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Short Communication

EFFECT OF CINNAMON BARK EXTRACT ON *In Vitro* RUMEN FERMENTATION AND METHANOGENESIS

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ABSTRACT

An experiment was conducted to examine the effect of cinnamon bark extract at three different dose levels (0.5 ml, 1.0 ml and 2.0 ml/ 30 ml buffered rumen fluid) using oats hay as substrate for *in-vitro* rumen fermentation and methanogenesis and compared with control having only rumen liquor and substrate. The rumen liquor was collected from fistulated Murrah buffaloes and used as source of inoculums. The experiment was conducted in 100 ml glass syringes using oats hay as substrate and rumen fermentation parameters and methanogenesis was measured after 24h of incubation. There was significant ($p<0.001$) reduction in total gas production and *in vitro* dry matter digestibility (IVDMD) at higher dose level as compared to other treatments. At high dose, a significant reduction ($p<0.05$) in all the volatile fatty acids production was evident. Therefore, it may be concluded that cinnamon bark extract at appropriate doses (0.5-1.0 ml/30 ml buffered rumen fluid) modified rumen microbial fermentation towards reduced methanogenesis and ammonia-N production without affecting feed digestibility and volatile fatty acids production, suggesting its possible application as anti methanogenic feed additive.

Key words: Cinnamon bark extract, rumen fermentation, methanogenesis, buffalo

In ruminants, mainly methanogens in rumen and hind gut are responsible for methane production. The livestock production operations mainly from ruminant animals are responsible for 37% of anthropogenic methane production in the atmosphere (FAO, 2009). Indian livestock produce about 14.3 million metric tons methane (Patra, 2014). Since methane is a greenhouse gas, the strategy for reduction in its production is of interest in ruminant physiology studies.

Interest in using plant bioactive compound as feed additives is due to their antimicrobial effect and ability to modulate rumen fermentation towards reduced methanogenesis by specifically inhibiting a group of microbes. Essential oils can interact with microbial cell membrane and inhibit the growth of some gram-positive and gram-negative bacteria. As a result of such inhibition, the addition of some plant extracts, garlic oil, cinnamaldehyde, eugenol, capsaicin to the rumen result in an inhibition of deamination and methanogenesis, resulting in lower ammonia N, methane and acetate and in higher propionate and butyrate concentrations (Calsamiglia *et al.,* 2007). Bioactive compounds of cinnamon are euginol, cinnameldehyde and cinnamic (Vangalapti *et al.,* 2012). As per a study, cinnamon bark contains 65-80% cinnamaldehyde and 5-10 % eugenol (Senanayake *et al*., 1978). Although these bioactive compounds have the potential to act as methane inhibiting agents, they were not explored to a great extent. Keeping in view of the above review, the present study was planned to examine the effect of cinnamon bark extracts on *in vitro* rumen fermentation and methane production.

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MATERIAL AND METHODS

Collection of plant parts and preparation of extract

The dalchini bark *(Cinnamomum zeylanium)* was purchased from market and for extraction, bark was grounded to make powder form and extracted in petroleum ether solvent through Soxhelt's apparatus for 72h using 15 gm sample and final volume was made to 100 ml.

Collection of rumen liquor and preparation of media

Rumen liquor (RL) was collected from three rumen fistulated adult male Murrah buffaloes maintained at animal farm of ICAR- CIRB, Hisar. The experimental animals were cared for in accordance with the guidelines of Institute's Animal Ethics Committee (IAEC protocol number 1/ 2011). The solid contents of rumen digesta were collected manually from different locations of rumen at different depths in the morning, before feeding and watering of the animals and hand squeezed to obtain rumen liquor. Equal volume of rumen fluid from each animal was pooled to completely fill 1-L pre-warmed thermos flask and brought to the laboratory. The rumen fluid was filtered through four layers of cheese cloth under continuous flow of CO_2 to use as source of inoculums for the *in vitro* studies. The buffer media was prepared as per the method of Menke and Steingass (1988).

