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Enteric methane mitigation and improved animal performance by feeding rumen modifier

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Abstract
The present investigation had been focused on evaluating the effect of feeding a rumen modifier which is mixture of RM-7 [neem seed cake (*Azadirachta indica*), mahua seed cake (*Madhuca longifolia*), fennel seed (*Foeniculum vulgare*), harad (*Terminalia chebula*), fruit pulp of bahera (*Terminalia bellirica*), fruit pulp of amla (*Phyllanthus emblica*) and ajwain seed (*Trachyspermum ammi*) in 2:2:2:1:1:1:1 proportion] and sodium sulphate on enteric methane production and improved animal performance. Twenty one growing buffalo calves divided into three groups of seven animals each were selected for the experiment. All the animals were fed iso nitrogenous diet with basal diet i.e. concentrate and roughage in 50:50 ratio. The control group (T1) was fed basal diet, the treatment group (T2) was fed basal diet plus rumen modifier (RM-7 at 2% DMI + sodium sulphate at 0.06%) and the treatment group (T3) was fed basal diet plus rumen modifier (RM-7 at 4% DMI + sodium sulphate at 0.06%) for a period of 120 days. Daily dry matter intake and body weight gain of the animal showed no significant ($P>0.05$) difference among treatment groups. The percent reduction in methane production was significantly ($P<0.05$) less in treatment groups when compared to control.

Keywords: Methane, ionophores, methanogenesis, plant secondary metabolites, sulphur

1. Introduction

A large number of microbes inhabited in the anaerobic environment of rumen include bacteria, archaea, protozoa, fungi, bacteriophage and mycoplasma, are responsible for feed fermentation. The end products of fermentation are volatile fatty acid, ammonia, amino acids, carbon dioxide, methane and hydrogen. Both CO_2 and CH_4 are active green house gases, which are responsible for global warming [1]. CO_2 has less potential towards global warming whereas methane (CH_4) is a highly potent. Methanogenesis decrease feed utilization efficiency, as 2-12% of gross energy of feed consumed by the animals is wasted in the form of methane [2]. Ruminants, especially cattle and buffalo are the target species for methane mitigation because these animals contribute 91.9% of total enteric methane emission. Among these two species annual growth rate in methane production by buffalo are 2.21 times more than cattle [3].

Due to climate change, eco-friendly and precision feeding practices are adopted in feeding to minimize methane production and avoid wastage of nutrients to maximize the animal production. Use of chemical compounds as feed additives, feed processing technologies [4], defaunation [5], supplementation of unsaturated fatty acid [4], ionophores, microbial feed additives and prebiotics [6] etc. have been tried, but most of them showed inconsistent results between *in vitro* and *in vivo* studies and their long term use showed microbial resistance.

Plant secondary metabolites (PSM) are natural components of herbs and spices which do not produce any adverse effect on animals or residual effect on the livestock products. These include saponins, tannins, lignins, alkaloids, essential oils etc. have antimethanogenic as well as antiprotozoal activity [7]. Various studies showed that 30-45% of methanogenic activity is related with ciliate protozoal population, because methanogens are externally attached with protozoa and undergo interspecies hydrogen transfer [8]. The sulphate reducing bacteria (SRBs) reduce sulphate into hydrogen sulphide by utilizing ruminal hydrogen but, main hurdle is its limited activity due to low sulphur concentration and low number of SRB in the rumen [9]. Therefore, sulphate along with PSMs can act as rumen modifiers to mitigate methane production and improved nutrient utilization. So the present study was conducted with the aim to reduce methane production without any harmful effects on the performance of animals.

2. Materials and Methods

2.1 Selection and management of buffalo and experimental design

The experiment was conducted on twenty one growing male buffaloes of about 15-18 months of age divided into three groups of seven animals each at the Animal Farm, Indian Veterinary Research Institute (IVRI), Izatnagar, India. The experiment was conducted for 120 days and animals were fed as per ICAR [10] feeding standards. The dietary treatments were divided into three groups. The first group (control, T1) was fed control diet containing wheat straw and concentrate in 50:50 ratio without rumen modifier. The second group (T2) was fed diet similar to that of control group along with rumen modifier mixed with the concentrate (RM-7 and sodium sulphate at 2% and 0.06% of DMI, respectively). The third group (T3) also was fed same diet of control group plus rumen modifier mixed with concentrate (RM-7 and sodium sulphate at 4% and 0.06% of DMI) respectively.

