MODULATING FEED DIGESTION AND METHANE PRODUCTION BY EUCALYPTUS (*Eucalyptus citriodora*) LEAVES ESSENTIAL OILS IN WATER BUFFALO (*Bubalus bubalis*)

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ABSTRACT

Farm ruminants are responsible for about 15% of total global methane emission due to enteric fermentation of feeds. Additives are being used to modify rumen fermentation towards reduced methanogenesis, however, hazards associated with feeding of antibiotics and other chemical compounds to livestock for improving animal performance and methane mitigation are growing public awareness and encourage the research towards exploiting natural products as feed additives. Therefore, an experiment was carried out to examine the potential of eucalyptus (*Eucalyptus citriodora*) leaves essential oils in modulating rumen fermentation for reduced methane production in water buffalo (*Bubalus bubalis*). Essential oils were extracted both from dried and fresh eucalyptus leaves, separately in Soxhlet's apparatus by organic solvent, hexane [15 g leaves extracted in 100 ml n-hexane (mol. wt. 86.18, assay 99.0% and density 0.658)] for 72 h and tested at three dose levels (0, 0.5 and 2.0 ml) in buffered rumen fluid (BRF). Oats hay (200±5 mg) was used as substrate and incubated with 30 ml BRF in 100 ml calibrated glass syringes at 39°C for 24 h following standard *in vitro* gas production protocol. Gas production was recorded by displacement of piston in the syringe. Methane in the gas phase and volatile fatty acids in the fermentation medium were estimated by Gas Chromatograph (GC). The total gas production and dry matter digestibility was increased ($P<0.05$) at low dose (0.5 ml) of eucalyptus leaves extract, irrespective of nature of leaves however, these were reduced ($P<0.05$) at higher dose (2.0 ml). Methane production was reduced ($P<0.05$), irrespective of doses. Volatile fatty acids (VFA) production were increased ($P<0.05$) at low dose but higher dose detrimentally reduced it. Rumen ammonia - N was not affected at low dose however, it was reduced at high dose of extract. Extracts from both dried and fresh leaves behaved similarly in modulating all the rumen fermentation parameters at corresponding dose levels. Results of this study revealed reduced methane production with increased feed digestibility and volatile fatty acids production by dosing extract of eucalyptus leaves. Therefore, it suggests using of either extract of eucalyptus (*Eucalyptus citriodora*) leaves (0.5 ml/

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30 ml rumen fluid) or its corresponding dose of leaves as feed additive in buffaloes.

Keywords: *Bubalus bubalis*, buffaloes, Eucalyptus leaves, essential oils, methanogenesis, rumen fermentation

INTRODUCTION

Methane is one of the important greenhouse gases, produced normally during the enteric fermentation of feeds in ruminants mainly. Domesticated ruminants contribute about 15% of total methane emission (FAO, 2010), which not only impact on environment but also lose a substantial (2 to 15% of gross energy) amount of feed energy (Johnson and Johnson, 1995), that otherwise could be converted to metabolizable energy for productive purposes. Therefore, reduction in methanogenesis is of interest of ruminant nutritionists since long for efficient utilization of feed and more recently from the perspective of global warming.

Plant bioactive compounds have the capability to modulate rumen fermentation through restructuring rumen microbial community and may be exploited to reduce enteric methane production. Essential oils are steam-volatile, terpenoids and phenylpropanoid compounds commonly derived from herbs and spices having wide range of anti-microbial activities (Calsamiglia *et al*., 2007). The diverse properties and activities of essential oils have focused their importance of using as animal feed additives to modify rumen microbial ecosystem for improving feed efficiency. The main constituents in eucalyptus essential oil are 1,8-cineole, α-pinene, γ-terpinene and *p*-cymene, which vary in composition depending upon species, season, geographical region and methods of extraction, capable to modify rumen fermentation.

Therefore, the present study was undertaken to examine the potential of eucalyptus (*Eucalyptus citriodora*) leaves extract rich in essential oils in modulating rumen fermentation for reduced methane production in buffalo with the aim to develop phytogenic feed additives.

MATERIALS AND METHODS

The leaves of eucalyptus (*Eucalyptus citriodora*) were collected from the institute campus. Half portion of the leaves was dried in electric oven at 45° to 50°C and ground to pass through 1 mm sieve. Other portion was crushed into small pieces afresh. Extracts (100 ml) were prepared using organic solvent, n-hexane, from 15 g sample of both dried and fresh, separately, and stored at 4°C for use.

Two separate *in vitro* experiments were conducted with extracts from either dried or fresh leaves and the extracts were tested at three levels (0, 0.5 and 2.0 ml) in three replicates for each treatment with oats hay as substrate. Rumen liquor was collected from fistulated buffaloes fed on standard diet, just before feeding and used as source of inoculum.

