Journal of Agricultural Science (2017), **155**, 171–183. © Cambridge University Press 2016
doi:10.1017/S0021859616000642

ANIMAL RESEARCH PAPER

Nutrient composition, rate of fermentation and *in vitro* rumen methane output from tropical feedstuffs

R. BHATTA*, M. SARAVANAN, L. BARUAH, P. K. MALIK AND K. T. SAMPATH

Energy Metabolism Laboratory, ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, India

(*Received 30 March 2016; revised 6 June 2016; accepted 27 July 2016; first published online 7 September 2016*)

SUMMARY

In vitro rumen methane output (IRMO) of over 200 feed/feed mix samples representing approximately 74 feed types was investigated in a series of completely randomized experiments. The samples comprised dry fodder, grass, tree leaves, cultivated grasses, cereal by-products, cereal grains, oilseed/meals, compound feeds and total mixed rations (TMRs) from the tropical regions. These samples were subjected to three *in vitro* gas production tests at 39 °C in 100 ml Heberle syringes. The first incubation was conducted with 200 mg dry matter (DM) substrate for 96 h to determine half-time gas production ($t^{1/2}$, h) value of each sample. The second and third incubations were carried out simultaneously. The second incubation was done with 200 mg DM substrate until $t^{1/2}$ time to determine IRMO and third with 500 mg DM to estimate *in vitro* dry matter digestibility (IVDMD) of each samples, respectively. The IRMO was expressed as ml/100 mg digestible substrate. Crude protein content (g/kg DM) was lowest in dry fodder samples and highest in oilseed meals, whereas it was similar in local grass and tree leaves. The IVDMD values ranged from 0·48 to 0·87; the lowest digestibility was recorded in tree leaves. The potential gas production (PGP, ml/200 mg DM) ranged from 9·76 to 61·3. The PGP from grasses and compound feeds was similar, whereas it was lowest in tree leaves. The rate constant (mg/h) was maximum in compound feed followed by oilseed meal. The rate constant was similar among other group of feedstuffs. The $t^{1/2}$ time ranged from 9·8 to 19·4 h. The highest $t^{1/2}$ time was recorded in local grass samples followed by dry fodder and cultivated grasses. However, they were similar among tree leaves, cereal grains, by-products and compound feeds. The methane % in the total gas varied from 9·79 (tree leaves) to 20·2 (local grasses). Among straw, IRMO varied from 3·88 (*Zea mays* fodder) to 12·0 (*Sorghum vulgare*) and it was lower in fruit tree leaves than cultivated grasses. Among protein and energy sources, IRMO was higher in cereal by-products as compared with cereal grains, oil meals and compound feed. The IRMO was similar among TMR, irrespective of the composition of the concentrate mixture. Nevertheless, it varied with the amount of concentrate in the TMR. This is the first exhaustive data on IRMO from the tropical region. Because of the substantial amount of dietary gross energy lost in methane, knowledge of the methane output from these feed ingredients will help in formulating low methane emitting diets for ruminants. Incorporation of tropical tree leaves in the diets and feeding TMR are potential strategies to reduce enteric methane emission in ruminants.

INTRODUCTION

Methane is an important greenhouse gas (GHG) produced from enteric fermentation of feed/fodder by ruminant animals. The productivity of livestock in the tropical and sub-tropical areas of developing countries is limited by lower nutritional conditions that are characterized by highly lignified, low digestible feed from poor quality, nitrogen (N)-limited native grass pastures and crop residues, or may also suffer from a general lack of feed during drought (Goel & Makkar 2012). This sub-standard productivity results in high absolute methane emissions resulting in a very high cost of methane emissions per unit of product (Aluwong *et al.* 2011). This is particularly true when straw-based forages are the main ingredient in ruminants' diets (Bhatta *et al.* 2008, 2009).

