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In vitro Methanogenesis and Fermentation of Feeds Containing Oil Seed Cakes with Rumen Liquor of Buffalo

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ABSTRACT : Eight feeds (mixture of wheat straw and oil seed cakes in 3:1 ratio) were evaluated for methane emission and fermentation pattern with buffalo rumen liquor as inoculum in an *in vitro* gas production test. The cakes tested were groundnut cake (GNC), soybean cake (SBC), mustard seed cake (MSC), cotton seed cake (CSC), karanj seed cake expeller extracted (KCEE), karanj seed cake solvent extracted (KCSE), caster bean cake expeller extracted (CBCEE) and caster bean cake solvent extracted (CBCSE). The gas production (ml/g dry matter) was significantly higher with SBC and MSC followed by CSC, GNC, KCSE, KCEE, CBCSE and was the lowest with CBCEE. Methane emission was significantly lower with KCEE, KCSE, CBCEE, CBCSE (20.32- 22.43 ml/g DM) than that with SBC, GNC, CSC (27.34-31.14 ml/g DM). Mustard seed cake was in-between the two groups of oil cakes in methane production. *In vitro* true digestibility was highest with SBC followed by GNC, CSC, MSC, KCSE, KCEE, CBCSE and CECEE. Ammonia nitrogen level was positively correlated with the amount of protein present in the cake. Total holotrich protozoa were significantly higher with SBC, whereas, large spirotrich protozoa tended to be lower than with other cakes. The counts of small spirotrich and total protozoa were similar with all the cakes. Total volatile fatty acid production and acetate to propionate ratio were significantly higher with SBC and significantly lower with KCEE as compared to the other cakes. Among the conventional oil cakes tested in the present experiment (GNC, SBC, MSC and CSC), mustard seed cake-based feed produced the minimum methane without affecting other fermentation characteristics adversely. (**Key Words :** Oil Cakes, Methane, Rumen Fermentation, Buffalo)

INTRODUCTION

Feed is fermented in the rumen by concerted activity of different groups of microorganisms to volatile fatty acids, which serve as an energy source for the animal. During the process of fermentation a large number of by-products are also generated including carbon dioxide and methane. Methanogenesis in the rumen is an essential metabolic process to maintain steady state fermentation as this scavenges the molecular hydrogen generated during fermentation. Methane produced in the rumen has 13.15 kcal/g of energy resulting in a 3-12% loss of gross energy intake depending upon the nature of feed (Lee et al., 2003). In addition, methane has a greenhouse effect and is responsible for 15% of global warming (Tyler, 1991). Globally, ruminants produce about 77×10^{12} g (77 Tg) methane annually, which constitutes about 15% of total atmospheric methane emission (Crutzen, 1995). Efforts have been made to reduce methane emission by using chemical and microbial feed additives (Nagaraja et al., 1997; Fievez et al., 1999) and recently some plant secondary metabolites have also been tested (Hosoda et al., 2005; Lila et al., 2005), but most of them either have adverse effects on nutrient utilization or are economically unfeasible. The chemical composition of feed influences the extent of methanogenesis and therefore, it appears that manipulation of feeding strategies might be the easiest mode of controlling enteric methane emission. Keeping this in view an experiment was conducted to study the methane production potential of different oil seed cakes either being used or proposed to be used for livestock feeding.

MATERIALS AND METHODS

Feed ingredients (wheat straw and oil seed cakes) were procured from the feed technology unit, IVRI, Izatnagar and ground in a Wiley mill to pass through a 2 mm sieve. The

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Table 1. Chemical composition of feed ingredients and their mixtures (g/100 g DM)

Ingredients	OM	CP	EE	TA	NDF	ADF	Hemi cellulose	Cellulose
Wheat straw	91.32	3.04	1.45	8.68	83.99	58.34	25.65	51.74
Ground nut cake	91.28	43.92	1.03	8.72	44.96	18.66	28.30	13.82
Soybean cake	91.83	43.78	1.51	8.16	23.33	12.49	10.84	12.17
Mustard seed cake	87.49	35.26	1.34	12.50	30.48	22.40	8.08	16.86
Cotton seed cake	91.49	37.44	1.38	8.51	46.42	15.62	30.79	11.99
Karanj seed cake (EE)	93.86	29.71	6.88	6.13	39.92	15.17	24.75	11.68
Karanj seed cake (SE)	94.05	32.87	0.98	5.94	35.39	12.58	22.80	9.00
Caster bean cake (EE)	93.52	31.82	5.40	6.47	55.06	39.06	15.99	9.90
Caster bean cake (SE)	87.66	33.81	0.86	12.33	49.65	37.10	12.54	14.69
Feed mixtures*								
Ground nut cake	91.31	13.26	1.34	8.69	74.23	48.42	26.31	42.26
Soybean cake	91.44	13.22	1.46	8.55	68.82	46.87	21.94	41.84
Mustard seed cake	90.36	11.09	1.42	9.63	70.61	49.35	21.25	43.02
Cotton seed cake	91.36	11.64	1.43	8.63	74.59	47.66	26.93	41.80
Karanj seed cake (EE)	91.95	9.70	2.80	8.04	72.97	47.54	25.42	41.72
Karanj seed cake (SE)	92.00	10.49	1.33	7.99	71.84	46.90	24.99	41.05
Caster bean cake (EE)	91.87	10.73	2.43	8.12	76.75	53.52	23.23	41.28
Caster bean cake (SE)	90.40	10.23	1.30	9.59	75.40	53.03	22.37	42.47

