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RESEARCH ARTICLE

EFFECT OF *TRIGONELLA FOENUM-GRAECUM- BRASSICA JUNCEA* ON METHANEPRODUCTION IN BUFFALO AND CROSS BREED CATTLE

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Abstract

Methane (CH_4) is a green house gas; it can trap heat 25 times more per molecule than Carbon dioxide and is expected to cause 15-17% of the global warming over the next 50 years. Livestock, particularly the ruminants accounts for a significant proportion of the global methane budget, about 65-100 million tons per Annam. Agricultural emission of CH_4 accounts for about 60% of the total CH_4 from anthropogenic sources, out of which 25% arises from enteric fermentation in livestock. We studied the effect of Fenugreek *(Trigonella foenum-graecum)*, and Mustard *(Brassica juncea)* with different concentrations (F_{20}, F_{60}, F_{80} and M_{20}, M_{60}, M_{80}) on methane production control in Cattle and buffalo by *invitro* experiment. Methane emission was reduced in Buffalo and Cattle by the addition of mustard in diet from 11.51% to 12.74% and 13.93% to 16.33% levels, respectively, whereas no significant reduction in methane % was observed with Fenugreek supplemented diet. More studies were required to validate the results under *in vivo* conditions.

Introduction

Methane produced from ruminant enteric fermentation has a significant role in global warming by trapping the infrared radiation in the form of heat. It contributes to 9% of total green house gasses (GHG). Methane is the second largest anthropogenic GHG, which contributed to 14.3% of total anthropogenic GHG emissions estimated in 2004 (Solomon et al., 2007). India has livestock wealth of 272.1 million cattle, 159.8 million buffaloes, 71.6 million sheep, 140.6 million goats and 13.1 million (GOI.2012,) other ruminants, which produce large amounts of CH_4 as a part of their normal digestive process. This constitutes about 20% of the world's ruminant population. The effects of GHG emissions on the ecological and socioeconomic vulnerability have already been noticed and will continue to grow regionally and globally in the years to come (Solomon et al., 2007). Carbon dioxide (CO_2), Methane (CH_4), Nitrous oxide, Hydro fluorocarbons, Per-fluorocarbons, and Sulphur hexafluoride are the important GHGs that are monitored by the United Nations Framework Convention on Climate Change (IPCC.2007) and have been listed in Annex A of Kyoto Protocol for their mitigation commitment. In 1948 the presence of CH_4 in the atmosphere was first discovered in the infrared absorption spectrum (Migeotte, 1948) and still it is monitoring in the atmosphere. Methane emission contributed from ruminants becoming to distressing as EC, (2010) reported. Methane emission from livestock was 83% of the greenhouse gases emitted within the agricultural sector. (Kebreab et al. 2008) posited that a 287 million tonnes of methane is released globally and yearly from anthropogenic sources, of which 50% is from agriculture and enteric fermentation from ruminants is said to be the largest biogenic source USEPA, 2006. (Aluwong et al., 2011) gave global anthropogenic methane emission from enteric fermentation and manure management as 35-40% of total emission. Methane emissions in ruminants also account for a 2% to 12%. The concentration of CH_4 has increased by about 1,059 ppbv (i.e. from 715 to 1,774 ppbv in 2005) since 1750 (Solomon et al., 2007) of gross energy loss of feed depending upon the type of diets (Johnson et al., 1995) Due to animal physiological stage also contributes to the variation in CH_4 Production within and between the different species of ruminants. Therefore, inhibition of CH_4 production in the rumen has been attempted for more than three decades to increase the utilization of feed and fodder energy for production purpose. Many strategies such as processing of forages (Takahashi, 2001; Santoso et al., 2003), increasing proportion of concentrates in the diet (Lee et al., 2003), and supplementation of some methane inhibitors such as halogenated compounds (Martin and Macy, 1985), ionophores (Van Nevel and Demeyer,1988), organic acids (Martin,1988), Saponin (Lila *et al*., 2003), unsaturated fatty acids (Czerkawaski et al., 1966) antibiotics have been used to reduce the methane production from enteric fermentation of ruminants. The rumen fermentation (Nagaraja, 1995), improving the some end product (propionate) and decreasing the production of methane (Stanier and Davis, 1981). Past few decades to a great extent of the research has focused on the effect of ionophores and antibiotics (Russell, 1987), but antibiotics have been banned by European Union's Agricultural ministry since January 2006, due to the risk of the presence of its residue in milk and meat which can affect human health. (Russell and Houlihan 2003). Therefore, safe and cost effective new alternatives are needed to maintain efficient animal production systems. But Plants and it parts contain high concentration of Secondary metabolite compounds and can be used as a safe means of ruminal fermentation modulators (Teferedegne, 2000). The plant extracts effective modulators to manipulate rumen fermentation favourably to reduce ruminal methane emission with minimum adverse effect on ruminal fermentation of feed so that these can be practically explore for economic and ecologically friendly livestock production (Santra, 2012). In present study we observed the effect of fenugreek and mustard on *invitro gas* and methane production in both buffalo and cattle.