Weighing of feed sample and greasing of syringes

The sample of oats hay was ground to pass through 1.0 mm screen and preserved for subsequent use as substrate for *in-vitro* studies. Accurately weighed

Table 1
Effect of Dalchini bark petroleum ether extract on *in vitro* rumen fermentation and methane production of oats hay

Attributes	Control	T 1	T 2	T 3	SEM	P value
Total Gas, ml	22.33 ^{b} ± 0.58	22.34 ^{b} ± 0.58	21.67 ^{b} ± 0.58	17.33 ^{a} ± 0.58	1.26	<0.001
Gas, ml/g DM	118.39 ^{b} ± 2.9	118.00 ^{b} ±2 .89	114.09 ^{b} ± 2.07	91.28 ^{a} ± 2.65	6.72	<0.001
Gas, ml/g DMD	168.00 ^{b} ± 4.19	168.58^{b} ± 5.56	165.57 ^{b} ± 2.69	154.92 ^{a} ± 4.59	4.84	<0.001
Methane Conc., %	10.21 ^{c} ± 0.05	9.37 ^{b} ± 0.19	9.39 ^{b} ± 0.44	2.94 ^{a} ±0.10	1.53	<0.001
Total Methane, ml	2.69 ^{c} ± 0.57	2.47^{b} ± 0.16	2.41 ^{b} ± 0.10	0.63 ^{a} ± 0.01	0.43	<0.001
Methane, ml/g DM	14.25 ^{c} ± 0.29	13.05 ^{b} ± 0.82	12.69 ^{b} ± 0.56	3.31 ^{a} ± 0.09	2.30	<0.001
Methane, ml/g DMD	20.22 ^{c} ± 0.56	18.68 ^{b} ± 0.32	18.41 ^{b} ± 0.67	5.62 ^{a} ± 0.17	3.08	<0.001
IVDMD, %	70.49 ^{b} ± 1.61	69.86 ^{b} ± 0.65	68.90 ^{b} ± 0.65	58.92 ^{a} ± 0.20	2.49	<0.001
Ammonia N, mg/dl	28.47 ^{c} ± 0.80	26.6 ^{b} ± 0.00	27.07 ^{b} ± 0.81	14.00 ^{a} ± 0.00	3.53	<0.001

Control, T_1, T_2 and T3 are treatment groups @ 0.0, 0.5, 1.0, 2.0 ml Dalchini bark extract (Petroleum ether) /30ml BRF, respectively. Mean values bearing a, b, c superscripts in a row varies significantly ($p<0.001$)
DM = Dry matter, DMD = Digested dry matter, IVDMD = *In-vitro* dry matter digestibility

about 200 mg of air-dried sample and transferred it into the syringes (Fortuna®, Germany). Plant extract at different dose levels (0.0, 0.5, 1.0 and 2.0 ml petroleum ether extracts of cinnamon bark /30ml of buffered rumen fluid represented as Control, T1, T2 and T3, respectively) was added to the substrate through micropipette from the top of syringes. After adding extracts, the piston was greased with paraffin soft white LR (Hi Media; M.P. 39-56° C) and pushed into the barrel of the syringe.

Preparation of buffer media

Buffer media was prepared as per the Menke and Steingass (1988) and then strained rumen liquor (RL) at required quantity was mixed with the media to make final concentration of 2:1 (Buffer: RL) for dispensing to the syringes.

***In vitro* parameters**

Estimation of total gas and Methane production

After 24 h of incubation, the total gas production was recorded by displacement of piston. Concentration of methane in head space gas was measured by taking sample (200 µl) in 1000 µl graduated Hamilton gas tight syringe (Hamilton, Switzerland) using gas chromatograph (Nucon -5700, Nucon Engineers, New Delhi) fitted with flame ionization detector (FID) and a column ((Porapak 'Q'). Simultaneously, a gas standard (Centurion Scientific, New Delhi) having methane and CO_2(50:50) was injected for comparison. The proportion of methane (%) was calculated as follows:

$$\text{Methane (\%)} = \frac{\text{Area covered by the sample x 50}}{\text{Area Covered by the standard of gas}}$$

Total methane production was calculated by multiplying methane concentration in the head space gas with the total gas produced and expressed as per g DM

Table 2
Effect of Dalchini bark extract (petroleum ether) on *in vitro* volatile fatty acid production

Attributes	Acetate (mM/100ml)	Propionate (mM/100ml)	Butyrate (mM/100ml)	A:P
Control	3.83 ^{b} ± 0.06	0.87 ^{b} ± 0.01	0.33 ^{b} ± 0.01	4.42 ^{a} ± 0.04
T1	3.76 ^{b} ± 0.35	0.90 ^{b} ± 0.02	0.33 ^{b} ± 0.01	4.20 ^{a} ±0.31
T 2	3.56 ^{b} ± 0.29	0.88 ^{b} ± 0.00	0.30 ^{b} ± 0.05	4.47 ^{a} ± 0.28
T 3	2.25 ^{a} ± 0.13	0.39 ^{a} ± 0.04	0.15 ^{a} ± 0.03	5.80 ^{b} ±0.28
SEM	0.177	0.131	0.02	0.078
P Value	0.008	<0.001	0.010	0.010

Control, T_1, T_2 and T_3 are treatment groups @ 0.0, 0.5, 1.0, 2.0 ml Dalchini bark (petroleum ether extract) /30ml of incubation media respectively. Mean values bearing a, b superscripts in a column varies significantly ($p<0.001$).

incubated as well as per g DM digested.

