Research Biotica 2020, 2(2):61-64 DOI: 10.54083/ResBio.2.2.2020.61-64

Research Article ***Article ID:*** RB0044

Efficacy of Sunflower Oil in Modulating Rumen Functions and Reducing Enteric Methane Production in Buffalo (*Bubalus bubalis*)

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Keywords

Buffalo, Fatty acids, Methanogenesis, Rumen fermentation, Sunflower oil

How to cite this article?

Dey *et al.*, 2020. Efficacy of Sunflower Oil in Modulating Rumen Functions and Reducing Enteric Methane Production in Buffalo (*Bubalus bubalis*). *Research Biotica* 2(2), 61-64.

Abstract

Enteric methane emission from ruminant livestock reduces the efficiency of feed energy utilization and contributes to global warming. An experiment was conducted to investigate the effects of sunflower (SFL) oil supplementation on methanogenesis, volatile fatty acids composition and feed fermentation pattern by *in vitro* gas production (IVGP) test. SFL oil was examined at three concentrations (0, 0.4 and 0.8 ml/ 30 ml buffered rumen fluid). *In vitro* incubation was carried out with sorghum hay (200 ± 5 mg) as substrate in 100 ml calibrated glass syringes following standard IVGP protocol. Addition of SFL oil resulted in increase ($p < 0.05$) in total gas production and decrease ($p < 0.05$) in methane concentration in head space gas, irrespective of level of inclusion. Linear decrease ($p < 0.001$) in feed degradability was evident with increasing doses of oil. Acetate production decreased ($p < 0.05$) without affecting propionate, however, butyrate production increased ($p < 0.05$) with addition of oil, irrespective of doses. The ratio of acetate to propionate was reduced ($p < 0.01$) with addition of oils. It is concluded that sunflower oil supplementation exerted inhibitory effects on methane production; however, dry matter degradability was also reduced. Further studies need to be carried out with lower dose levels for their practical application in animal feeding practices.

1. Introduction

Methane (CH_4) is produced in digestive tract of ruminant livestock by fermentation of feeds with anaerobic microorganisms (bacteria, protozoa, fungus, archaea) and emitted to the environment via burping mainly. Since CH_4 has no nutritional value to the animals, its production represents a loss of 2-12 % dietary gross energy intake (Johnson and Johnson, 1995). Methane production not only reduces the efficiency of feed energy utilization but also contributes to global warming. Ruminant animals contribute about 18% of the global anthropogenic greenhouse (GHG) gas emissions mostly due to anaerobic enteric fermentation of feeds. About 37-44 % of global methane emissions are contributed by ruminant livestock and are a major source of methane production in the agriculture sector (IPCC, 2007). Therefore, reducing enteric methane production by dietary modulation of livestock feeding is one of the major strategies not only in view of clean animal production, but also increasing the utilization efficiency of feed energy in productive purpose. Vegetable oils, a source of poly-unsaturated fatty acids, have the potential to reduce enteric methane production. However, characteristics of oils, level of inclusion and composition of basal diet influences their efficacy. Therefore, this study was aimed to investigate the effects of sunflower (SFL) oil on methanogenesis, volatile fatty acids composition and feed fermentation pattern by *in vitro* gas production (IVGP) test with rumen liquor of buffaloes for abatement of environmental pollution from livestock production.

2. Materials and Methods

The study was carried out at the Rumen Microbiome Laboratory, ICAR- Central Institute for Research on Buffaloes, Hisar (29.1203° N, 75.8069° E). The guidelines of the Institute Animal Ethics Committee (IAEC) were complied with respect to rumen liquor collection and the care of the rumen fistulated animals.

2.1 Rumen Inoculum

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

Article History

RECEIVED on 17th May 2020 RECEIVED in revised form 13th June 2020 ACCEPTED in final form 14th June 2020

mixture and green oats fodder 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.

2.2 Experimental Procedure

Three concentrations (0, 0.4 and 0.8 ml/ 30 ml buffered rumen fluid) of sunflower oil (SFL) were investigated in a randomized block design for this *in vitro* study. Sorghum hay (200 mg ± 5 mg) was used as substrate and incubated with 30 ml of buffered rumen fluid (2:1, buffer: rumen liquor) in 100 ml calibrated glass syringes and placed in a ventilated incubator at 39 °C for 24 h (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.

