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Efficacy of different plant part combinations as rumen fermentation modulator in wheat straw based diet evaluated *in vitro*

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ABSTRACT

The in vitro gas production technique was used to evaluate effect of different plant powder combinations on rumen fermentation parameters and methane production. Neem (Azadiracta indica); Mehndi (Lawsonia inermis) and Eucalyptus (Eucalyptus globulus) plant parts were used for the preparation of different combinations (1:1) i.e Mehndi + Neem (T_1), Neem + Eucalyptus (T_2), Mehndi + Eucalyptus (T_3) and Neem+ Mehndi + Eucalyptus (T_4). The different combinations (20 mg) were incubated with in vitro substrate (200 mg, 50R:50C) and 30ml buffered rumen fluid for 24 h. Results show that methane production, protozoa population were reduced significantly on addition of T_3. T_3 also increase DDM (mg), propionate concentration and partition factor on incubation with wheat straw based diet. Among the all treatments, T_4 reduced pH and protozoa population significantly. The present results demonstrate that plants powder combination is a promising feed additive in wheat straw based diet. They have the potential to modulate the methane production, dry matter digestibility, propionate concentration and microbial biomass synthesis.

Key words: Plant powder combination, DDM, methane, microbial biomass, *In vitro* gas production technique.

INTRODUCTION

In India methane emission from the livestock is the major contributor to the global warming. In 2009, India livestock methane-emission was 11.75 million metric tons per year higher than the 9 million metric tons estimated in 1994[1]. To mitigate methane emission is considered as an international goal in order to reduce global warming as methane is considered to be a potent green house gas. Level of methane emission from the ruminants is affected by a number of

factors such as level of feed intake, addition of lipids and ionophores in their diet, change in rumen microbial environment and the level of animal productivity have been identified[2,3,4].

Plants having secondary metabolites have been thought to play an important role in reducing methanogenesis in rumen [5]. Saponins or saponin-like substances and tannins have been reported to suppress methane production, reduce rumen protozoa counts, and modulate fermentation pattern [6,7,8]. However, effectiveness of plants or plant extracts having high content of saponins and tannins varied depending upon the source, type and level of secondary metabolite present in it. Several secondary compounds contained in plants can be used as a safe means of ruminal fermentation modulators. However, only a small number of plant species have been tested to date, and only few studies have dealt specifically with the possibility of decreasing methane production using phytogenic additives. The present experiment was planned to evaluate the potential of several plant parts in combination on rumen fermentation and methane reduction under *in vitro* conditions.

MATERIALS AND METHODS

Procedure of plant powder combinations preparation

The investigator herbal plant parts were Neem (*Azadiracta indica*); Mehndi (*Lawsonia inermis*) and Eucalyptus (*Eucalyptus globulus*) manually collected from National Dairy Research Institute, Karnal, INDIA. The plants materials were dried at 70^0C and ground in mills to pass a 1 mm sieve. Finely grounded plant parts were used for the preparation of different combinations (1:1) i.e. Mehndi + Neem (T_1), Neem + Eucalyptus (T_2), Mehndi + Eucalyptus (T_3) and Neem+ Mehndi + Eucalyptus (T_4).

Preparation of diet

To evaluate the effect of different plant powder combination diet was prepared by taking roughage concentrate ratio of 50:50. The roughage part composed of wheat straw and the concentrate part composed of maize (33%), GNC (21%), mustard cake (12%), wheat bran (20%), deoiled rice bran (11%), mineral mixture (2%) and salt (1%) respectively.

Treatments and experimental design

Twenty milligram (% of DM basis) of each treatments were added to the diet sample in glass syringe (100ml) containing 200 mg of milled (1mm) wheat straw based diet. All the treatment combinations were arranged in RBD with three replicates**.** Sets was also incubated devoid of substrate with and with out plant powder combinations which served as blanks for particular treatment and values were corrected for different parameters with these blanks.