* To whom all correspondence should be addressed. Email: ragha0209@yahoo.com

During anaerobic digestion, ruminal microbes usually convert major portions of the carbohydrate (CHO) and protein in feeds to useful end-products such as volatile fatty acids and microbial protein, as well as waste products; mainly methane and carbon dioxide (CO_2). The pattern and concentration of these end products depends mainly on the chemical components of the diet (i.e. CHO and protein fractions), their digestibility and intake. Fermentation of plant materials containing low amounts of cell walls results in lower methane production (Johnson & Johnson 1995), as well as a decrease in the molar proportion of acetate and an increase in the molar proportion of propionate (Widiawati & Thalib 2007). Fermentation of diets containing high amounts of plant cell walls is likely to produce a higher molar proportion of acetate than propionate (Bhatta *et al.* 2008). Methane from enteric fermentation represents a loss of dietary energy in ruminants up to 12% of gross energy intake (McCrabb & Hunter 1999), and depends primarily on the quantity and quality of the diet as it affects rate of ruminal digestion and passage (Van Soest 1994; Beauchemin *et al.* 2008). Decreased forage digestibility is generally accompanied by decreased forage intake and increased ruminal acetate: propionate ratio, which favours increased methane production per unit forage consumed (McAllister *et al.* 1996). Tamminga (1992) reported a decrease in methane losses [as a proportion of digestible energy (DE)] with increasing N content in fresh grass and this decrease was hypothesized to be linked to its lower fibre content. Protein degradation *in vitro* has been shown to be associated with lower methane production than fermentation of CHOs (Cone & Van Gelder 1999; Jentsch *et al.* 2007), although increasing dietary N concentrations might also stimulate ruminal methanogenesis (Kurihara *et al.* 1999). Enteric methane production could be influenced by the nature of CHOs fermented, such as cellulose, hemicelluloses and soluble residues of the diet (Takahashi 2001; Santoso *et al.* 2003). Moss (1994) reported that digestible acid detergent fibre (ADF), cellulose and hemicellulose are important fibre fractions influencing methane production in the rumen. The information on rumen methane output of feeds from tropical region is largely unknown. *In vitro* experiments could be used to obtain methane production data from diverse feeds/fodder for further use to estimate methane production from ruminants/livestock fed different feeds/fodder or diets. The objective of the present work was to develop a database on methane production for common feed ingredients and diet combinations fed to ruminants so that rations could be formulated with lowest methane emission.

MATERIALS AND METHODS

Samples

The experiment was conducted at the ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, India. The samples were collected from different parts of Karnataka state, India.

Collection and processing of samples

Samples comprised dry fodder (14 samples), grass (two), tree leaves (five), cultivated grasses (11), cereal by-products (three), cereal grains (five), oilseed meals (eight), compound feed (five) and total mixed ration (TMR, 21). The TMRs were prepared using locally available feedstuffs, mimicking the feeding practices followed in this region.

The dry fodder samples were collected after harvesting their grain. The samples from different regions were pooled by combining equal portions into a representative sample. The local grass and cultivated grass were sampled from three random sites using a 1 m^2 quadrat to create three field replicates during the pre-flowering stage. Leaf samples (leaves + fine stem < 6 mm diameter) were collected from three trees to get representative samples. Cereal by-products, cereal grains, oilseed meals and compound feed samples were collected from different stalls in local markets and likewise pooled by combining equal portions. The TMR was formulated in the laboratory by mixing the required ingredients. All the samples were oven-dried at 60 °C for 48 h and then ground to pass through a 1 mm sieve in a Wiley mill. Ground samples were stored for chemical and biochemical analysis.

Chemical analysis

The tree leaf samples were analysed in triplicate for crude protein (CP) (AOAC 1997), neutral detergent fibre (NDF) and ADF (Van Soest *et al.* 1991). The NDF was analysed in samples without sodium sulphite and amylase. Both NDF and ADF were expressed with residual ash. Other samples were analysed according to the standard methods of AOAC

(1995) for dry matter (DM; 976·63) and N (984·13). Lignin (sa) was determined by solubilization of cellulose with sulphuric acid in the ADF residue (Van Soest *et al.* 1991).

In vitro incubation

Initial incubations were performed to determine the time to achieve the half-time gas production ($t^{1/2}$ time) of the substrate. For this, rumen liquor was collected from two cannulated Holstein Friesian crossbred bulls fed a TMR (160 g/kg CP and 9·0 MJ/kg DM of metabolizable energy) containing finger millet (*Elusine coracana*) straw and commercial concentrate mixture in 1 : 1 ratio. The rumen liquor, strained through muslin cloth, was pooled and used as the source of inoculum. A total of 200 mg air-equilibrated sample was incubated with 30 ml of buffered rumen inoculum (Menke *et al.* 1979) in 100-ml calibrated syringes and placed in a water bath maintained at 39 °C. The incubations were conducted in triplicate for each sample on two successive days and these incubations were performed three times. Incubations without samples served as the blanks with every set. The difference in composition and activity of the rumen inoculum among incubations, if any, was controlled by parallel incubation of reference concentrate and hay standard from Hohenheim University, Germany as suggested by Menke *et al.* (1979). The gas volumes were recorded at 2, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96 h. This data were subjected to a graph pad prism program to determine their potential gas production (PGP, ml/200 mg DM), rate constant (k) and $t^{1/2}$ (h) time.