MATERIALS AND METHOD

PREPARATION OF INOCULUMS

Rumen liquor was collected separately from two fistulated Cattle (*Karan Swiss*) and Buffalo (*Murrah*) after feeding the animals. Rumen liquor was collected into a thermal flask to maintain anaerobic conditions. Inoculums was prepared by repeated filtration through muslin cloth and followed by centrifugation at 4°C and 10,000 × *g* for 20 minutes. The supernatant obtained was used as inoculum for further experiment.

PREPARATION OF SYRINGES

In vitro experiment was carried out in a 100 ml calibrated glass syringe (Sigma. U.S.A) the syringes were incubated in water bath at 39 ± 0.50^0C for methane production. (Blummel and Orskov E.R., 1993). Wheat straw and concentrate mixture (60:40) was milled and used as a substrate. 200 mg of substrate was placed into the bottom of the plastic syringe. The piston was lubricated with petroleum jelly and pushed inside the glass syringe. The medium mixture solution was prepared by mixing 500 ml distilled water, 0.125 ml micro mineral solution, 250 ml buffer solution and 250ml macro mineral solution, 1.25 ml Resazurin solution, and 50 ml reducing solution (prepared fresh and added prior to incubation). The medium mixture solution was prepared by passing CO_2 and maintained at 39^0C to maintain anaerobic environment. Once the medium mixture solution becomes colourless, and the filtered rumen liquor was added (30ml) using auto dispenser (Thermo Scientific). The proportion of medium mixture solution to rumen liquor was 2:1 (Menke and Steingass, 1988).

The syringes were shaken gently and residual air or air bubbles were removed and outlet was closed. The level of piston was recorded and syringes were placed in water bath (39± 0.50ºC). The syringes were shaken every one hour from the start of the incubation up to 10 hour of incubation. These trials were conducted along with respective blank and control in triplicate. The control syringes were without Fenugreek (F) (*Trigonella foenum-graecum*), Mustard (M) *(Brassica juncea*) and in test syringes Fenugreek and Mustard separately with different concentrations F_{20}, F_{60}, F_{80} and M_{20}, M_{60}, M_{80} was added. Total gas and methane were estimated at 24 hr post incubation.

ESTIMATION OF GAS AND METHANE.

After 24 hour of incubation, volume of gas was withdrawn from the tip of the incubation syringe and analyzed methane produced in syringe with the help of Gas chromatography (Nucon 5700, India) fitted with stainless steel column packed with Porapak- Q (length 6';o.d.1/8" i.d. 2mm; mesh range 80-100) and Flame ionizing detector (FID).The temperature of injection port, column and detector was 40º, 50º and 500ºC respectively. Volume of gas taken for injecting was 200µl with the help of Hamilton syringe (Sigma U.S.A). The flow rate of carrier gas (Nitrogen) through the column was 30ml/min and H_2 was 30ml/min and air was 300 ml/min. The standard gas for methane estimation (Spantech calibration gas, Surrey, England) composed of 50% Methane (CH_4) and 50% Carbon dioxide (CO_2). The peak of methane gas was identified on the basis of retention time of standard methane gas and the response factor obtained was used to calculate methane percentage in the gas sample. The methane produced from substrate during 24 hour incubation was corrected for the blank values. The volumes of methane produced were calculated by following formula.

Methane production (ml) = Total gas produced (ml) × % Methane in the sample.

Results and Discussion

The physical and chemical composition of wheat straw based diet was shown in Table: **1**. the results of the effect of Mustard (M), Fenugreek (F) on *in vitro gas* and methane production (ml/g of substrate, l/100g of substrate and g/100g substrate) in Buffalo and Cattle were shown in Table: **2,** and **3,** Fig: **1** and **2** respectively. In this study, results were analyzed by Descriptive statistics and the results showed that Mean CH_4 % and Mean Total gas production were affected due to the addition of mustard and fenugreek. The results were more or less in accordance with previous studies. Total gas production increased on supplementation of fenugreek and mustard though the difference was not significant in Cattle and Buffalo. Bakshi et al. (2004) also reported higher total gas production with herbs in the diet at 0.4% level of supplementation. The increase could be attributed to the availability of glucogenic (water soluble) components of the seeds (Gupta et al, 1984). The fenugreek and mustard did not have any significant impact on the methane production. In case of mustard, slight decrease in methane production in both the cases of buffalo and cattle. Methane % was reduced from 11.51% 12.74% to in buffalo and 13.93% to 16.33% in cattle as the concentration of treatment (mustard) increased from M_{20} to M_{80} (20mg to 80mg). But in case of fenugreek, there was increase in % of methane from 13.36% to 15.97% in Buffalo and 15.45% to 16.54% in Cattle as the concentration of treatment (Fenugreek) increased from F_{20} to F_{80} (20mg to 80mg).