In vitro gas production

The buffer and rumen liquor were prepared as described by [9]. Rumen samples were obtained after manual mixing of rumen contents from three rumen fistulated mature male buffalo (*Bubalus bubalis*). The buffalo were kept on a standard diet comprising concentrate and roughage in a ratio 50:50.

Total gas production and methane estimation

After 24 h incubation, total gas production was estimated by the displacement of piston during incubation. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank syringe (containing no substrate, but only the inoculum and buffer) from total gas produced in the syringe containing substrate and inoculum and buffer. For methane estimation, representative gas was sampled from the headspace of syringe in an airtight syringe and injected into Nucon-5765 gas chromatograph equipped with flame ionization detector (FID) and stainless steel column packed with Porapak-Q (length 6';o.d.1/8" i.d. 2 mm; mesh range 80-100). Temperatures were 40, 50 and 50^0 C, in injector oven, column oven and detector respectively and the flow rates of carrier gas (nitrogen), hydrogen and air were 30, 30 and 300 ml/min, respectively. For methane estimation, each gas sample (250µl) was manually injected using Hamilton airtight syringe. Methane content in sample was calculated by external calibration, using a certified gases mixture with 50% CH_4 and 50% CO_2 (Spantech calibration gas, Surrey, England). The volume of methane produced was calculated as follows:

Methane production (ml) = Total gas produced (ml) × % methane in the sample

Partitioning factor and microbial biomass yield

The PF is calculated as the ratio of substrate truly degraded *in vitro* (mg) to the volume of gas (ml) produced by it. The MBM yield was calculated by using the degradability of substrate and gas volume and stoichiometrical factor [10].

Microbial mass (mg) = Substrate truly degraded - (gas volume × stoichiometrical factor)
Where the stoichiometrical factor used was 2.25.

Total volatile fatty acid (TVFA) estimation

TVFA concentration (mM/100 ml) in the supernatant was estimated according to prescribed method [11].

Individual volatile fatty acid (IVFA) estimation

Individual volatile fatty acid estimated by gas chromatograph according to the prescribed method [12].

Estimation of ammonia nitrogen

The supernatant of each syringe including that of blank was used for NH_3-N estimation. Supernatant (5 ml) was mixed with 1 N NaOH (2 ml) and steam passed on this using KEL PLUS - N analyzer (Pelican, India) and the NH_3 evolved was collected in boric acid solution having mixed indicator and titrated against N / 100 H_2SO_4.

Protozoa counting

The protozoa in fermentation fluid were counted by Haemocytometer as per the prescribed method [13].

***In vitro* true DM degradability**

To estimate true DM degradability of feed sample of each syringe containing residues after incubation was estimated as per the prescribed method [14].

Proximate analysis and Cell wall constituents

The proximate analysis (Organic matter, Crude protein, Ether extract, Total Ash) of substrate was carried out as per the methods of AOAC [15]. The Neutral detergent fibre of substrates were determined according to prescribed method [14] and other cell wall components like Acid detergent fiber (ADF) and Hemicellulose (HC) as per the method [16] .

Statistical analysis

Experimental data of different parameters were analyzed in randomized block design with three replicates for analysis of variance [17].

RESULTS AND DISCUSSION

Chemical composition of diet presented in table 1. Results of incubating the wheat straw based diet *in vitro* during 24 h with different plant powder combinations on *in vitro* rumen fermentation and methanogenesis is shown in Table 2 and 3.