In vitro rumen methane output and *in vitro* dry matter digestibility

Two sets of samples were incubated simultaneously, each in triplicate. Samples in the first set were incubated with 200 mg substrate and 30 ml buffered rumen fluid, and the second with 500 mg substrate and 40 ml double-strength buffered rumen fluid, under identical conditions as described earlier. Each sample was incubated until its $t^{1/2}$ time as determined earlier and total gas volume was recorded and analysed for methane concentration, again as described earlier.

After terminating the incubation of the 500 mg samples by chilling the syringes in an ice bath, the syringe contents were transferred to a spoutless 600 ml beaker. The syringes were washed with neutral detergent (ND) solution (100 ml), boiled for 1 h, filtered, washed and dried to determine their DM digestibility.

Methane estimation

After terminating the incubation, the volume of fermentation gas produced was recorded from visual assessment of the calibrated scale on the syringe. Net gas production was calculated as the difference between the total gas produced and the gas produced in blank syringes (ml gas in sample syringe – ml gas in blank syringe). For methane estimation, 1·0 ml of gas was sampled with an airtight syringe (Hamilton Company, Reno, NV, USA) from the head space of the syringe (having one outlet) using a specialized adopter fitted to the silicon tubing and injected into a Thermo fisher gas chromatograph equipped with thermal conductivity detector and stainless steel column packed with Porapak-Q. The temperatures of injector oven, column oven and detector were 60, 100 and 110 °C, respectively (Kajikawa *et al.* 2007). Before analysis of unknown samples, the gas chromatograph was calibrated with standard known samples of methane and a standard curve was prepared with suitable regression equation. After injection of gas from each unknown sample, the area under the curve of peaks occurring at the same retention time of the methane standard was recorded and methane concentration was calculated from the standard curve by linear regression. Based on the methane percentage estimated in the gas produced, methane production in ml was calculated in each sample [methane volume (ml) = methane % × total gas produced (ml)]. The *in vitro* rumen methane output (IRMO) was expressed as methane in ml/100 mg digestible DM.

Statistical analysis

Analysis of variance for chemical analysis of nutrient content, fermentation pattern, *in vitro* dry matter digestibility (IVDMD) and IRMO was carried out by one-way analysis (SAS Institute 2002) using the model $Y_{ij} = \mu + Fi + E_{ij}$, where Y_{ij} represents the individual observations of the variable and F_i is the fixed effect of the ith feed ingredient/diet combination (i = 1–10). The overall mean is expressed as μ and E_{ij} is the random error associated with Y_{ij} not accounted in the fixed effect. Significant differences of feed ingredient/diet combination were considered at the $P < 0{\cdot}05$ level.

RESULTS

Composition

Crude protein content (g/kg DM) was least in dry fodder (70·1) and highest in oilseed meals (320), whereas it was similar in local grass and tree leaves (90·7). Cultivated grasses, cereal grains and their by-products contained 115 (g/kg DM) CP. The NDF and ADF contents were highest in dry fodder (711 and 459, respectively) and lowest in oil meals (458 and 213, respectively). Tree leaves contained higher (142) acid detergent lignin [ADL (sa)] than dry fodder (66·4) and local grasses (64·2). In TMR, CP and fibre fractions varied with R : C ratio (Table 1).

The IVDMD figures ranged from 0·48 to 0·87, with the lowest digestibility recorded in tree leaves (0·48). The digestibility of dry fodder was higher (0·508) than tree leaves (0·475) but lower than local grasses (0·557). The digestibilities of cereal by-products and compound feeds were similar (0·61), whereas those of oilseed meals (0·69) were lower than cereal grains (0·87). The nutrient composition of the TMR varied with the level of concentrate in the diet.

