

EFFECTS OF VEGETABLE OILS SUPPLEMENTATION ON *IN VITRO* RUMEN FERMENTATION, VOLATILE FATTY ACID COMPOSITION AND METHANE PRODUCTION IN BUFFALOES

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ABSTRACT

This study appraised the effects of vegetable oils on *in vitro* methanogenesis, gas production, fatty acid composition and feed fermentation in buffaloes. Sesame (SOL) and mustard (MOL) oils were added individually to a basal diet of sorghum hay at the rate of 0, 0.4 and 0.8 ml per 30 ml of incubation fluid in 100 ml calibrated glass syringes. Rumen inoculums was obtained from four fistulated buffalo steers and incubated with 200 mg of substrate for 24 h at 39°C. Gas production was recorded by displacement of piston. Methane in the gas phase and volatile fatty acids in the fermentation medium were estimated by gas liquid chromatography. *In vitro* true degradability of dry matter (TDDM), neutral detergent fibre degradability (NDFD) was estimated, and microbial biomass production (MBP) and partitioning factor (PF) were calculated. Increasing doses of SOL and MOL linearly decreased ($P<0.05$) methane production without any variation between oil types. Both oils linearly decreased TDDM and NDF digestibility of feeds as well as MBP, however, the effects were more ($P<0.05$) pronounced with MOL. Acetate production was not affected ($P>0.05$), but increased production of propionate and butyrate and reduced

($P<0.05$) acetate to propionate ratio was evident with addition of oils. Mustard oil exerted greater ($P<0.05$) inhibitory effects on degradability and microbial biomass production at corresponding doses. Therefore, sesame oil is better than mustard oil as methane inhibiting agent without adversely affecting feed digestibility and may be considered as valuable feed supplement.

Keywords: *Bubalus bubalis*, buffaloes, sesame oils, mustard oils, methanogenesis, rumen fermentation, fatty acid composition

INTRODUCTION

Methane, a colourless, odourless gas primarily produced in the rumen of livestock species emitted into the environment by eructation mainly and accounts for not only environmental pollution by contributing to the global green house gas emission, but also reduce the efficiency of feed energy utilization by ruminants. Primary digestive microorganisms (bacteria, protozoa and fungi) hydrolyze proteins, starch and plant cell wall polymers into amino acids and sugars. These simple products are then fermented to volatile fatty acids, hydrogen and carbon dioxide by rumen

microorganisms. Acetate, propionate, and butyrate, which are the major VFA, are then absorbed and utilized by the host animal. The major producers of H_2 are the organisms which produce acetic acid in the fermentation pathway (Hegarty and Gerdes, 1998). Although methanogens are only directly involved in the very terminal stages of fermentation, they are very important because they are capable of effectively utilizing electrons in the form of H_2 to reduce CO_2 to CH_4 , thereby maintaining low H_2 pressure in the rumen. Thus, in their absence, organic matter could not be degraded as effectively in the gut (McAllister *et al.*, 1996). However, since CH_4 has no nutritional value to the animals, its production represents a loss of 2 to 12% dietary gross energy intake (Johnson and Johnson, 1995).

Currently, India possesses the world's largest livestock population (13% of the global livestock population, with 57% of the world's buffalo and 16% cattle). Buffalo contributes 42% of total CH_4 emissions from enteric fermentation of Indian livestock (Chhabra *et al.*, 2009). Reducing enteric CH_4 emissions has been identified as one key way of lowering global CH_4 emissions. Therefore, ruminant nutritionists are exploring various technologies to mitigate enteric methane emissions. Fat supplementation is one of the dietary interventions to decrease enteric methane emissions. However, efficacy on rumen methanogenesis varies with the concentration, type and fatty acid composition of fats or oils (Beauchemin *et al.*, 2008). Sesame oil (SOL) contains very high concentrations of oleic and linolenic acids, in comparison to mustard oil (MOL). Therefore, the present study was undertaken to investigate the efficacy of SOL and MOL on inhibiting ruminal methanogenesis with their associated effects on gas production, fermentation pattern and volatile

fatty acid production by *in vitro* gas production technique with rumen liquor of buffaloes.