2.2 Experimental diet

Experimental diet was prepared from plant parts brought from local market of Izatnagar, Bareilly. The seed kernel of neem *(Azadirachta indica)* contains bitter principles like nimbin, nimbidin and azadirachtin. Dried fruits of harad *(Terminalia chebula)* and bahera *(Terminalia bellirica)* are rich in tannins, whereas seeds of mahua *(Madhuca longifolia)* are a rich source of saponin. Fruit of amla *(Phyllanthus emblica)*, seeds of ajwain *(Trachyspermum ammi)* and fennel *(Foeniculum vulgare)* are rich in essential oils. The parts were dried, powdered to prepare a blend (RM-7) in a proportion of mahua (20g/100g), fennel (20g/100g), neem (20g/100g), amla (10g/100g), ajwain (10g/100g), harad (10g/100g) and bahera 117 (10g/100g). The concentrate mixture consisted of maize, 35; solvent extracted soybean meal, 24; wheat bran, 38; mineral mixture, 2 and salt, 1%. The substrate comprised of concentrate mixture and wheat straw in 1:1 ratio. The proximate composition of wheat straw, concentrate and rumen modifier are presented in Table 1.

Table 1: Chemical composition (% of DM) of concentrate mixture, wheat straw and RM-7 fed to growing buffaloes

Attributes	Concentrate mixture	Wheat straw	RM-7
Dry matter	96.68	95.09	91.33
Total ash	10.69	11.07	10.04
Organic matter	89.30	88.92	89.96
Crude Protein	18.50	2.90	10.04
Ether extract	3.51	1.90	2.80
Neutral detergent fibre	27.83	89.35	43.63
Acid detergent fibre	11.02	54.23	38.73

RM-7: mixture of neem, fennel, mahua, harad, bahera, ajwain and amla in 2:2:2:1:1:1:1 proportion

2.3 Feed intake

Feed intake by animals and leftovers of previous day feed were recorded daily. The dry matter content of offered and residual feed was also estimated to calculate the total dry matter intake by the animals.

2.4 Fortnightly body weight changes

Fortnightly changes in body weight of growing buffaloes was monitored to check the growth rate, performance and feed conversion efficiency of the animal. The individual animal was weighed using an electronic weighing scale in the morning, prior to feeding and watering and recording was done fortnightly for a period of 120 days.

2.5 Estimation of methane emission by open circuit respiration chamber

The animals were shifted in open circuit respiration chamber and after acclimatization, methane emission was measured. The procedure involved observation of flow rate, temperature of dry and wet bulb and atmospheric pressure. Methane was measured by an Infrared Gas Analyzer (Analytical Development Co. Ltd. Hoddesdon, England, model 300) by measuring flow rate and the total volume of air coming to the chamber and going out from the chamber. Atmospheric pressure was recorded by an electronic device. sampling air pump set at a flow rate of 250l/min and provided with a by-pass arrangement. The chamber was opened every 22 h to offer feed and to collect residues of feed and feces.

2.6 Methane measurements

The concentration of methane in the chamber air was recorded after running it for one hour. A known amount of methane gas was flowed into the chamber and concentration was recorded by carbon dioxide analyzer till the increased methane reached to the initial concentration. Analysis of outgoing air was done by pre-calibrated infrared methane analyzer till the recovery was completed.

CH_4 recovery (%) = [V_{STP} (M_f - M_i)/ Vol. of CH_4 introduced] × 100

Where,
M_f = Methane present in outgoing air from the chamber
M_i = Methane present in incoming air into the chamber
V_{STP} = Volume at standard temperature, and pressure
Therefore, the total volume of methane produced was computed as per the following

formula:

CH_4 (L) = V_{STP} (M_f - M_i)/100

2.7 Statistical Analysis

The data of were statistically analyzed by using one way ANOVA with Tukey's post hoc testing to compare experimental groups. For all statistical analyses, probability values less than 0.05 were considered as significant.