Fermentation kinetics

Potential gas production (ml/200 mg DM) ranged from 9·76 to 61·3. The PGP of grasses and compound feeds was similar (39·7), whereas it was least in tree leaves (29·8) (Table 2). The rate constant (mg/h) was maximum in compound feed (0·19) followed by oilseed meal (0·08). The rate constant was similar among the other groups of feedstuffs (0·05).

The $t^{1/2}$ time ranged from 9·8 to 19·4 h for local grass (Table 2). The $t^{1/2}$ time for dry fodder was 16·5 h and 14·0 h for cultivated grasses; values were similar among tree leaves, cereal grains, by-products and compound feeds at 10·5 h.

In vitro rumen methane output

Methane composition of the total gas varied from 9·79 (tree leaves) to 20·2% (local grasses). The IRMO was expressed as ml methane/100 mg truly digested substrate. Among the straws, IRMO varied from 3·88 (*Zea mays*) fodder to 12·0 (*Sorghum vulgare*) with a mean of 6·01. It was 4·67 among the grasses (Table 2). The IRMO was lower (1·34) in fruit tree leaves than cultivated grasses (2·83). Among protein and energy sources, IRMO was higher in cereal by-products (5·92) as compared with cereal grains (2·44), oil meals (2·47) and compound feed (1·12). The IRMO was similar (3·5) among TMR, irrespective of the composition of the concentrate mixture. However, it varied with the level of concentrate in the TMR.

DISCUSSION

The main objective of the current study was to assess the IRMO of a range of feeds with contrasting chemical characteristic and nutrient composition. Chemical composition of feeds and forages was influenced by factors such as crop type, variety, fertilizer, stage of harvest and environment. Based on their CP contents, dry fodder and local grasses cannot be fed to ruminants as sole diets without supplementation. Higher contents of lignin (sa) in legume straw than in the cereal forages and grasses were recorded because legumes synthesize more lignin for strength and rigidity of plant walls. Nutrient contents of most of the feedstuffs investigated in the present study were within the range of values reported earlier (Singh *et al*. 2002; Chaurasia *et al*. 2006; Bhatta *et al*. 2008). Jung & Allen (1995) described the plant cell characteristics affecting intake and digestibility of forages in ruminants. Higher digestibility of legume straw than cereal straw and stovers may be attributed to their lower NDF, ADF, cellulose and lignin contents. The higher DM digestibility of legume straw (by 10%) than cereal straw reported earlier by Bhatta *et al.* (2008) is in agreement with the present findings. Further, DM digestion of forages is highly dependent on structural factors such as the relative proportion of cell types present in the plant tissues and the existence of factors restricting microbial access to walls. The low IVDMD of cereal straw in the present study may be attributed to low microbial activity, due to inadequate protein supply to meet their requirements during incubation. The $t^{1/2}$ time of local grass was lower as compared with dry fodder due to higher lignification. Cereal by-products, cereal grains and oil cakes were degraded in similar time frames (similar $t^{1/2}$).

Methane concentration and IRMO differed significantly among feedstuffs. Such variation in *in vitro* methane was recorded mainly from straw and agricultural by-products. Variation in methane production from dry roughage may be attributed to significant

Table 1. *Composition* (*g/kg DM*) *and* in vitro *dry mater digestibility* (*IVDMD*) *of feed ingredients and total mixed ration* (*TMR*)