MATERIALS AND METHODS

Collection of rumen inoculum

Fresh rumen fluid was collected before morning feeding from four rumen cannulated Murrah buffalo steers (avg. age 2.5 years, 380 ± 14 kg B.W.) fed on a basal diet of wheat straw offered *ad libitum* and a limited amount of standard concentrate mixture and green oats in the morning (09:30 h) to meet their nutrient requirements for maintenance (Paul and Lal, 2010). Clean drinking water was provided free choice to the animals housed in well-ventilated shed with provision of individual feeding. Rumen inoculum was collected before feeding and watering of the animals from both solid and liquid phase between 09:00 and 09:30 h according to standard procedures. Equal volume of rumen liquor from each animal was pooled and brought to the laboratory in a pre-warmed thermos flask for further use. All laboratory handling of rumen liquor was done under continuous flushing with CO_2 to maintain anaerobic conditions.

Experimental procedure

Three concentrations (0, 0.4 and 0.8 ml/ 30 ml buffered rumen fluid) each of SOL and MOL were investigated in a randomized block design for this *in vitro* study. Sorghum hay ($200 \text{ mg} \pm 5 \text{ mg}$) was used as substrate and incubated with 30 ml of buffered rumen fluid in 100 ml calibrated glass syringes and placed in a ventilated incubator at 39°C for 24 h (Menke *et al.*, 1979; Menke and Steingass, 1988). Three syringes without substrate were incubated as blank. The syringes were

regularly shaken by hand during the incubation period to prevent the plunger from picking up substrate and proper mixing of feeds with rumen inoculum. Each treatment was replicated four times in each run and every set was repeated twice to get consistent observations in the study.

Estimation of gas and methane production

After 24 h of incubation, the gas production was recorded by the displacement of piston during incubation. The net gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank syringe. For methane estimation, 200 μ l gas was sampled from the head space of syringe in an airtight Hamilton syringe and injected into NUCON-5700 gas chromatograph equipped thermal conductivity detector (TCD) and stainless steel column packed with Porapak-Q. The column pressure for carrier gas hydrogen was put to 10 PSI and temperature of column oven, injector and detector were 60°C, 140°C and 150°C, respectively. A 50:50 mixture of methane and carbon dioxide (Centurion Scientific, New Delhi) was used as standard.

Volatile fatty acid estimation

After 24 h incubation 1 ml of the supernatant of each syringe content was taken in a micro centrifuge tube containing 0.20 ml metaphosphoric acid (25%, v/v). The mixture was allowed to stand for 2 h at room temperature and centrifuged at $5,000 \times g$ for 10 minutes to get clear supernatant. The supernatant (1 μ l) was injected into NUCON-5700 gas chromatograph equipped with flame ionization detector (FID) and glass column packed with chromosorb 101 as described by Cottyn and Boucque (1968). The column pressure for hydrogen and zero moisture air were 20 PSI and 10 PSI, respectively. Temperature of

column oven, injector and detector were 170°C, 240°C and 250°C, respectively.

In vitro dry matter degradability and microbial protein synthesis

The content of the syringes were transferred to 500 ml spout less beakers by repeated washings with neutral detergent solution. After refluxing the contents for 1 h, the residue was recovered in pre-weighed filter crucibles (G1). After drying the crucibles to the constant weight, ashing was done at 550°C. Truly degradable dry matter (TDDM) and organic matter (TDOM) was estimated and microbial biomass production (MBP) and partitioning factor (PF) was calculated (Blummel *et al.*, 1997).

Chemical and statistical analysis

Samples of sorghum hay and fermentation residue were analyzed following the methods of Association of Official Analytical Chemists (AOAC, 1995) to determine DM by the oven drying method (934.01) and organic matter by muffle furnace incineration (967.05). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the methods of Van Soest *et al.* (1991).

Data obtained were subjected to analysis of variance (ANOVA) using SPSS 11.0 software and treatment means were ranked using Duncan's multiple range tests according to Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The effects of dietary fats depend on source, fatty acids composition and their concentrations in the rumen fluid (Steele and Moore, 1968). Dosing

of both the oils resulted an apparent increase in total gas production, however, it was significant ($P < 0.05$) only with MOL. The result is in contrary with previous workers (Kongmun *et al.*, 2010; Adeyemi *et al.*, 2015), where supplementation of various oils either did not affect or reduced the gas production. However, our results get support from Getachew *et al.* (2001); Narimani-Rad *et al.* (2011), where addition of yellow grease or sunflower oil significantly increased gas production under *in vitro* rumen fermentation system (Figure 1).