3. Results and Discussion

3.1 Plane of nutrition and Body weight

The effects of feeding rumen modifier on body weight change, dry matter intake and feed conversion ratio are presented in Table 2. Dry matter intake (kg/day) in rumen modifier supplemented groups was found similar to control. The intake through concentrate mixture and wheat straw revealed that the 50:50 ratio was maintained throughout the feeding trial. Net body weight gain (kg) and average daily weight gain (g/d) during 120 d feeding trial was 94.66 and 676.2; 102 and 728.6; 105.1 and 751.2 in T1, T2 and T3, respectively. No significant (P>0.05) difference was observed in growth performance of the rumen modifier fed animals (T2 and T3) and the control animals (T1). No difference in feed intake and body weight gain resulted in similar feed conversion ratio in all the three groups. Similarly no changes in growth performance were reported by various workers with the administration of different rumen modifiers among different species. Rao *et al.*, [11] The feeding of neem seed cake to growing kids at 34g and 68 g per kg diet led to no change in the DMI in any of the groups. There was also no

(P>0.05) effect on DMI, ADG and FCR with ajwain oil (1 and 2 ml/h/d) supplementation [12]. No effect on the growth and feed intake was reported after feeding of equal proportion of harad, seeds of fennel, fruits of amla, bulbs of garlic and rhizomes of ginger @1 and 2% of DMI [13].

Table 2: Effect of feeding rumen modifier on body weight change and feed conversion ratio of growing buffaloes

Attributes	T1	T2	T3	SEM	P value
Initial wt (kg)	238.0±9.43	237.8±10.2	234.8±9.97	13.9	0.968
Final wt (kg)	326.6±12.8	339.8±12.2	340.0±10.3	16.5	0.881
ADG (g)	676.2±0.03	728.6±0.03	751.2±0.02	0.04	0.277
Concentrate DMI (kg)	2.83±0.00	2.83±0.00	2.83±0.00	0.00	0.16
WS DMI (kg)	3.09±0.11	3.19±0.08	3.15±0.05	0.12	1.00
WS: Concentrate	1.06±0.04	1.12±0.03	1.07±0.02	0.04	0.111
DMI (kg/d)	5.92±0.13	6.02±0.09	5.98±0.17	0.19	0.892
DMI (%BW)	2.91±0.04	3.02±0.06	2.95±0.03	0.08	0.47
Total DMI (kg)	791±15.8	807±12.2	798±20.6	23.4	0.791
Feed conversion ratio	6.34±0.32	5.97±0.27	5.72±0.25	0.40	0.330

T1, Control; T2, RM-7 + sodium sulphate @ 2 and 0.06% of DMI; T3, RM-7 + sodium sulphate @ 4 and 0.06% DMI; RM-7: mixture of neem, fennel, mahua, harad, bahera, ajwain and amla in 2:2:2:1:1:1:1 proportion.

3.2 Effect of rumen modifier on methane emission

The effect of feeding rumen modifier to the growing buffalo calves on methane emission is presented in Table 3. During chamber studies, the DMI was not affected indicating that the animals were not in stress. There was similar DMI in all the three groups. The values for methane production (l/d) ranged from 147.6 to 182.5 and was significantly low (14 and 19 per cent) in the T2 and T3 groups fed rumen modifier supplemented diet as compared to control. The methane production when expressed in terms of l/kg DMI was also reduced (P=0.002) by 18 per cent in the animals of T2 and T3 groups as compared to control (Fig 1). But when the methane production was expressed as l/kg DDMI significant reduction (14.9 per cent) was there only in T3 group as compared to control, whereas, T2 was similar to control as well to T3 group.