2.3 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 µl gas was sampled from the head space of syringe in an airtight Hamilton syringe and injected into NUCON-5700 gas chromatograph equipped flame ionization detector (FID) and stainless-steel column packed with Porapak-Q. Hydrogen was used as carrier gas at 10 PSI column pressure. The column injector, detector and oven temperature were 140 °C, 150 °C and 60 °C, respectively. A 50:50 mixture of methane and carbon dioxide (Centurion Scientific, New Delhi) was used as standard.

2.4 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 could stand for 2 h at room temperature and centrifuged at 5,000 × g for 10 min to get clear supernatant. The supernatant (1 µl) 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). Temperature of column oven, injector and detector were 170 °C, 240 °C and 250 °C, respectively. The column pressure for hydrogen and zero moisture air were 20 PSI and 10 PSI, respectively.

2.5 In vitro Dry Matter Degradability and Microbial Protein Synthesis

The content of the syringes was 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 (G-1). After drying the crucibles to the constant weight, ashing was done at 550 °C. Truly degradable dry matter (TDDM) and neutral detergent fibre degradability (NDFD) was estimated and microbial biomass production (MBP) and partitioning factor (PF) was calculated as the ratio of substrate *in vitro* truly degraded to gas volume produced by it (Blummel *et al*., 1997).

2.6 Chemical Analysis

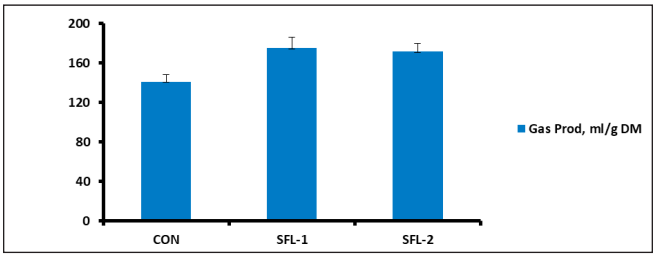
Samples of sorghum hay and fermentation residue were analysed following the methods of Association of Official Analytical Chemists (AOAC, 1995) to determine DM by the oven drying method (934.01) and organic matter by muffle furnace incineration (967.05). Neutral detergent fibre (NDF) and acid detergent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

2.7 Statistical Examination

Data obtained were subjected to one-way analysis of variance (ANOVA) using SPSS 17.0 software and treatment means were ranked using Duncan's multiple range tests according to Snedecor and Cochran (1994) and significant difference was stated when $p < 0.05$.

3. Results and Discussion

Addition of sunflower oil to the fermentation fluid resulted in increase ($p < 0.05$) in total gas production, irrespective of concentration (Figure 1). The effects of dietary fats depend on source, fatty acids composition and their concentrations in the rumen fluid (Steele and Moore, 1968). While examining the effect of vegetable oils on rumen fermentation, Dey *et al.* (2018) demonstrated increased total gas production with addition of mustard oil, however no effect was evident with addition of sesame oil. In contrary, Adeyemi *et al*. (2015) reported reduced the gas production with supplementation of oils. Comparable to our study, Narimani-Rad *et al.* (2011) described significantly increased gas production under *in vitro* rumen fermentation system with addition of sunflower oil.



[CON = Control, SFL-1 and SFL-2 are sunflower oil (0.4 ml and 0.8 ml/ 30 ml buffered rumen fluid, respectively)]

Figure 1: Effect of sunflower oil on in vitro gas production of sorghum hay

The degradability of dry matter and fibre on dietary supplementation of oils depends on various factors viz. sources, degree of saturation, chain length of fatty acid, amount of free fatty acids, rate and extent of hydrolysis etc. (Bateman *et al*., 1996). Jenkins (1993) 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 SFL (Table 1).