The effects of supplementary fat on dry matter and fibre digestibility failed to yield a consistent result due to a variety of factors viz. source of fats, degree of saturation, chain length of fatty acid, amount of free fatty acids, rate and extent of hydrolysis of fats that determine their activity on rumen microbes (Jenkins, 1994; Bateman *et al.*, 1996). Devendra and Lewis (1974) reported that dietary oil forms a coating on the surface of fiber which creates a physical barrier preventing bacteria from having access to the fiber. Besides, dietary

oil could cause growth depression or death of cellulolytic bacteria. Jenkins (1993) also reported that unprotected fat could form insoluble calcium soap which reduces calcium availability for fiber digestion and microbial activities. In present study, a linear ($P < 0.001$) decrease in degradability of DM and NDF was evident with increasing doses of oils, however, the effects were more pronounced with MOL (Table 1).

The variation in effects with same concentration of oil could be due to deviations in fatty acid composition and their properties. In contrary, Bateman and Jenkins (1998) did not report any effect on dry matter and fibre digestibility of diet supplemented with soybean oil. As there is inverse relationship between gas production and microbial biomass production, the MBP was significantly ($P < 0.001$) reduced irrespective of oil type and dose. Partitioning factor (PF), a reflection of truly degradable dry matter per unit gas production was also reduced with oil supplementation. Reduction in substrate degradability in present study

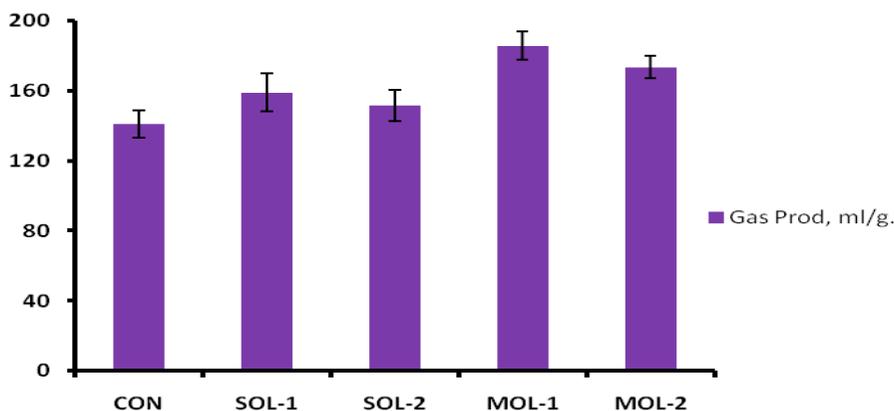


Figure 1. Effect of sesame and mustard oils on *in vitro* gas production of sorghum hay.

CON = Control, SOL-1 and SOL-2 are sesame oil and MOL-1 and MOL-2 are mustard oil (0.4 ml and 0.8 ml/30 ml, respectively).

corroborate with the findings of others (Ikwuegbu and Sutton, 1982; Kongmun *et al.*, 2010) could be the reason of reduced microbial protein production and partitioning of nutrients.

A decrease ($P < 0.05$) in methane production (ml/ g DM) and methane concentration (%) of head space gas were evident with supplementation of both the oils. However, no difference ($P > 0.05$) in methane production was recorded between SOL and MOL at corresponding doses. But, methane concentration was lower ($P < 0.001$) in MOL than

that of SOL. Methane production was reported to be lower in diets supplemented with fats compared to control (Machmueller *et al.*, 1998). The reason for suppressing methane production of fats could be through a direct influence on the rumen methanogenic microbes (Figure 2).

Acetate production was not affected (Table 2), but propionate and butyrate production was increased ($P < 0.05$) with addition of oils, irrespective of type and doses. The ratio of acetate to propionate (A : P) was reduced ($P < 0.01$) with

Table 1. Effect of sesame and mustard oils on *in vitro* substrate degradation and microbial biomass production.