In the present study, rumen modifier fed to T2 and T3 group reduced methane emission (l/kgDMI) significantly (P<0.05) by 18.0 and 17.9% respectively, as compared to control. This inhibition in methane production might be due to the presence of array of the PSM in the different components of the rumen modifier used which might affect the microbial activity in the animals. Reports of 20–26% reduction in enteric methane emission through the supplementation of three selected tanniniferous tropical tree leaves of *Ficus benghalensis*, *Artocarpus heterophyllus* and *Azadirachta indica* in straw based diet [14]. Reduction in methanogenesis might be due to direct inhibitory effect of sulphur on methanogenic archaea [15] and change in archeal community or reduced activity of methanogens by essential oils [16].

Table 3: Effect of feeding rumen modifier on methane emission in growing buffaloes

Attributes	T1	T2	T3	SEM	P value
Body weight (kg)	301.8±3.89	300.2±8.82	306.3±10.06	11.38	0.85
Body weight (kgW$^{0.75}$)	72.4±0.70	72.1±1.60	73.2±1.80	2.05	0.86
DMI (kg/d)	6.05±0.21	6.32±0.20	6.50±0.24	0.315	0.397
Methane (l/d)	182.5^{b}±3.30	156.2^{a}±6.78	147.6^{a}±2.84	5.09	0.001
Methane (l/kg DMI)	29.97^{b}±0.64	24.50^{a}±0.42	24.52^{a}±1.3	0.90	0.002
Methane (l/kg DDMI)	48.45^{b}±0.60	43.79^{ab}±1.83	41.27^{a}±1.75	1.19	0.024

T1, Control; T2, RM-7 + sodium sulphate @ 2 and 0.06% of DMI; T3, RM-7 + sodium sulphate @ 4 and 0.06% of DMI; RM-7: mixture of neem, fennel, mahua, harad, bahera, ajwain and amla in 2:2:2:1:1:1:1 proportion. ^{ab}different superscripts within a row differ significantly

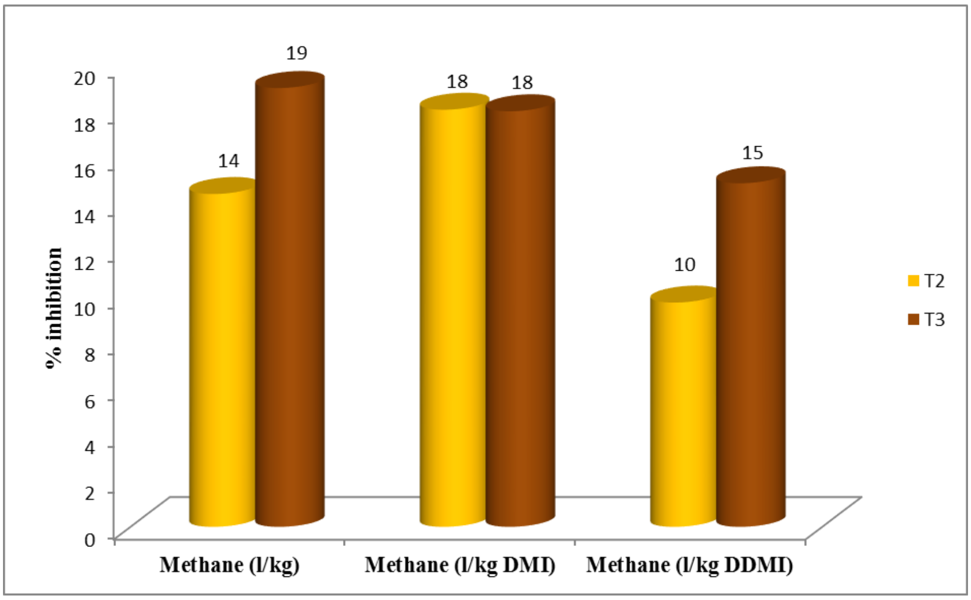

Fig 1: Percent reduction in enteric methane production by feeding of rumen modifier

4. Conclusion

Rumen modifier could be fed to animals without any adverse effect on feed intake, growth rate and overall performance of animals as well as being cost effective. Feeding of rumen modifier at 2% and 4% had significant reductions in level of methane production. Thus the rumen modifier can be incorporated in diet of animals.

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