Table 1: Physical Composition of diet used as substrate in *invitro* incubations.

INGREDIENT OF DIETS

	g/Kg on DM Basis	
DIETS	**WHEAT STRAW**	**CONCENTRATE**
HFD	600	400

INGREDIENT OF CONCENTRATE

PARTICULARS	**g/Kg on DM Basis**
Maize	330
Ground nut cake	210
Mustard cake	120
Wheat bran	200
De oiled rice bran	110
Mineral mixture	20
Salt	10

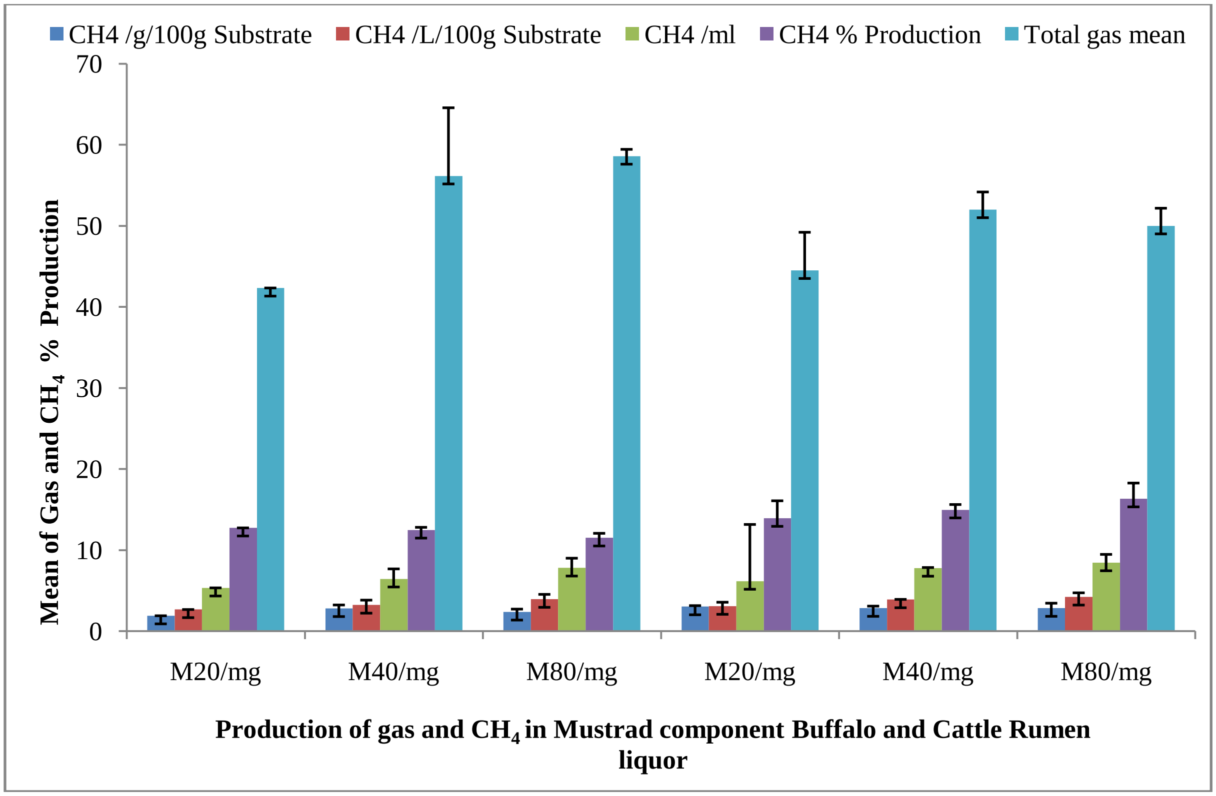

Fig.1

Table 2: Effect of Mustard on Gas and % of Methane in Buffalo and Cattle.

Parameters	Buffalo *(Mean+S.E)*			Cattle*(Mean+S.E)*		
	M_{20}	M_{40}	M_{80}	M_{20}	M_{40}	M_{80}
CH_4/g /100 g substrate	1.9±0.43	2.8±0.35	2.37±0.13	3.02±0.26	2.83±0.62	2.83±0.62
CH_4/L /100 g substrate	2.67±0.61	3.22±0.60	3.94±0.49	3.08±0.03	3.89±0.51	4.22±0.36
CH_4 production/ml	7.8±6.99	6.45±1.20	5.34±1.23	6.17±0.07	7.78±1.02	8.45±0.73
Mean CH_4 %	11.51±2.15	12.48±0.56	12.74±0.33	13.93±0.65	14.97±1.94	16.33±0.58
Mean Total gas production	42.33±8.4	56.16±0.83	58.6±4.7	44.5±2.17	52.0±2.17	50.0±4.5

*M_{20}: Mustard (20mg); M_{40}: Mustard (40mg); M_{80}: Mustard (80mg)

Table 3: Effect of Fenugreek on Gas and % of Methane in Buffalo and Cattle.