Individual Volatile Fatty Acids (IVFAs)

After 24 hr incubation, 1ml of the supernatant of each syringe content was taken in a micro centrifuge tube containing 0.20 ml metaphosphoric acid (25% w/v). The mixture was allowed to stand for overnight at room temperature and centrifuged at 5,000 g for 10 minutes to get clear supernatant. The supernatant (1µL)was injected into gas chromatograph equipped with flame ionization detector (FID) and glass column packed with chromosorb- 101as described by Cottyn and Boucque (1968).

Estimation of *in-vitro* dry matter digestibility (IVDMD)

IVDMD estimated as per method of Van Soest *et al*. (1992).

Estimation of ammonia nitrogen (NH_3-N)

For estimation of NH_3-N concentration, conway disc method was applied (Conway, 1965).

RESULTS AND DISCUSSION

The trial was conducted to study the effect of dalchini *(Cinnamomum zeylanium)* bark extract (petroleum ether extracted) on *in-vitro* rumen fermentation and methane production of oats hay and the results are given in Table 1. There was significant ($p<0.001$) reduction in total gas production and IVDMD in T_3 as compared to other treatments, however these parameters remained similar ($p>0.05$) in T_1 and T_2 as compared to control. Methane concentration in head space gas, total methane production and ammonia N concentration were reduced in all the treatment groups as compared to control. There was no significant ($p>0.05$) difference in all these parameters between treatments T_1 and T_2. The essential oil contents of dalchini bark are cinnamaldehyde, cinnamic acid, cinnamate and eugenol, which possess antimicrobial activity (Rao and Gun, 2014). Although the mechanism of action of cinnamaldehyde as an antimicrobial remains poorly understood, some authors suggested that the carbonyl group might be the active site (Helander *et al*., 1998). Similarly to other secondary plant metabolites, gram-positive bacteria are more sensitive to the inhibition by cinnamaldehyde (Smith-Palmer *et al*., 1998), although some studies have demonstrated that purified cinnamaldehyde was also highly effective on gram-negative bacteria (Helander *et al*., 1998; Kim *et al*., 2004). The reduction in methane and ammonia N concentration by dalchini bark extract, in the present study could be due to inhibitory effects of bioactive components of essential oil to methanogenic archaea and hyper ammonia producing bacteria in the fermentation fluid (Castillejos *et al*., 2006; Busquet *et al*., 2006).

A similar experiment was conducted by Goel *et al*. (2011) with methanolic extract of cinnamon and concluded that methane concentration was decreased with increasing the extract dose without affecting the digestibility. In a further study, Pawar *et al*. (2014) reported that at 167 µl l^{-1} of incubation medium cinnamon bark oil reduce methane production by 34.9% without affecting the feed digestibility. Antimicrobial activity of cinnamon leaf oil (CIN) was also established by Fraser *et al.,* (2006) who found lower protozoa population by inclusion of CIN in rumen simulation technique. Thus, these experiments support the present study that at low dose, there was only reduction in methane production without affecting the digestibility due to antimicrobial action of cinnamon oils.

The concentration of acetate, propionate and butyrate production and the ratio of acetate to propionate showed no difference between the T_1 and T_2 treatment groups as compared to control (Table 2), while in T_3 treatment group, there was significant reduction ($p<0.05$) in acetate, propionate and butyrate concentration as compared to control. Busquet *et al*. (2006), while examining graded dose responses of various plant essential oils observed that upto 300mg/L incubation media, either cinnamaldehyde or cinnamon oil has no effect on total volatile fatty acids (TVFA) production, however, adverse effect was evident only at high (3,000 mg/L) level. The reduction of VFA concentration at high dose indicated that feed fermentation was drastically suppressed which is harmful to animal if expressed in *in-vivo.*

Cinnamon bark extract had important anti methanogenic activity and decreased methane and ammonia-N production along with feed digestibility and volatile fatty acids concentrations. At appropriate doses (0.5-1.0 ml/30 ml incubation media), cinnamon bark extract modified rumen microbial fermentation towards reduced methanogenesis and ammonia-N production without affecting feed digestibility and volatile fatty acids production, suggesting its possible application as anti methanogenic feed additive. Further *in vivo* studies are required to define the optimal doses and effects of cinnamon bark extracts on rumen microbial fermentation.

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