OM: organic matter; CP: crude protein; EE: ether extract; TA: total ash; NDF: neutral detergent fibre; ADF: acid detergent fibre.
* Wheat straw and oil cakes in the ratio of 3:1.

oil seed cakes tested in an *in vitro* gas production test were groundnut cake (GNC), soybean cake (SBC), mustard seed cake (MSC), cotton seed cake (CSC), karanj seed cake expeller extracted (KCEE), karanj seed cake solvent extracted (KCSE), caster bean cake expeller extracted (CBCEE) and caster bean cake solvent extracted (CBCSE). Two adult male fistulated buffaloes (393 kg body weight) fed on wheat straw and concentrate mixture in a 50:50 ratio were used as donors for rumen liquor. Substrate (0.2 g of a mixture of wheat straw and oil seed cake in 3:1 ratio) was incubated for 24 h with buffer and buffalo rumen liquor as inoculum (30 ml) in a 100 ml capacity gas syringe (Menke and Steingass, 1988). For methane and total gas production, there were 6 replicates. Out of 6 syringes for each treatment, the contents of three syringes were used for ammonia, protozoa and VFA analyses and the remaining three were used for the estimation of *in vitro* true digestibility.

Sample analyses

After 24 h incubation the displacement of the syringe piston indicated gas production. For methane estimation, 100 μl gas from the headspace of each syringe was injected into a Gas Chromatograph (NUCON 5765, AIMIL, India) equipped with a stainless steel column packed with Porapak-Q and a flame ionization detector (FID). The calibration gas (Spancan, Spantech Products Ltd., England) consisting of 50% methane and 50% CO_2 was used as a standard. Flow rate of nitrogen, hydrogen and air were kept as 20, 30 and 320 ml/min and the oven, injector and detector temperatures were 40, 50 and 50°C, respectively.

Ammonia nitrogen was estimated by the method of Weatherburn (1967). For volatile fatty acids, a 1.0 ml sample was mixed with 0.2 ml of 25% metaphosphoric acid and after 2 h was centrifuged at 5,000×g to obtain a clear supernatant. The supernatant (1 μl) was injected into a Nucon-5765 gas chromatograph equipped with a flame ionization detector and a chromosorb 101 column as described by Cottyn and Boucque (1968). Flow rate of nitrogen, hydrogen and air were kept as 30, 30 and 320 ml/min and the oven, injector and detector temperatures were 172, 270 and 270°C, respectively. Equal quantities (1 ml each) of the sample and methyl green formal saline solution were mixed and incubated for 24 h at room temperature for microscopic counting of protozoa numbers as described by Kamra et al. (1991).

The contents of the other three syringes from each treatment were transferred separately to spoutless beakers by repeated washing with 100 ml neutral detergent solution. The flask contents were refluxed for 1 h and filtered through pre-weighed Gooch crucibles (Grade G1). The dry matter content of the residue was weighed and *in vitro* true digestibility of feed was calculated as follows (van Soest and Robertson, 1988):

True digestibility (TD)

$$= \frac{\text{(Initial DM of feed taken-NDF residue)} \times 100}{\text{(Initial DM of feed taken)}}$$

The feed samples were analysed for total ash, crude protein, ether extract (AOAC, 1995) and for fiber fractions (van Soest et al., 1991). The data were analyzed by one-way ANOVA and differences between means were compared by Duncan's Multiple Range Test as per SPSS (1996).