Common name	No. samples screened	Botanical name	Crude protein	Neutral detergent fibre	Acid detergent fibre	Acid detergent lignin	IVDMD
Dry fodder							
Ragi straw	(6)	*Oryza sativa*	57·6	765	505	43·2	0·410
Paddy straw	(7)	*Elusine coracana*	52·3	755	531	42·2	0·409
Jowar straw	(5)	*Sorghum vulgare*	45·4	722	370	65·5	0·478
Jowar Kadbi	(3)	*Sorghum vulgare*	52·4	802	704	92·3	0·443
Maize fodder	(3)	*Zea mays*	100	693	236	60·6	0·736
Sorghum fodder	(4)	*Sorghum vulgare*	52·4	782	506	46·2	0·519
Black gram fodder	(3)	*Vigna mungo*	152	576	393	37·4	0·427
Hybrid Jowar fodder	(3)	*Sorghum vulgare*	34·9	752	452	39·0	0·563
Sorghum powder	(3)	*Sorghum vulgare*	122	447	105	51·0	0·476
Gram straw	(2)	*Cicer arietinum*	119	659	538	135	0·485
Groundnut straw	(2)	*Arachis hypogea*	99·8	659	505	115	0·674
Bajra straw	(3)	*Pennisetum typhhoids*	22·8	753	506	75·4	0·470
Barley straw	(2)	*Hordeum vulgare*	20·6	796	519	62·2	0·505
Wheat straw	(3)	*Triticum aestivum*	48·0	798	548	63·7	0·516
		Mean	70·1	711	459	66·4	0·508
		S.D.	41·0	100	146	29·6	0·942
		S.E.M.	11·0	26·8	39·1	7·92	0·252
Local grasses							
Local grass	(8)	–	111	553	395	75·7	0·590
Forest grass	(3)	–	61·1	736	437	52·6	0·522
		Mean	86·5	645	416	64·2	0·557
		S.D.	35·8	129	295	16·3	0·048
		S.E.M.	25·4	91·8	20·9	11·6	0·034
Tree leaves							
Amla	(2)	*Phyllanthus emblica*	103	412	322	137	0·231
Mango	(3)	*Mangifera indica*	73·2	599	478	166	0·581
Tamarind	(3)	*Tamarindus indica*	121	426	287·1	146	0·588
Custard apple	(3)	*Annona squamosa*	50·7	443	335·9	144	0·389
Guava	(2)	*Psidium guajava*	105	522	321·9	113	0·588
		Mean	90·7	481	349	142	0·475
		S.D.	28·3	78·8	74·3	18·9	0·161
		S.E.M.	12·6	35·3	33·3	8·43	0·072
Cultivated grasses							
Para grass	(3)	*Brachiaria mutica*	139	628	375	51·3	0·670
Hybrid Napier	(3)	*Pennisetum purpureum*	144	746	441	56·6	0·711
Cowpea leaf	(2)	*Vigna sinesis*	97·9	458	355	39·9	0·882
Black gram leaf	(2)	*Vigna mungo*	151	432	311	54·5	0·856
Green gram leaf	(3)	*Phaseolus aureus*	169	435	231	54·9	0·421
Lucerne	(4)	*Madicago sativa*	172	616	490	34·7	0·585
Gram leaf	(2)	*Cicer arietinum*	171	659	538	55·6	0·750
Bajra leaf	(3)	*Pennisetum typhoids*	107	665	536	49·4	0·741
Jowar leaf	(2)	*Sorghum* spp.	67·9	704	360	36·2	0·720
Maize green	(3)	*Zea mays*	96·8	585	381	41·0	0·644
Sugarcane leaf	(2)	*Saccharum officinarum*	20·6	750	471	70·8	0·425
		Mean	122	608	408	49·5	0·673
		S.D.	48·5	118	96·2	10·8	0·150
		S.E.M.	14·6	35·5	29·0	3·30	0·045

https://doi.org/10.1017/S0021859616000642 Published online by Cambridge University Press

Table 1. (*Cont.*)

