Full Length Articles

Methane production potential of feed ingredients estimated by *in vitro* gas production test

M.Ramachandran, A. Bharathidhasan and V.Balakrishnan
Tamil Nadu Veterinary and Animal Sciences University
Department of Animal Nutrition, Madras Veterinary College,
Vepery, Chennai – 600 007, India.

ABSTRACT

This study was conducted to investigate methane production potential of feed ingredients to develop a database on methane production. Feed ingredients such as cereal grains, cereal by-products and protein supplements were tested for methane production potential using *in vitro* gas production technique. *In vitro* true digestibility (IVTD) of cereal grains ranged from 60.1 to 96.7% and oats grain (76.2%) and distiller's grain (60.1%) had lower (P<0.05) values than other cereal grains. Among the cereal by-products, wheat bran showed highest (P<0.05) IVTD (74.9%) than rice bran (42.7%). IVTD of cottonseed oil cake, black gram and sunflower oil cake was lower (P<0.05) than other protein supplements. Methane production potential of cereal grains at half life (t1/2) ranged from 0.66 to 2.85 ml/100 mg truly digested substrate and the difference was significant (P<0.05), however, maize grain, sorghum grain, bajra and broken rice did not vary among themselves. Average methane production potential of cereal by-products at half life (t1/2) and 24 hrs was 1.27 and 1.81 ml/100 mg truly digested substrate, respectively. Average methane production potential of protein supplements at half life (t1/2) and 24 hrs was 1.39 and 1.75ml/100 mg of truly digested substrate, respectively and the difference was statistically significant (P<0.05). Maximum (P<0.05) methane production potential at half life (t1/2) was recorded for black gram (4.07 ml/100 mg truly digested substrate). Lowest methane production potential both at half life (t1/2) and 24 hrs were recorded in fish meal and spirulina. It can be concluded that among cereal grains, methane production potential was higher (P<0.05) in oats grain at half life (t1/2) and all the cereal grains had similar methane production potential at 24 hrs. Among cereal by-products, wheat bran had higher (P<0.05) methane production potential both at half life (t1/2) and 24 hrs. Among protein supplements, black gram had significantly (P<0.05) higher methane production potential at half life (t1/2) and horse gram had significantly (P<0.05) higher methane production potential at 24 hrs.

Key Words: Methane, database*, in vitro* true digestibility

*Corresponding author: Professor and Head, Livestock Farm Complex, Veterinary College and Research Institute, TANUVAS, Orathanadu, Thanjavur – 614 625, India

INTRODUCTION

Methane is second major gas after carbon dioxide responsible for the warming of environment and ozone layer depletion. It is a potent green house gas as it has 23 times higher global warming potential than carbon dioxide (IPCC, 1996). Estimates of global methane production ranged between 350-820 Tg/year (Khan *et al*., 2001). Ruminants contribute about 30% of the world total methane production. Global warming and ozone layer depletion due to increased emission of green house gases in the atmosphere have drawn worldwide attention with an alarming stage of iceberg melting, increased ocean level, local and global eco-system upsets, changes in the rainfall patterns, changes in pathogenesis of plants, animals and human beings and alteration in life of the people (Kumar *et al.,* 2008). Several reports of the United Nations inter-governmental panels on climate changes indicated the urgency of the problem. IPCC (2001) has warned that by the mid of this century the globe's temperature will rise just like anything up to 5.8^{o}C.

Livestock are one amongst the largest single source of methane emission with 80–115 million tonnes per year, equivalent to 15–20% of total anthropogenic methane (IPCC, 2001). Ruminal microorganisms are responsible for the emission of methane from livestock (cattle, buffalo, sheep, goats, camel, *etc.*). The global cattle population is responsible for 73% of methane emissions of all livestock (McCrabb and Hunter, 1999). Tropical grasses are of low to moderate digestibility (on average 13% lower dry matter (DM) digestibility than temperate grasses) and are often deficient in critical nutrients such as protein and phosphorus. Under such conditions, methane produced during ruminal fermentation represents a loss of 10–11% of gross energy intake. The enteric methane contributes approximately 30–40 per cent of total methane produced from agricultural sources (Moss *et al*., 2000). Methane from enteric fermentation by ruminants is not only an important greenhouse gas associated with environmental problems, but it also represents a loss of feed energy (20–150 kJ/MJ) intakes (Singh *et al*., 2005). Therefore, developing feeding strategies to minimize methane emission is desirable in long-term mitigation of emission of greenhouse gases into the atmosphere and for short-term economic benefits.

