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SHORT COMMUNICATION

Effect of Rumen Modifier on Methanogenesis and Feed Digestibility under *in Vitro* Conditions

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ABSTRACT

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 fruit of amla (*Phyllanthus emblica*) and ajwain seed (*Trachyspermum ammi*) were dried, powdered and mixed in a ratio of 2: 2: 2: 1: 1: 1: 1 to prepare a mixture of rumen modifier-7 (RM-7) and was tested at 0 (control) 5, 10, 15 and 20% of the substrate along with 0.06% of sodium sulphate (except control) to study their effects on rumen fermentation *in vitro.* Inclusion of RM-7 at 5, 10, 15 and 20% of substrate along with 0.06% of sodium sulphate did not affect gas production but there was significant (P<0.01) reduction (18.5 and 34.1%) in methane production as well as feed digestibility (60.88 to 52.71%)). Total volatile fatty acids (VFA), individual VFA (acetate, propionate and butyrate) and ammonia N levels were not affected by any of the treatments. Hence, inclusion of rumen modifier (RM-7 at 5, 10, 15 and 20% + 0.06% sodium sulphate of substrate) did not affect total gas production but there was significant reduction in methane production upto 10% level.

Key words: *In vitro* true digestibility, Methane, Rumen modifier, Sodium sulphate

The feed consumed by ruminants is fermented by synergistic activity of bacteria, protozoa, fungi, archaea and bacteriophages as a result of which the polysaccharides of feed are converted into volatile fatty acids and microbial protein, the two main sources of energy and protein for the host animals. But during feed fermentation in the rumen, CO_2 and H_2 gases are produced as by products and rumen methanogenic archaea reduce CO_2 into methane by utilizing H_2 which is eructated out through mouth. In rumen, methanogenesis is an essential metabolic process to maintain a low H_2 pressure but wasteful as 2-12% dietary energy (Johnson and Johnson, 1995) is wasted in the form of methane. Also, methane being a potent green house gas having 23 times more global warming potential than CO_2. Enteric methane production contributes significantly to global warming. Hence, the inhibition of methanogenesis has been of great concern for nutritionists since long back and now a days due to global warming, in the perspective of green house gas emission.

A number of feed additives like antibiotics, ionophores, defaunating agents and antimethanogenic compounds have been experimented to manipulate rumen fermentation to improve feed utilization efficiency with a reduction in methanogenesis in the rumen. But due to increasing awareness of the adverse effects associated with these chemical feed additives their use is being discouraged owing to several reasons. Several plant secondary metabolites (PSM) such as essential oils (EOs), saponins, tannins and flavonoids have been documented for their ability to reduce methane emission and improve feed utilization efficiency by modulating rumen microbial fermentation process (Inamdar *et al.,* 2015). PSMs are quite effective in methane mitigation at higher doses but fibre digestibility is also reduced (Pawar *et al.,* 2014). Hence, an *in vitro* experiment was designed to find out a combination of PSMs and sulphate to achieve maximum methane inhibition with no adverse effect on other fermentation parameters.

For this study, neem seed cake (*Azadirachta*

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indica), mahua seed cake (*Madhuca longifolia),* fennel seed (*Foeniculum vulgare*), harad (*Terminalia chebula)*, fruit pulp of bahera (*Terminalia bellirica*), fruit pulp of fruit of amla (*Phyllanthus emblica*) and ajwain seed (*Trachyspermum ammi*) were dried, powdered and mixed in a ratio of 2: 2: 2: 1: 1: 1: 1 to prepare a mixture of rumen modifier-7 (RM-7). This RM-7 was used at the rate of 0, 5, 10, 15 and 20% along with sodium sulphate at 0.06% of the substrate. The plant parts selected in this study were those which showed promising results in the series of *in vitro* experiments conducted in the author's laboratory using several plant parts rich in PSMs (Patra *et al.* 2006a). The estimations were done in triplicates. *In vitro* gas production tests were conducted as per the procedure of (Menke and Steingass, 1988). The substrate (200 mg per syringe) used was wheat straw and concentrate mixture (maize grain 32, de-oiled soyabean meal 26, wheat bran 39, mineral mixture 2 and salt 1%) in 1 :1 ratio and rumen liquor was collected from two fistulated buffaloes fed on the same diet. The rumen liquor was then pooled together and used as inoculum. Incubation medium (30mL) was dispensed anaerobically in each syringe. Each set of syringe comprised of one treatment in triplicate and two blank syringes (without substrate). Three such sets were run for each percentage of RM-7. An incubation period of 24 h at 39°C for each set of syringes was allowed to study rumen fermentation parameters.

After 24 h of incubation, total gas production was estimated by piston displacement. For methane estimation, 100 µL of gas sampled from headspace of caliberated syringe was injected into Nucon-5765 gas chromatograph equipped with PorapakQ column and flame ionization detector (Agarwal *et al.,* 2008). A mixture of 50% carbon dioxide and 50% methane (Spancan; Spantech Products Ltd, Godstone, UK) was used as standard. For VFA estimation, 0.5 mL fermented medium was mixed with 0.1 mL of 25% metaphosphoric acid and allowed to stand at room temperature for 1 h. The mixture was centrifuged at 10,000 rpm for 10 minute and 1 µL of the supernatant was injected on Nucon-5765 gas chromatograph equipped with chromosorb 101 column and FID as per the procedure (Cottyn and Boucque, 1968) with some modifications (Agarwal *et al.,* 2008). Fermented medium was analyzed for NH_3-N (Weatherburn, 1967). The proximate principles (AOAC, 1995) and cell wall constituents (Van Soest *et al.,* 1991) in wheat straw and concentrate mixture and RM-7 were estimated. The means were compared using Tukey's test if the main effect was significant (*i.e.,* $P<0.05$) using SPSS Version 16.0 (Inc, Chicago, USA).