Common name	No. samples screened	Botanical name	Crude protein	Neutral detergent fibre	Acid detergent fibre	Acid detergent lignin	IVDMD
Cereal by-products							
Wheat bran	(8)	*Triticum aestivum*	143	654	150	27·3	0·643
Rice bran	(9)	*Oryza sativa*	99·1	693	192	48·8	0·498
Rice polish	(4)	*Oryza sativa*	78·6	601	183	24·2	0·703
		Mean	107	649	175	33·4	0·645
		S.D.	33·1	46·3	22·3	13·4	0·105
		S.E.M.	19·1	26·8	12·9	7·74	0·061
Cereal grains							
Maize grain	(4)	*Zea mays*	111	520	47·6	13·4	0·787
Ragi grain	(5)	*Elusine coracana*	175	451	232	20·9	0·921
Bajra grain	(2)	*Pennisetum typhoids*	99·0	452	301	31·0	0·976
Jowar grain	(3)	*Sorghum bicolour*	78·0	472	105	21·0	0·771
Barley grain	(3)	*Hordeum vulgare*	115	452	92·0	24·0	0·911
		Mean	115	469	156	22·1	0·873
		S.D.	36·1	29·7	106	6·30	0·090
		S.E.M.	16·1	13·3	47·6	2·84	0·040
Oilseed meal							
Groundnut cake	(6)	*Arachis hypogaea*	408	448	117	33·0	0·699
Cottonseed cake	(2)	*Gossypium* spp.	250	521	357	45·0	0·557
Sunflower cake	(4)	*Helianthus annus*	298	485	326	53·5	0·698
Kum kum cake	(2)	*Crocus sativus*	166	517	200	83·9	0·580
Subabul seeds	(2)	*Leucaena leucocephala*	253	521	220	30·4	0·507
Soybean meal	(3)	*Glycine max*	480	518	138	19·2	73·3
Til cake	(2)	*Sesamum indicum*	363	412	185	38·4	0·856
Mustard cake	(3)	*Brassica* spp.	347	244	165	29·0	0·878
		Mean	320	458	213	41·6	0·688
		S.D.	99·7	95·4	86·0	20·0	0·135
		S.E.M.	35·3	33·7	30·4	7·10	0·048
Compound feed							
Compound pellet-1	(3)	–	271	435	145	45·2	0·653
Compound pellet – 2	(3)	–	236	495	148	43·5	0·653
Mash feed	(3)	–	218	763	161	49·7	0·644
Creeper ration	(3)	–	192	570	393	37·4	0·602
Home-made feed	(8)		159	535	313	70·5	0·533
		Mean	215	559	232	49·3	0·617
		S.D.	42·3	125	114	12·7	0·051
		S.E.M.	18·9	55·7	50·2	5·70	0·023
Total mixed ration							
RS + Feed1(90 : 10)*	–		61·2	665	417	58·5	0·483
RS + Feed1(80 : 20)	–		96·0	662	381	55·6	0·500
RS + Feed1(70 : 30)	–		96·1	794	354	49·4	0·533
RS + Feed1(60 : 40)	–		113	705	331	47·6	0·570
RS + Feed1(50 : 50)	–		130	636	301	42·6	0·602
RS + Feed1(40 : 60)	–		148	651	253	33·0	0·652
RS + Feed1(30 : 70)	–		157	628	223	33·1	0·701
		Mean	114	676	322	45·7	0·577
		S.D.	33·6	57·2	69·3	10·1	0·080
		S.E.M.	12·7	21·6	26·2	3·81	0·030
RS + Feed2(90 : 10)†	–		87·2	766	409	53·2	0·463

Table 1. (*Cont.*)

Common name	No. samples screened	Botanical name	Crude protein	Neutral detergent fibre	Acid detergent fibre	Acid detergent lignin	IVDMD
RS + Feed2(80 : 20)	–		78·7	651	379	47·3	0·482
RS + Feed2(70 : 30)	–		114	663	371	53·7	0·512
RS + Feed2(60 : 40)	–		114	646	350	50·9	0·546
RS + Feed2(50 : 50)	–		105	603	320	47·2	0·572
RS + Feed2(40 : 60)	–		135	663	295	42·3	0·601
RS + Feed2(30 : 70)	–		157	550	235	37·7	0·635
		Mean	112·9	648·6	336·9	47·5	0·544
		S.D.	27·0	65·8	58·8	5·9	0·629
		S.E.M.	10·2	24·9	22·2	2·21	0·238
RS + Feed3(90 : 10)‡	–		75·9	699	392	40·1	0·443
RS + Feed3(80 : 20)	–		78·6	703	421	66·1	0·477
RS + Feed3(70 : 30)	–		92·6	686	367	66·5	0·504
RS + Feed3(60 : 40)	–		135	708	380	58·5	0·551
RS + Feed3(50 : 50)	–		127	771	321	70·7	0·587
RS + Feed3(40 : 60)	–		183	608	359	67·1	0·615
RS + Feed3(30 : 70)	–		197	597	365	64·0	0·629
		Mean	127	68·2	372·2	61·9	5·436
		S.D.	48·7	60·7	60·7	10·3	0·714
		S.E.M.	18·4	22·9	22·9	3·89	0·269

RS-finger millet straw (*E. coracana*).

* Feed 1: crushed maize 45 parts + soybean meal 27 parts + wheat bran 25 parts + mineral mixture 2 parts + salt 1 part.

† Feed 2: crushed maize 45 parts + peanut extract 27 parts + wheat bran 13 parts + de-oiled rice bran 10 parts + mineral mixture 2 parts + salt 1 part.