This study was conducted to investigate *in vitro* methane production potential of different feed ingredients to develop a database on methane production to estimate the methane emission from ruminant livestock precisely and to develop methane mitigation strategies to reduce global warming and enhance the efficiency of nutrient utilization.

MATERIALS AND METHODS

Collection, processing and chemical analysis of feed ingredients

Feed ingredients such as cereal grains, cereal by-products and protein supplements were collected from Tamil Nadu and these samples were dried in a hot air oven at about 50-60^{0}C and ground using 1 mm sieve. Total ash (TA) and ether extract (EE) content were estimated as per the procedure of AOAC, (1995). Organic matter (OM)

was calculated based on the total ash (TA) content. Neutral detergent fibre (NDF) content was analyzed as per the procedure of Goering and Van Soest, (1970). The OM, EE and NDF content of different feed ingredients is given in Table 1.

Table 1: Organic matter (OM), Ether extract (EE) and Neutral detergent fibre (NDF) content of feed ingredients (% Dry matter basis) (Mean ±SE)

Feed ingredient	OM (%)	EE (%)	NDF (%)
Cereal grains			
Maize	98.2±0.15	5.06±0.35	14.5±1.35
Sorghum	98.2±0.13	3.75±0.41	14.4±0.71
Ragi	97.0±0.10	1.10±0.06	17.0±0.99
Bajra	96.5±0.12	4.16±1.50	11.1±1.89
Oats	96.7±0.05	3.55±0.13	30.8±0.75
Broken rice	94.8±0.65	1.43±0.01	42.3±1.44
Distiller's grain	94.6±0.03	1.90±0.02	49.8±0.51
Cereal by-products			
Rice bran	85.0± 1.51	4.22±0.81	68.7±3.81
Wheat bran	91.5±0.89	2.67±0.65	45.3±2.35
Protein supplements			
Groundnut oil cake	92.4±1.05	6.72±0.46	17.6±3.25
Coconut oil cake	93.6±0.35	12.6±0.80	35.5±1.89
Soybean meal	92.6±0.74	1.40±0.25	20.5±2.67
Cottonseed oil cake	95.2±0.20	9.91±0.31	45.9±3.32
Sunflower oil cake	91.0±0.37	0.96±0.04	50.4±2.34
Gingely oil cake	93.1±0.22	1.22±0.08	14.2±1.10
Linseed	97.7±0.01	43.5±0.07	22.6±0.94
Horse gram	95.9±0.01	0.94±0.01	53.8±0.50
Fish meal	58.9±0.01	6.08±0.14	16.5±0.40
Green gram	94.7±0.01	1.32±0.04	31.0±2.77
Black gram	95.6±0.09	1.68±0.03	46.2±1.22
Spirulina	92.8±0.06	1.13±0.04	1.68±0.06

In vitro gas production

Collection of rumen inoculum

Rumen content was collected from male calves fed on paddy straw based rations using stomach tube and strained through 4 layers of muslin cloth. The strained rumen liquor (SRL) was transported to the laboratory in a cud transport container having the facility for CO_2 flushing and temperature maintenance.

In vitro gas production test

Five media solutions were prepared individually and were mixed later as specified by Menke and Steingass, (1988). Total volume of buffer required was calculated based on the number of samples incubated. The required quantity of water, micro, macro, buffer and resazurin were mixed in a flat bottom flask and kept in the incubator at about 39°C.

Ground samples (1mm) of about 200 mg were weighed and transferred carefully in to the 100 ml calibrated glass syringes. After weighing all the samples, vaseline was applied to the piston and inserted in to the syringes. The nozzles of the syringes were closed with rubber cork. The syringes were kept in an incubator at 39°C a day before the incubation.