The substrate was milled to pass through 1 mm sieve and weighed (200±5 mg) in glass syringes of 100 ml capacity. The incubation medium (Menke and Steingass, 1988) and inoculums (2:1) was dispensed (30 ml) anaerobically in each syringe and kept at 39°C for 24 h. Gas production was recorded by displacement of piston in the syringe. Methane in the gas phase and VFA in the fermentation medium was estimated by Nucon-5700 gas chromatograph (Cottyn and

Boucque, 1968). *In vitro* true degradability of dry matter (TDDM) was determined (Van Soest *et al*., 1991). Ammonia nitrogen (NH_3-N) concentration was estimated by Conway disc method (Conway, 1962).

Data obtained were analyzed using General Linear Model procedure of SPSS (1996) as a randomized block design and means were compared using Tukey's test.

RESULTS AND DISCUSSION

There was significant (P<0.001) increase in total gas in T_1, however, it was reduced (P<0.001) in T_2, in comparison to control, irrespective of extracts either prepared from fresh or dry (Table 1) eucalyptus leaves. Similarly, gas ml/g dry matter (DM) and gas ml/g digested dry matter (DMD) were significantly (P<0.001) increased in T_1 and reduced (P<0.001) in T_2, in comparison to Control group. There was significant (P<0.001) reduction in methane production in terms of methane concentration (%) in the head space gas, methane ml/g DM and methane ml/g DMD in all treatments, in comparison to control, irrespective of type of extracts. *In vitro* dry matter digestibility was significantly (P<0.001) increased in T_1 with reduction (P<0.001) in T_2, in comparison to control.

Essential oils (EO) produced by different plant species can vary in chemical structures as well as bioactive activities (Burt, 2004). In the present study, the increased gas production and IVDMD with concomitant reduction of methane production at lower dose (0.5 ml/ 30 ml buffered rumen fluid) of eucalyptus leaves (Figure 1), irrespective of nature of leaves, could be due to specific activity of bioactive compounds, EO present in the extracts against some of the rumen microbes (Evans and Martin, 2000) which stimulated the fibrolytic activities and reduced the methanogenic archeal population. But at higher dose (2.0 ml) level, the bioactive compounds might be detrimental for rumen microbes, because of their natural antimicrobial properties (Benchaar *et al*., 2008) which ultimately reduced the feed digestibility and gas production.

Ammonia nitrogen (NH_3-N) concentration remained similar (P>0.05) in T_1 but reduced (P<0.001) in T_2 (Table 2.) by addition of extracts from either dry or fresh leaves. Analysis of volatile fatty acids (VFAs) showed significant (P<0.01) increase in acetate, propionate and butyrate production with decrease (P<0.001) in acetate and propionate (A/P) ratio in T_1 in comparison to control. However, all the VFA production was significantly reduced in T_2. Thus, the first Treatment Group (T_1), either from fresh or dry leaves was found to be better dose of extract for methane inhibition with increased DM digestibility and VFA production.

Sallam *et al.* (2009) also reported reduced methane production and gas production by dosing (25 to 150 µl/75 ml incubation media) pure eucalyptus oil *in vitro* without affecting digestibility. However, Beauchemin and MacGinn (2006) reported reduced digestibility of dry matter as well as fibre at higher dose levels. Our results corroborate with the findings of others with improvement of feed digestibility by stimulating microbial activity and gas production at lower dose and reduction at higher dose as the effects of EO are dose dependent (Benchaar *et al*., 2008; Dey *et al*., 2016). Reduction in ammonia nitrogen concentration at higher dose level could be due to inhibition of hyper-ammonia producing (HAP) bacteria (Patra and Saxena, 2009), but at lower dose level, it might not be effective in inhibiting those bacteria. Our study gets support of Sallam *et*

Table 1. Effects of Eucalyptus leaves (fresh /dry) extracts on *in vitro* total gas production, methane production and feed digestibility in buffalo.

Attributes	Control	Fresh leaves		Dry leaves		SEM	P-value
		T_1	T_2	T_1	T_2		
Total gas, ml	26.67^b	33.33^c	16.33^a	32.67^c	13.33^a	3.295	<0.001
Gas, ml/g DM	140.44^b	174.98^c	86.31^a	172.62^c	70.69^a	17.26	<0.001
Gas, ml/g DMD	200.03^b	219.33^c	168.04^a	275.45^c	146.9^a	17.26	0.008
Methane conc., %	10.81^c	8.78^b	2.85^a	8.88^b	2.96^a	2.335	<0.001
Total methane, ml	3.09^c	2.54^b	0.52^a	2.40^b	0.20^a	0.72	<0.001
Methane, ml/g DM	16.29^c	13.36^b	2.78^a	12.68^b	1.10^a	3.8	<0.001
Methane, ml/g DMD	23.22^c	16.73^b	5.42^a	15.83^b	3.53^a	4.975	<0.001
IVDMD, %	70.19^c	79.77^b	51.44^a	80.16^c	48.07^a	7.735	<0.001

Control, T_1 and T_2 are Treatment Groups 0.0, 0.5, 2.0 ml of Eucalyptus fresh leaves extracts in hexane /30 ml of buffered rumen fluid (BRF), respectively. Mean values bearing a, b, c superscripts in a row vary significantly (P<0.01). DM = Dry matter; DMD = Digested dry matter; IVDMD = *In vitro* dry matter digestibility

Table 2. Influence on ammonia-N and volatile fatty acids production by supplementation of Eucalyptus leaves extracts in buffalo.