Attributes	Treatments					SEM	P value
	CON	SOL-1	SOL-2	MOL-1	MOL-2		
TDDM, %	64.27 ^c	52.36 ^b	48.26 ^{ab}	47.11 ^a	44.06 ^a	3.42	<0.001
NDFD, %	52.36 ^c	36.49 ^b	31.01 ^{ab}	29.49 ^a	25.41 ^a	4.56	<0.001
Gas prod, ml/g DM	141.06 ^a	159.01 ^{abc}	151.50 ^{ab}	185.83 ^c	173.43 ^{bc}	9.19	0.027
MBP, mg/g DM	332.38 ^d	190.48 ^c	155.97 ^b	62.31 ^a	57.01 ^a	47.10	<0.001
PF	4.59 ^c	3.32 ^b	3.20 ^b	2.54 ^a	2.55 ^a	0.36	<0.001

CON = Control, SOL-1 and SOL-2 are sesame oil and MOL-1 and MOL-2 are mustard oil (0.4 ml and 0.8 ml/30 ml, respectively).

^{a,b,c,d} means with different superscripts within a row differ significantly

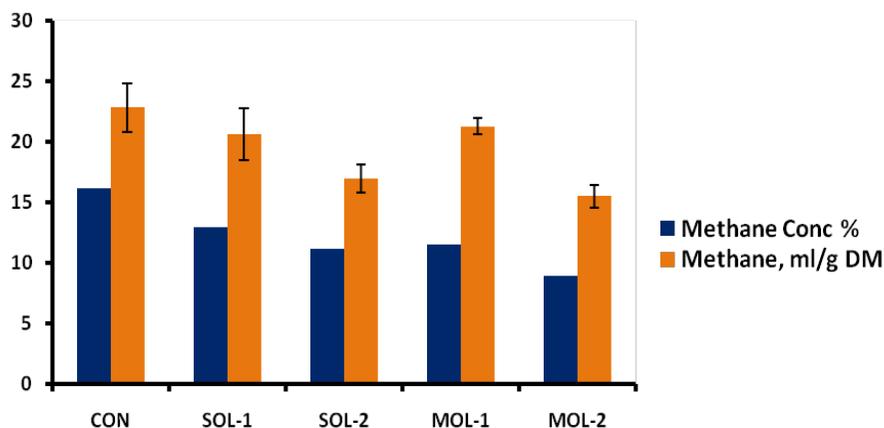


Figure 2. *In vitro* ruminal methanogenesis of sorghum hay supplemented with vegetable oils.

Table 2. Composition of volatile fatty acids of rumen fluid incubated with sorghum hay supplemented with sesame and mustard oils.

Attributes	Treatments					SEM	P value
	CON	SOL-1	SOL-2	MOL-1	MOL-2		
Acetate, mM/dl	3.19	2.87	3.03	3.00	2.98	0.10	0.626
Propionate, mM/dl	0.74 ^a	0.84 ^{ab}	0.91 ^b	0.81 ^{ab}	0.80 ^{ab}	0.04	0.051
Butyrate, mM/ dl	0.19 ^a	0.25 ^{ab}	0.32 ^b	0.29 ^b	0.28 ^b	0.02	0.021
A : P ratio	4.29 ^b	3.44 ^a	3.34 ^a	3.73 ^a	3.73 ^a	0.18	0.009

CON = Control, SOL-1 and SOL-2 are sesame oil and MOL-1 and MOL-2 are mustard oil (0.4 ml and 0.8 ml/30 ml, respectively).

^{a,b,c,d} means with different superscripts within a row differ significantly.

addition of oils, however, no difference between SOL and MOL was noted. A shift in VFA production from acetate to propionate, thereby reduced acetate to propionate ratio corroborate with the findings of earlier workers (Getachew *et al.*, 2001; Jacob *et al.*, 2012). In contrary, no significant effects on total VFA and propionate production was reported with supplementation of carotino, soybean, cotton seed or linseed oils (Kim *et al.*, 2007; Adeyemi *et al.*, 2015).

It is concluded that both SOL and MOL can reduce methane production; however, feed degradability is also affected with increasing doses. Mustard oil exerted greater inhibitory effects on feed degradability and microbial biomass production. Hence, sesame oil is better than mustard oil as methane inhibiting agent. Further studies need to be taken up to find out optimal dose and combination of oils for feeding to buffaloes for improved animal production in addition to reduced methane production.

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