The chemical composition of concentrate mixture, wheat straw and RM-7 has been presented in Table 1. The total gas production in 24 h ranged from 138.4 to 141.6 mL/g DM. No significant increase in gas production was observed by inclusion of increasing level of RM-7 as compared to the control (Table 2). Arif *et al.* (2015) tested various plant parts and few oil cakes rich in different secondary metabolites like tannins, saponins, essential oils, flavonoids *etc.* There was no effect on *in vitro* gas production by inclusion of any of the plant parts at the rate of 10% of the substrate. Phuong (2012) reported significant ($P<0.05$) reduction in gas production by inclusion of sulphur (as sodium

Table 1. Chemical composition (% DM basis) of concentrate mixture, wheat straw and RM-7

Attribute	Concentrate mixture	Wheat straw	RM-7
Dry matter	93.78	94.18	92.13
Organic matter	89.12	90.92	88.90
Crude Protein	17.95	3.01	9.44
Ether extract	3.59	2.13	2.69
Neutral detergent fibre	28.38	86.50	43.31
Acid detergent fibre	12.20	53.38	37.63

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

sulphate) @ 0.4 and 0.8%.

Unlike gas production, methane production decreased (P<0.01) linearly by including graded levels of the rumen modifier. The reduction in methane production (mL/g DM) was 8.41, 22.97, 31.34 and 25.84% at 5, 10, 15 and 20% of substrate, respectively when compared to control. Similar results were obtained by Kumar *et al.* (2016) using fenugreek seeds @ 1, 2 and 3% level under *in vitro* conditions. Chaturvedi *et al.* (2016) reported that methane production (mg/g of substrate DM) reduced (P<0.05) by the inclusion of combinations of amla and neem. The significant reduction in level of methane production was due to the various components present in the rumen modifier. The RM 7 contained mixture of plant parts rich in tannins, saponins and essential oils which have already been tested for their antimethanogenic activity in the author's laboratory (Kumar *et al.* 2011; Patra and Yu, 2014). *In vitro* inclusion or feeding of sulphur resulted in reduction in methane production confirming its role as alternate electron acceptor (Van Zijderfeld *et al.,* 2010). The rumen modifier (RM-7 + sodium sulphate) also exhibited antimethanogenic activity in a dose dependent manner.

A significant linear decrease (P<0.01) in the feed IVTD was observed with increasing level of rumen modifier with maximum reduction at 20% level when compared to control. The values of IVTD ranged from 52.7 to 60.8%. In the present study, RM-7 was used which contained tannins (amla, harad and bahera), saponin (mahua), essential oils (ajwain and fennel), azadiractin and oils (Agarwal *et al.,* 2008). The ingredients were selected in a manner to achieve maximum methane inhibition with either increase in feed IVTD or no effect. Lila *et al.* (2003) reported decreased IVDMD with sarsaponin (methanol extract) at the level of 1.2-3.2 g/L of incubation medium. Gupta *et al.* (2017) reported increase in feed IVTD by including sodium sulphate either alone or in combination with blend of plant parts in the incubation mixture.

No significant differences in the level of TVFA, its fractions and ammonia were observed due to increasing level off RM-7. Gupta *et al.* (2017) found changes in TVFA and its fractions by inclusion of a blend of plant parts but were not affected by inclusion of sodium sulphate. The difference in the two experiments might be due to difference in the two blends and also the levels of the blend of plant parts. Ammonia nitrogen concentration in the fermented medium was not affected either by inclusion of the blend of plant parts or sodium sulphate alone or in combination (Gupta *et al.,* 2017) indicating no change in nitrogen metabolism due to addition of feed additive.

Therefore, rumen modifier had no significant effect on total gas production but was potent inhibitor of methane up to 10% level under *in vitro* conditions,

Table 2. Effect of rumen modifier on *in vitro* rumen fermentation parameters

Attribute	RM-7 (% of substrate)					SEM
	0 (control)	5.0	10.0	15.0	20.0	
Gas production(mL/g DM)	138±1.30	129±3.15	133±2.91	128±4.75	141±4.49	5.01
Methane(mL/g DM)	28.69^{b}±0.83	23.37^{b}±0.87	21.48^{a}±1.02	18.93^{a}±1.02	21.65^{a}±0.84	1.30
IVTD (%)	60.88^{c}±0.87	57.95^{b}±0.93	58.91^{b}±1.39	54.94^{ab}±1.47	52.71^{a}±0.81	1.60
NH_3-N (mg/dL)	9.52±0.47	10.54±0.15	9.81±0.32	10.87±0.84	9.84±0.59	0.85
TVFA (mM/L)	47.99±0.07	49.43±0.08	46.81±0.12	46.71±0.18	49.05±0.10	0.17
Acetate (%)	76.51±0.53	74.06±1.34	77.89±1.46	75.88±1.03	75.18±0.76	1.53
Propionate(%)	15.68±0.76	17.86±1.63	13.77±1.07	15.10±1.23	16.60±0.81	1.62
Butyrate (%)	7.62±0.30	8.39±0.73	7.76±0.73	8.07±0.79	8.81±0.64	0.94
A: P Ratio	4.96±0.29	4.32±0.48	5.38±0.28	5.25±0.47	4.75±0.18	0.57

[a,b,c]Mean values bearing different superscripts in a row differ significantly (P<0.001)

however, *in vivo* trials need to conducted for valid conclusions.

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