Table 2. Effect of oil seed cakes on methane and gas production, *in vitro* true digestibility (IVTD) and ammonia nitrogen in an *in vitro* gas production test

Wheat straw+cake (3:1)	Total Gas (ml/g DM)	Methane (ml/g DM)	IVTD (%)	Ammonia-N (mg/dl)
Ground nut cake	133.90±3.45^{b}	27.34±1.65^{ab}	57.77±1.54^{ab}	23.35±0.73^{a}
Soybean cake	144.03±2.90^{a}	31.14±1.34^{a}	59.95±0.75^{a}	23.35±1.00^{a}
Mustard seed cake	138.30±2.52^{ab}	25.05±1.40^{bc}	56.18±0.92^{b}	21.98±0.71^{a}
Cotton seed cake	134.19±1.10^{b}	28.19±1.70^{ab}	56.60±0.73^{b}	21.56±1.00^{a}
Karanj seed cake (EE)	118.77±1.10^{c}	20.32±0.55^{d}	50.82±0.57^{c}	16.56±0.76^{b}
Karanj seed cake (SE)	122.72±3.05^{c}	21.21±1.01^{cd}	55.52±0.74^{b}	16.86±0.29^{b}
Caster bean cake (EE)	107.93±1.15^{d}	21.85±1.26^{cd}	49.06±1.53^{c}	21.83±0.50^{a}
Caster bean cake (SE)	115.90±2.37^{c}	22.43±1.16^{cd}	50.73±0.72^{c}	20.33±0.77^{a}

$^{a, b, c, d}$ Means with different superscripts in a column differ significantly ($p<0.05$).

Table 3. Effect of oil cakes on protozoa counts ($\times 10^{3}$/ml) in rumen liquor of buffalo in an *in vitro* gas production test

Wheat straw+cake (3:1 ratio)	Total holotrichs	Large spirotrichs	Small spirotrichs	Total spirotrichs	Total protozoa
Ground nut cake	0.91±0.47^{b}	2.35±0.39^{ab}	60.74±4.23	63.10±3.97	64.0±4.40
Soybean cake	1.82±0.26^{a}	1.04±0.13^{b}	50.16±3.28	51.21±3.17	54.08±4.07
Mustard seed cake	0.78±0.22^{b}	1.30±0.72^{b}	60.41±10.10	61.72±1.07	62.50±11.0
Cotton seed cake	0.78±0.22^{b}	1.69±0.91^{ab}	58.39±4.00	60.09±3.97	60.61±3.98
Karanj seed cake (EE)	0.78±0.22^{b}	1.04±0.47^{b}	53.76±1.47	54.80±1.82	55.58±2.05
Karanj seed cake (SE)	0.78±0.22^{b}	3.65±0.91^{a}	56.56±3.62	60.22±3.67	61.00±3.82
Caster bean cake (EE)	1.17±0.22^{b}	2.61±0.85^{ab}	54.86±6.10	57.48±6.95	58.65±6.98
Caster bean cake (SE)	1.17±0.59^{b}	3.26±0.47^{ab}	58.52±4.10	61.79±3.63	62.96±4.11

$^{a, b}$ Means with different superscripts in a column differ significantly ($p<0.05$).

RESULTS AND DISCUSSION

The chemical composition of wheat straw, oil seed cakes and their mixtures in the ratio of 3:1 is presented in Table 1. Groundnut cake and soybean cake had the highest crude protein while karanj seed cake and caster bean cake had the lowest. Ether extract was around 1% in all solvent extracted cakes, but expeller extracted cakes had significantly higher ether extract (6.88% and 5.40% in karanj seed cake and caster bean cake). In the present experiment a mixture of wheat straw and oil seed cake in a 3:1 ratio was used as substrate, which compared well with the conventional animal feed given to ruminants under field conditions. The feed mixtures had a crude protein content between 10-11%, except for the feed mixtures based on GNC and SBC, where the crude protein was 13.26% and 13.22%, respectively. As expected, the feed mixtures containing expeller extracted oil seed cakes had a higher either extract.

Soybean cake based feed produced the highest (133.90 ml/g DM) and caster bean cake (EE) the lowest (107.93 ml/g DM) gas during 24 h incubation (Table 2). Higher gas production in SBC might be due to higher cell content of the ration. There was a significant ($p<0.05$) negative correlation (-0.747*) between gas production and neutral detergent fiber (NDF) content of the feed mixture. Caster bean cake based feed supported lower gas production, indicating lower microbial activity which might be due to the more fibrous nature of the feed as indicated by higher NDF and ADF contents in this feed mixture. Maximum methane synthesis was observed with soybean cake based feed followed by cotton seed cake, groundnut cake, mustard seed cake based feeds. The caster bean cake and karanj seed cake based feeds caused significantly lower ($p<0.05$) methane synthesis as compared to the other feeds tested in this experiment. High content of readily fermentable substrates in soybean cake appears to be the possible reason for higher methanogenesis. Lee et al. (2003) also reported higher methane production in oil cakes having more fermentable substrates.