Attributes	Control	Fresh leaves		Dry leaves		SEM	P-value
		T_1	T_2	T_1	T_2		
Ammonia N, mg/dl	14.9^b	13.53^b	11.93^a	14.0^b	11.67^a	1.305	<0.001
Acetate, mM/dl	4.54^b	4.96^c	4.09^a	5.15^c	3.44^a	0.34	0.005
Propionate, mM/dl	1.05^b	1.61^c	0.77^a	1.40^c	0.73^a	0.195	<0.001
Butyrate, mM/dl	0.33^b	0.53^c	0.27^a	0.50^c	0.24^a	0.07	<0.001
Acetate: propionate ratio	4.33^b	3.09^a	5.36^c	3.69^a	4.79^c	0.445	<0.001

Control, T_1 and T_2 are Treatment Groups 0.0, 0.5, 2.0 ml of Eucalyptus fresh leaves extracts in hexane /30 ml of buffered rumen fluid (BRF), respectively. Mean values bearing a, b, c superscripts in a row vary significantly (P<0.01). DM = Dry matter; DMD = Digested dry matter; IVDMD = *In vitro* dry matter digestibility.

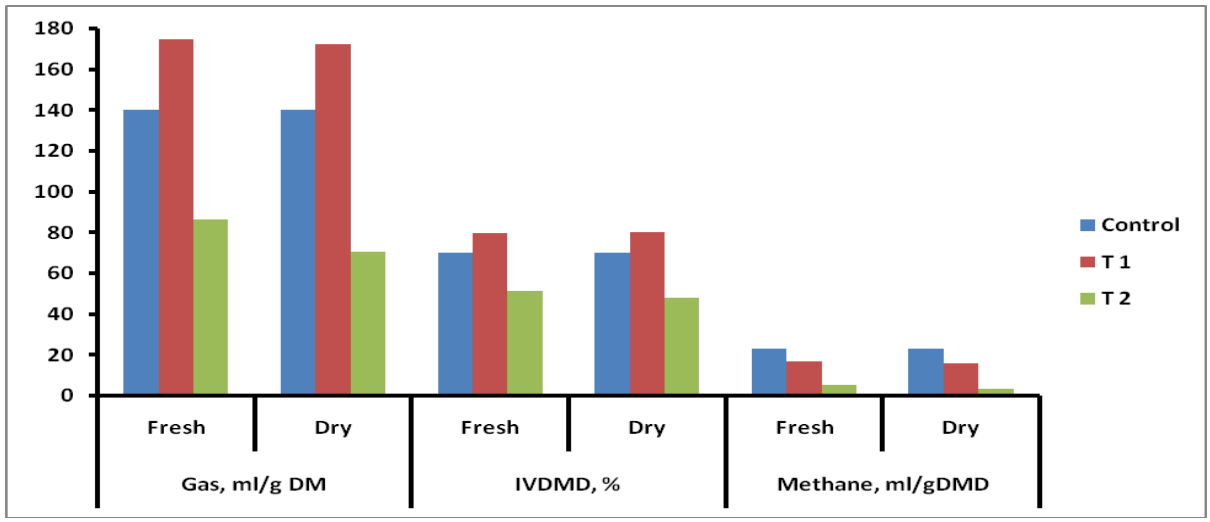

Figure 1. Modulation of gas production, methanogenesis and feed digestibility by extracts of Eucalyptus leaves rich in essential oils.

al. (2010), who also reported reduction in ammonia nitrogen concentration in fresh and dry leaves of eucalyptus as compared to alfalfa hay.

The increased concentration of acetate, propionate, and butyrate with decreased acetate to propionate ratio at low dose in present study, are in agreement with those of Castillejos *et al.* (2006), who reported modulation of VFA profile without decreasing total VFA concentration in a study with eugenol in batch fermentation and thymol in continuous culture. However, the decrease in all the fatty acids with higher dose as compared to control suggested detrimental effects on rumen microbes (Evans and Martin, 2000; Busquet *et al*., 2006).

Results of this study revealed reduced methane production with increased feed digestibility and volatile fatty acids production by dosing extract of eucalyptus leaves. Therefore, it suggests using of either extract of eucalyptus (*Eucalyptus citriodora*) leaves or its corresponding dose of leaves as phytogenic feed additive to improve feed utilization and reduce enteric methane production in buffaloes.

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