Results show that pH was affected by these treatments and found similar among T_1, T_2 and T_3 combinations, except in T_4 combination in which pH was significantly reduced. Digestible dry matter (DDM) content increased significantly in all treatment groups. Surprisingly the digestibility of DM was not decreased rather increased in most of the treatment combinations by addition of these powder in wheat straw based diet. The maximum increase in DDM (mg) was noticed 44.60% in T_3 in comparison with control diet. Further increase was 36.70%, 35.00% and 20.40% in T_1, T_2 and T_4 combinations, respectively. Similarly, partition factor value was highest in T_3, here the increase was 39.3%. Microbial biomass (MBM) yield was also highest in T_3 i.e. 143 percent in comparison to control diet. Maximum reduction in methane (mM/g DM) was observed up to 12 percent in T_3 combination and propionate concentration also increased significantly (21.8%) in this combination. Increase in propionate concentration was 9.80%, 14.00%, and 10.00% in T_1, T_2 and T_4 combinations, respectively. Results also showed about 45% decrease in protozoal population in T_3 and T_4 combinations. The present results demonstrate that T_3 (mehndi leaves + eucalyptus leaves) combination seems to be promising feed additive in wheat straw based diet. It addition in diet increases dry matter digestibility, propionate concentration and microbial biomass synthesis and decreased methane productions. Further research with different diets is required to assess the dietary conditions that influence the effectiveness of these plant parts combinations.

Table1. Chemical composition of total mixed ration (50% Roughage: 50%Concentrate)

Parameters	Diet (50R:50C)
OM	87.84
CP	12.53
EE	3.04
NDF	60.45
ADF	32.95
HC	27.50
Cellulose	21.80
ADL	5.06
Total Ash	12.16

OM= Organic matter, CP= Crude protein, EE= Ether extract, NDF= Natural detergent fiber, ADF= Acid detergent fiber, HC= Hemicelluloses, ADL= Acid detergent lignin, Roughage=Wheat Straw, Concentrate= Normal farm concentrate

Table2. Effect of different plant powder combinations on rumen fermentation and methane production in wheat straw based diet (50 R: 50 C)

Parameters	Treatment					
	Control	(Mehndi + Neem) T_1	(Neem + Eucalyptus) T_2	(Mehndi + Eucalyptus) T_3	(Neem+ Mehndi + Eucalyptus) T_4	SEM
pH	7.13	7.10	7.08	7.10	6.99	0.02
DDM (mg)	96.33	131.67	130.00	139.33	116.00	3.38
PF	3.21	3.24	3.94	4.47	3.41	0.15
MBM (mg)	28.83	40.17	55.75	69.21	39.12	4.23
CH_4 (mM/gDM)	2.65	3.74	2.52	2.20	2.49	0.20
NH_3-N (mg/100ml)	21.47	20.35	18.29	18.39	17.27	0.72

DDM= Digestible dry matter, PF= Partition factor, MBM= Microbial biomass, NH_3-N= Ammonia nitrogen, CH_4=Methane, SEM=standard error of means.

Table3. Effect of different plant powder combinations on short chain fatty acids in wheat straw based diet (50 R: 50 C)

Parameters	Treatment					
	control	(Mehndi + Neem) T1	(Neem + Eucalyptus) T2	(Mehndi + Eucalyptus) T3	(Neem+ Mehndi + Eucalyptus) T4	SEM
TVFA (mM/100ml)	67.00	70.83	77.50	77.83	74.83	4.87
Acetate (mM/100ml)	53.39	56.30	61.69	61.59	60.03	NS
Propionate (mM/100ml)	10.99	12.07	12.54	13.39	12.11	NS
Butyrate (mM/100ml)	2.62	2.47	3.27	2.85	2.69	NS
No. of Protozoa/ml(10^4)	1.83	1.67	1.67	1.00	1.00	NS

TVFA= Total volatile fatty acids

CONCLUSION

In ruminants, many ionophores**,** antibiotics have been used to improve the rumen fermentation, improving the some end product (propionate) and decreasing the total amount of methane. Since, January 2006, European Union banned the use of antibiotics as a feed additive due to the risk of its residue in animal products (e.g.: milk and meat) and its subsequent effects on human health. Therefore, safe and cost effective new alternatives are needed to maintain efficient animal production systems. Plants or plants parts are very good natural alternatives of synthetic compounds and plants products use as a productivity enhancer provides cheaper, safer and more consumer- acceptable alternatives. This study suggested that the plant powder have the potential to affect ruminal fermentation efficiency.

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