In vitro true digestibility (%IVTD) was the highest with soybean cake (59.95) and statistically similar to groundnut cake based feeds. The lowest IVTD was reported with CBCSE (50.73), which was similar to that of CBCEE and KCEE based feeds. The difference in IVTD of different feed mixtures might be due to variation in ADF content of the feeds because ADF was found to have a significantly negative correlation (-0.721*) with IVTD. The ammonia nitrogen (mg/dl) of different feed mixtures was proportional to the crude protein in the ration (correlation coefficient = 0.798*). Ammonia nitrogen was the highest in soybean cake and groundnut cake and the lowest in karanj seed cake based feeds.

Total and differential protozoal counts in the medium with different oil cake based feeds are presented in Table 3. When soybean cake based feed was used as substrate there was a significantly ($p<0.05$) higher number of holotrich protozoa in comparison to the other groups. This might be

Table 4. Effect of oil cakes on production of volatile fatty acids in an *in vitro* gas production test

Wheat straw+cake (3:1 ratio)	TVFA (mM/100 ml)	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)	Ac:Pr ratio
Ground nut cake	3.83±0.14[ab]	70.30±0.65	21.79±0.33[ab]	7.89±0.36	3.22±0.07[ab]
Soybean cake	4.43±0.18[a]	71.36±0.53	21.20±0.42[b]	7.43±0.11	3.36±0.09[a]
Mustard cake	4.33±0.02[ab]	70.41±0.47	21.71±0.46[ab]	7.87±0.23	3.24±0.08[ab]
Cotton seed cake	3.73±0.25[b]	68.14±0.86	23.48±0.83[ab]	8.37±0.18	2.91±0.14[ab]
Karanj cake (EE)	3.82±0.07[ab]	68.13±1.08	23.86±0.55[a]	7.99±0.55	2.85±0.10[b]
Karanj cake (SE)	4.01±0.12[ab]	69.48±1.03	21.93±0.56[ab]	8.57±0.47	3.17±0.13[ab]
Caster bean cake (EE)	3.97±0.04[ab]	71.38±0.23	21.38±0.14[ab]	7.23±0.10	3.33±0.03[ab]
Caster bean cake (SE)	4.00±0.16[ab]	70.42±0.69	22.31±0.67[ab]	7.27±0.03	3.16±0.12[ab]

[a, b] Means with different superscripts in a column differ significantly ($p<0.05$).

due to high cell content of the feed, since holotrichs are soluble sugar utilizing protozoa. Total protozoa and spirotrichs were similar ($p>0.05$) in feeds containing different oil cakes. The feed with expeller extracted cakes showed less numbers of protozoa in comparison to solvent extracted oil cakes which might be due to the toxic effect of polyunsaturated fatty acids on ciliate protozoa (Dohme et al., 2001).

The production of total and individual volatile fatty acids in different feeds is presented in Table 4. The TVFA level was the highest in SBC containing feed and the lowest with cotton seed cake containing feed. In other feeds it varied between these two extremes. There were no differences in acetate and butyrate levels between the different feeds tested. The per cent of propionate was the highest ($p<0.05$) with karanj seed cake (EE) and was the lowest ($p<0.05$) with SBC, resulting in a significantly lower acetate : propionate ratio in karanj seed cake (EE) and a significantly higher ratio in SBC based feed. The changes in propionate level corresponded with the level of methane production indicating that the reduction in methanogenesis was due to a shift in relative concentration of different VFA fractions. Similar changes in VFA fractions accompanied by inhibition of *in vitro* methanogenesis using extracts of saponin rich plants have been reported by Patra et al. (2005, 2006) and Agrawal et al. (2006). The extraction procedure of cakes did not affect rumen fermentation pattern, as the expeller extracted cakes, which are rich in fat content compared to solvent extracted cakes, did not have any impact on any of the parameters studied.

The results of the present experiment indicated that among the conventional oil cakes used for livestock feeding (GNC, SBC, MSC and CSC), mustard seed cake based feed produced the minimum methane without affecting other fermentation characteristics adversely. The other two cakes tested (karanj seed cake and caster bean cake) in this experiment caused a still lower methanogenesis (about 33% lower than that with soybean cake based feed), but it was accompanied by an adverse effect on *in vitro* true digestibility of feed which might be due to the presence of anti-nutritional factors in these two cakes. Therefore, further studies are required on these two cakes to explore their effects on animal physiology and on the quality of the livestock products resulting from prolonged feeding of animals.

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