Parameters	Buffalo*(Mean+S.E)*			Cattle*(Mean+S.E)*		
	F_{20}	F_{40}	F_{80}	F_{20}	F_{40}	F_{80}
CH_4/g/100 g substrate	2.37±0.13	1.45±0.92	2.23±0.75	2.57±0.06	2.47±0.31	2.74±0.54
CH_4/L/100 g substrate	2.87±0.18	2.45±0.98	2.30±0.65	3.45±0.04	3.49±0.11	4.03±0.47
CH_4production/ml	6.65±0.38	4.07±2.58	6.26±2.12	6.37±0.53	6.98±0.23	8.07±0.95
Mean CH_4 %	13.36±0.71	13.99±0.31	15.97±0.75	15.45±0.93	16.37±0.44	16.54±1.76
Mean Total gas production	47.5±1.75	52.5±0.5	56.8±2.3	41.6±1.96	42.3±0.33	48.6±1.01

*F_{20}: Fenugreek (20mg); F_{40}: Fenugreek (40mg); M_{80}: Fenugreek (80mg)

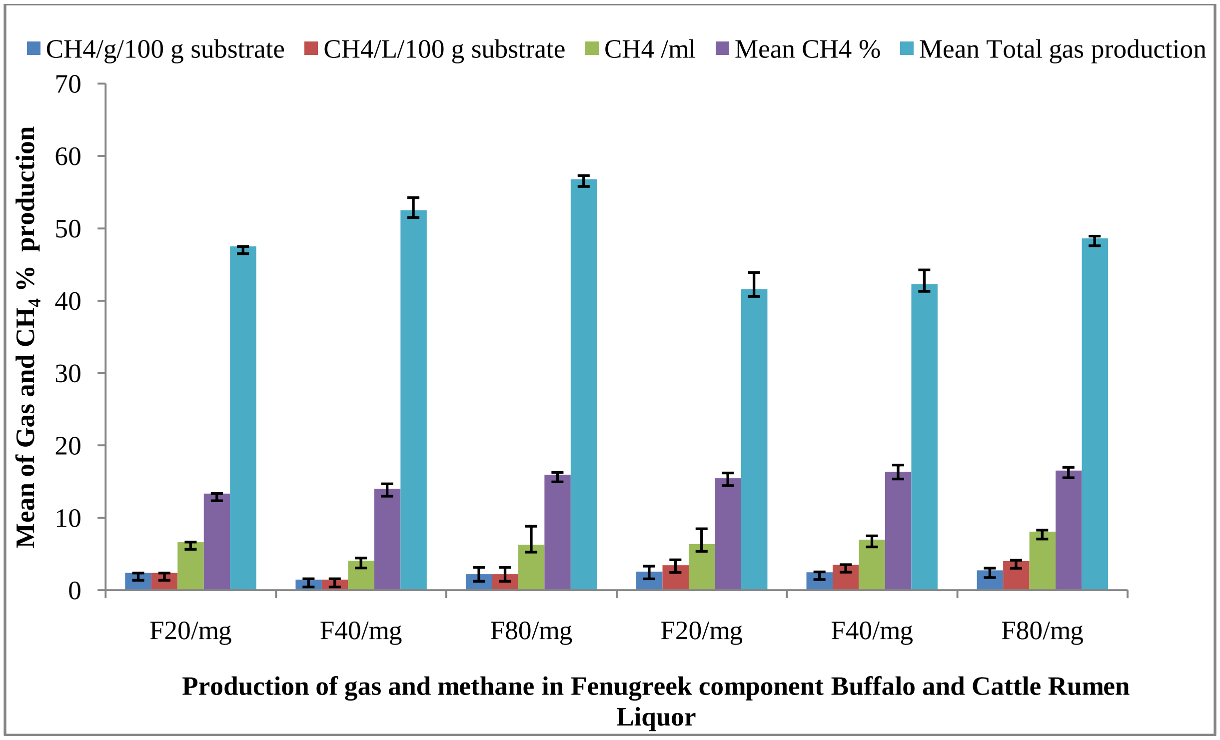

Fig.2

Conclusions

In the present study, it was concluded that the mustard addition in wheat straw containing diets decrease Methane (%) in both the cases of Cattle and Buffalo, and there was increase in total gas production. But more studies are required to validate the results under *in vivo* conditions.

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Conflict of interest

Authors declare that there is no conflict of interest

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