The required volume of strained rumen liquor was measured and added to the medium in the flask. Carbon dioxide was flushed through the medium. Exactly 30 ml of rumen inoculum was dispensed into the syringes through silicone tube fitted to the nozzle. After removing the silicone tube the nozzle was closed using a rubber cork after removing the gas bubbles. Then the syringes were incubated in a water bath maintained at 39°C in triplicates. The gas production was measured at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 hours interval and corrected with blank. The gas production at different intervals was analysed using Graph Pad Prism (version 5.0) to estimate half life (t1/2).

Estimation of *in vitro* true digestibility (IVTD)

Ground samples (1mm) of about 500 mg were weighed and transferred carefully in to the 100 ml calibrated glass syringes. Media solution for the estimation of *in vitro* true digestibility was prepared as per the procedure of Makkar *et al*. (1995). Exactly 40 ml of rumen inoculum with double strength buffer was dispensed into the syringes through silicone tube fitted to the nozzle. After removing the silicone tube the nozzle was closed using a rubber cork after removing the gas bubbles. Then the syringes were incubated in a water bath maintained at 39°C in triplicates. The experiments for the estimation of *in vitro* true digestibility and methane emission were carried out simultaneously.

After recording total gas production, *in vitro* true digestibility was estimated at 24 hours of incubation. After the end of incubation, the contents were carefully transferred into spoutless beaker by repeated washing with neutral detergent solution (NDS). The contents were refluxed for one hour using Fibertec equipment and filtered through pre-weighed Gooch crucible (Grade I). The residues were dried in the hot air oven and weighed.

$$\text{True digestibility (\%)} = \frac{\text{DM of feed taken for incubation - NDF residue}}{\text{DM of feed taken for incubation}} \times 100$$

Estimation of methane emission

Ground samples (1mm) of about 200 mg were weighed and transferred carefully in to the 100 ml calibrated glass syringes. Exactly 30 ml of rumen inoculum was dispensed into the syringes through silicone tube and the nozzle was closed using a rubber cork after removing the gas bubbles. Then the syringes were incubated in a water bath maintained at 39°C in triplicates and the experiment was repeated on 3 different days. Total gas production was recorded both at half life and 24 hours for feedstuffs with less than 16 hours half life. For other feed ingredients gas samples were collected at half life after recording the total gas production. Gas samples were collected in vacuum container to estimate the concentration of methane using Gas Chromatography.

Estimation of methane concentration using Gas Chromatograph

Methane concentration in different gas samples collected during the *in vitro* studies was estimated using Gas Chromatograph (Claurus 500, Perkin Elmer) fitted with Flame Ionization Detector (FID) and capillary column (30 meter length and 250 micrometer dia) using a calibration gas consisting of 22.53% methane, 1.05% hydrogen, 33.30% carbon dioxide and 43.12% nitrogen. Helium was used as carrier gas with oven temperature at 60° C, injector temperature at 100°C and detector temperature at 200°C.

Based on the true digestibility, methane production potential per 100 mg truly digested substrate was calculated for all the feed ingredients.

Statistical analysis

All the *in vitro* experiments adopted a completely randomized design (CRD). The methane production potential was statistically analyzed using one way analysis of variance (One Way - ANOVA) to compare the means as per the procedure of statistical analysis system (SAS/ SPSS version 15.0 for windows). When significant difference was detected the multiple range test was used to separate the mean value.

RESULTS AND DISCUSSION

The results of *in vitro* true digestibility (IVTD), half life and methane production potential of feed ingredients is given in Table 2. Results revealed that IVTD of cereal grains ranged from 60.1 to 96.7% and oats grain (76.2%) and distiller's grain (60.1%) had lower ($P<0.05$) values than other cereal grains. Lower ($P<0.05$) IVTD in oats grain and distiller's grain might be due to the higher content of structural carbohydrate (NDF) (Table 1) which is not easily available for microbial digestion. High digestibility of maize, sorghum, ragi, bajra and rice is attributed to high content of easily digestible carbohydrate. Similar IVTD for maize and sorghum grains were also reported by Hervas *et al.,* (2004). Cereal by-products comparatively had lower ($P<0.05$) IVTD than cereal grains

because of higher cell wall carbohydrate (NDF) (Table 1).