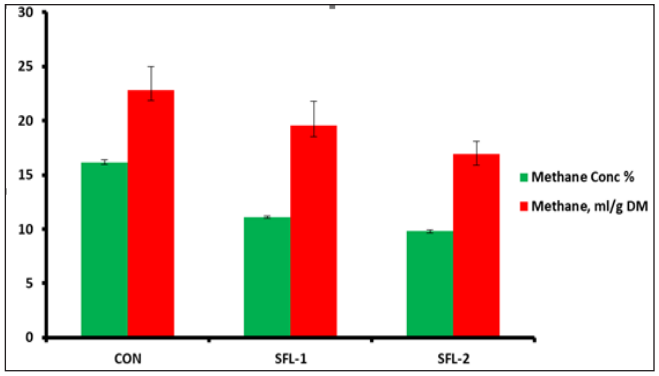
Table 1: Effect of sunflower oil supplementation on *in vitro* ruminal substrate degradation and fermentation pattern

Attributes	Treatments			SEM	P value
	CON	SFL-1	SFL-2		
TDDM, %	64.27^{c}	52.76^{b}	47.47^{a}	1.46	< 0.001
NDFD, %	52.36^{c}	37.01^{b}	29.96^{a}	1.95	< 0.001
Gas prod, ml/g DM	141.06^{a}	174.85^{b}	171.53^{b}	3.82	0.040
MBP, mg/g DM	332.38^{c}	142.92^{b}	97.33^{a}	21.11	< 0.001
PF	4.59^{b}	3.03^{a}	2.77^{a}	0.17	0.001
Methane, ml/g DM	22.84^{b}	19.53a^{b}	16.89^{a}	0.62	0.040
Methane Conc, %	16.14^{b}	11.10^{a}	9.80^{a}	0.62	0.006
Acetate, mM/dl	3.19^{b}	2.88^{a}	2.99^{a}	0.03	0.042
Propionate, mM/dl	0.74	0.75	0.76	0.01	0.764
Butyrate, mM/ dl	0.19^{a}	0.25^{b}	0.28^{b}	0.01	0.002
A:P ratio	4.29^{b}	3.86^{a}	3.82^{a}	0.05	0.007

[CON = Control, SFL-1 and SFL-2 are sunflower oil (0.4 ml and 0.8 ml/ 30 ml, respectively) a, b, c, d means with different superscripts within a row differ significantly]

As gas production and microbial biomass production are inversely related, the MBP was significantly ($p < 0.001$) reduced irrespective of dose. Partitioning factor (PF), which is a ratio of truly degradable dry matter per unit gas production was also reduced with oil supplementation. Reduction in substrate degradability and MBP production in present study support the findings of our earlier studies (Dey *et al.,* 2018) and other researchers (Kongmun *et al*., 2010).

A decrease ($p < 0.05$) in methane concentration (%) of head space gas was evident with supplementation of oil at both the doses, however, methane production (ml/g DM) was reduced ($p < 0.05$) at higher dose level (0.8 ml/ 30 ml) only (Figure 2). Lowered methane production was demonstrated in diets supplemented with fats (Machmueller *et al*., 1998). The suppression of methane production on supplementation of fats could be through a direct inhibition of rumen methanogenic microbes or reduction of feed digestibility.



[CON = Control, SFL-1 and SFL-2 are sunflower oil (0.4 ml and 0.8 ml/ 30 ml buffered rumen fluid, respectively)]

Figure 2: *In vitro* ruminal methanogenesis of sorghum hay supplemented with graded levels of sunflower oil

Acetate production was reduced (Table 1) without affecting propionate production, while butyrate production was increased ($p < 0.05$) with addition of oil, irrespective of doses. The ratio of acetate to propionate (A:P) was reduced ($p < 0.01$) with addition of oil. Kim *et al*. (2007) reported no significant effects on total VFA and propionate production with supplementation of soybean or cotton seed oils. The reduction in VFA production due to supplementation of SFL in the present study demonstrated decrease in feed degradability, which could be owing to inhibition of fibrolytic rumen microbes.

4. Conclusion

The supplementation (0.4 ml/ 30 ml rumen fluid) of sunflower oil to sorghum hay-based substrate exerted inhibitory effects on methane production under *in vitro* system. However, dry matter degradability was also reduced, envisaging lowered feed utilization. Further studies need to be carried out with lower dose levels for their practical application in animal feeding system for reducing environmental pollution.

5. Acknowledgement

The authors sincerely acknowledge the Indian Council of Agricultural Research, New Delhi, India for granting the fund in the form of IRC project for conducting the research work.

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