. The mean values of all haemato-biochemical parameters revealed non-significant effect of herb supplementation in complete feed. The results were in agreement with the findings of Randhawa *et al.*, 1995; Hosoda *et al.*, 2006 and El-Alamy *et al.*, 2001 who also reported non-significant effect of herbal preparation in the diet on haemato-biochemical parameters.

At the end, from the results of study undertaken regarding supplementation of herbs *viz.* Shatavari, Brahmi, Bhringraj and Jiwanti as feed additive in complete feed taking into account rumen fermentation pattern and ancillary observations of haemato-biochemical parameters it could be concluded that supplementation of Shatavari herb at 4% level and Brahmi, Bhringraj and Jiwanti each at 3% level in a viable proportion to improve the nutrient utilization efficiency in sheep. Further, looking to the improvement in rumen fermentation of the rams on account of supplementation of herbs, it appears that it could be a practical viable technology that can be adopted to improve feed utilization efficiency and to have sustainable sheep production system in arid and semi-arid areas.

Conclusion

Supplementation of herbs viz. Shatavari, Brahmi, Bhringraj and Jiwanti in wheat straw based complete feed has a positive effect as evidenced by increasing concentration of rumen metabolites in SRL of Marwari

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rams. It appears that supplementation of herbs could be a viable proposition to improve the nutrient utilization efficiency in Marwari rams; however, more studies are required.

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