Results of IVTD of protein supplements indicated that significant difference (P<0.05) was found among various protein supplements. Groundnut oil cake, soybean meal, gingely oil cake, linseed, fishmeal, horse gram and spirulina had similar IVTD values. *In vitro* true digestibility of cottonseed oil cake, black gram and sunflower oil cake was lower (P<0.05) than other protein supplements which may be attributed to high cell wall content (Table 2).

Table 2: *In vitro* true digestibility (%), half life (hr) and methane production potential (ml) of feed ingredients

Name of the feed ingredient	*In vitro* true digestibility (IVTD) (%)	Half life ($t_{1/2}$) (hr)	Methane production potential (ml/100 mg truly digested substrate)	
			Half life ($t_{1/2}$)	24 hrs
Cereal grains				
Maize	89.4 ± 0.30^{cd}	12.2	0.95 ± 0.13^{ab}	1.75 ± 0.33^{a}
Sorghum	96.7 ± 0.54^{d}	9.47	0.82 ± 0.14^{a}	2.31 ± 0.72^{a}
Ragi	87.1 ± 0.39^{c}	15.6	0.66 ± 0.14^{a}	1.59 ± 0.10^{a}
Bajra	87.5 ± 0.50^{cd}	13.4	1.62 ± 0.12^{abc}	3.71 ± 0.24^{a}
Oats	76.2 ± 1.02^{b}	14.9	2.85 ± 0.66^{c}	3.77 ± 0.76^{a}
Broken rice	88.2 ± 0.27^{cd}	12.6	1.72 ± 0.13^{abc}	2.10 ± 0.40^{a}
Distiller's grain	60.1 ± 1.75^{a}	14.4	2.41 ± 0.19^{bc}	3.78 ± 0.47^{a}
Average	**83.6**	**13.2**	**1.58**	**2.72**
Cereal by-products				
Rice bran	42.7 ± 0.63^{a}	10.4	0.55 ± 0.13^{a}	0.91 ± 0.33^{a}
Wheat bran	74.9 ± 0.93^{b}	12.4	1.98 ± 0.32^{b}	2.71 ± 0.46^{b}
Average	**58.8**	**11.4**	**1.27**	**1.81**
Protein supplements				
Groundnut oil cake	93.8 ± 0.58^{ef}	5.81	1.05 ± 0.05^{abc}	2.00 ± 0.08^{bcd}
Coconut oil cake	84.0 ± 0.99^{d}	7.52	1.38 ± 0.18^{abc}	2.47 ± 0.17^{cd}
Soybean meal	95.0 ± 0.05^{f}	6.51	0.96 ± 0.09^{abc}	1.70 ± 0.09^{abc}
Cottonseed oil cake	45.9 ± 0.82^{a}	24.0	0.75 ± 0.13^{abc}	-
Sunflower oil cake	70.4 ± 1.14^{c}	10.6	0.72 ± 0.21^{abc}	1.28 ± 0.23^{abc}
Gingely oil cake	95.5 ± 0.25^{f}	6.99	1.18 ± 0.17^{abc}	2.01 ± 0.20^{bcd}
Linseed	87.4 ± 0.42^{de}	17.6	1.35 ± 0.06^{abc}	-
Horse gram	90.4 ± 0.21^{def}	14.2	1.66 ± 0.57^{bcd}	3.13 ± 0.64^{e}
Fish meal	94.6 ± 0.66^{f}	11.3	0.40 ± 0.27^{a}	0.62 ± 0.33^{a}
Green gram	85.2 ± 3.18^{d}	19.0	2.54 ± 0.18^{d}	-
Black gram	54.0 ± 3.29^{b}	33.2	4.07 ± 0.07^{e}	-
Spirulina	96.2 ± 0.37^{f}	9.38	0.59 ± 0.07^{ab}	0.77 ± 0.10^{ab}
Average	**82.7**	**13.8**	**1.39**	**1.75**

^{abcde} Means with different superscripts in a column with respect to cereal grains/cereal by-products/ protein supplements differ significantly (P<0.05).

Methane production potential of cereal grains at half life (t1/2) ranged from 0.66 to 2.85 ml/100 mg truly digested substrate and the difference was significant (P<0.05). However, maize grain, sorghum grain, bajra and broken rice did not vary among themselves. Oats grain produced maximum methane at half life (t1/2) (2.85 ml/100 mg truly digested substrate) compared to all other cereal grains which may be due to high NDF (30.8%) and low digestibility (76.2%). Lowest methane was produced by ragi grain at 24 hrs (1.59 ml/100 mg truly digested substrate) and highest methane was produced by bajra grain, oats grain and distiller's grain at 24 hrs (3.71, 3.77 and 3.78 ml/100 mg truly digested substrate respectively), however, there was no significant difference found among the cereal grains. Average methane production potential of cereal grains both at half life (t1/2) and 24 hrs was 1.58 and 2.72 ml/100 mg truly digested substrate, respectively.

High methane production of cereal grains compared to cereal by-products and protein supplements might be attributed to high contents of easily fermentable starch, sugars or hemicellulose as substrate to rumen microbes for fermentation. Cereal grains contain high amount of NFE which is readily fermented by ruminal microbes and provide the large amount of substrates to microbes for methane production. Besides the high amount of easily fermentable substrates, Bonhomme, (1990) reported that grains rich in soluble carbohydrates increase the population of cilliate protozoa and stimulate their hydrogen transfer to methanogens resulting in high methane production. Lee *et al*. (2003) reported that the methane production potential of corn at 6 and 24 hrs was 4.03 and 10.33 ml/0.2g DM, respectively. Methane production potential of oat grain at 6 and 24 hrs was 4.34 and 6.87 ml/0.2 g DM, respectively (Lee *et al*., 2003).

Methane production potential both at half life (t1/2) and 24 hrs were maximum (P<0.05) in wheat bran (1.98 and 2.71 ml/100 mg truly digested substrate, respectively). Similarly, Lee *et al*. (2003) reported that methane production potential of rice bran was lower than wheat bran. Average methane production potential of cereal by-products at half life (t1/2) and 24 hrs was 1.27 and 1.81 ml/100 mg truly digested substrate, respectively. Rice bran contains high concentration of unsaturated fatty acid. Czerkawski *et al*. (1966) reported that unsaturated fatty acids are hydrogenated by rumen microbes resulting in low pressure of hydrogen which is pre-requisite for reduction in methane production. In addition, fat, itself, is considered to inhibit methane production by stimulating propionate production and inhibiting the protozoa activity as well as inhibitory effects on cellulolytic bacteria and feed digestion in rumen.

Average methane production potential of protein supplements at half life (t1/2) and 24 hrs were 1.39 and 1.75 ml/100 mg truly digested substrate, respectively and the difference was significant (P<0.05). Maximum (P<0.05) methane production

potential at half life (t1/2) was recorded in black gram (4.07 ml/100 mg truly digested substrate). Lowest methane production potential both at half life (t1/2) and 24 hrs were recorded in fish meal and spirulina. Lower methane production potential of protein supplements compared to cereal grains might be due to high crude protein generally more than 20% and low amount of fibre. Protein is degraded to ammonia in rumen and it combines to carbon dioxide resulting in production of ammonium carbonate (Getachew *et al*., 1998) resulting in its lower methane production.

It can be concluded that among cereal grains, methane production potential was higher ($P<0.05$) in oats grain at half life (t1/2) and all the cereal grains had similar methane production potential at 24 hrs. Among cereal by-products, wheat bran had higher ($P<0.05$) methane production potential both at half life (t1/2) and 24 hrs. Among protein supplements, black gram had significantly ($P<0.05$) higher methane production potential at half life (t1/2) and horse gram had significantly ($P<0.05$) higher methane production potential at 24 hrs.

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