‡ Feed 3: commercial concentrate feed.

differences in NDF and ADF fractions and IVDMD, as recorded in the present study. Klevenhusen *et al.* (2008) recorded greater methane outputs from high starch/sugar rather than high fibre feeds when fermented *in vitro* in a continuous culture system. This is in agreement with the findings of the present study in which feeds with relatively high proportions of non-structural CHOs gave rise to greater methane output than high-fibre feeds such as straw and stover. Getachew *et al.* (2005) reported 16% methane (in forages, concentrate ingredients and by-product feeds), which seems to be comparable with dry fodder, cereal by-products and oil meals, and lower in local grasses, home-made feed and higher than other feedstuffs. Among dry fodder, high IRMO was recorded in *S. vulgare* and *Arachis hypogea*. These feedstuffs form the bulk of the roughage component in ruminant feeds in the northern Karnataka state in India. If efforts are to be made to ameliorate enteric methane production, then a proportion of *S. vulgare* and *Arachis hypogea* should be replaced with feedstuffs having a higher nutritive value in the diet. The methane concentration and IRMO of cultivated grasses and cereal grains were similar. Boadi *et al.* (2004), Beauchemin *et al.* (2008) and Navarro-Villa *et al.* (2011) reported lower methane from legumes than grasses. Navarro-Villa *et al.* (2011) attributed less methane in legumes *v.* grasses to less extensive *in vitro* fermentation of legumes.

The lowest IRMO was recorded in tree leaves, mainly due to the presence of tannin. It is well established that tannin present in tropical leaves significantly reduces methanogenesis. Efforts have been made to screen these leaves for their methane suppression properties, so that they can be incorporated in ruminant diets (Bhatta *et al.* 2012, 2013*a*, *b*, *c*).

The IRMO of compound feed was higher than oil meals and lower than cereal by-products. This was attributed to the type of samples that were collected at the farm gate level. There are various types of compound feeds available for different categories of animals depending on their milk yield.

Table 2. *Potential gas production (PGP, ml/200 mg DM), rate constant (mg/h), $t^{1/2}$ (h) and IRMO of feed ingredients and diet combinations*

Common name	Botanical name	PGP production (ml/200 mg DM)	Rate constant (mg/h)	$t^{1/2}$ (h)	Methane proportion in the total gas	IRMO Methane ml/100 mg truly digested substrate
Dry fodder						
Ragi straw	*Oryza sativa*	46·0	0·040	18·4	0·19	5·26
Paddy straw	*Elusine coracana*	49·7	0·035	19·7	0·20	6·24
Jowar straw	*Sorghum vulgare*	51·3	0·039	17·9	0·15	6·91
Jowar Kadbi	*Sorghum vulgare*	48·3	0·047	14·6	0·18	12·0
Maize fodder	*Zea mays*	54·2	0·046	14·9	0·18	3·88
Sorghum fodder	*Sorghum vulgare*	45·5	0·020	34·5	0·18	4·41
Black gram fodder	*Vigna mungo*	32·8	0·073	9·39	0·22	2·37
Hybrid Jowar fodder	*Sorghum vulgare*	48·4	0·032	21·6	0·16	4·03
Sorghum powder	*Sorghum vulgare*	65·4	0·112	6·15	0·22	7·52
Gram straw	*Cicer arietinum*	45·2	0·081	8·08	0·10	5·40
Groundnut straw	*Arachis hypogea*	41·7	0·070	8·85	0·21	11·8
Bajra straw	*Pennisetum typhhoids*	34·0	0·040	15·7	0·10	4·05
Barley straw	*Hordeum vulgare*	35·6	0·040	14·7	0·10	5·69
Wheat straw	*Triticum aestivum*	45·0	0·021	26·3	0·11	4·52
	Mean	45·9	0·050	16·5	0·16	6·01
	S.D.	8·50	0·031	7·64	0·05	2·84
	S.E.M.	2·27	0·030	2·04	0·01	0·76
Local grasses						
Local grass	–	32·5	0·047	17·1	0·21	4·56
Forest grass	–	46·5	0·032	21·6	0·19	4·77
	Mean	39·5	0·041	19·4	0·20	4·67
	S.D.	9·90	0·010	3·25	0·01	0·15
	S.E.M.	7·70	0·008	2·29	0·01	0·11
Tree leaves						
Amla	*Phyllanthus emblica*	26·9	0·072	9·53	0·07	2·05
Mango	*Mangifera indica*	26·2	0·077	8·99	0·10	0·64
Tamarind	*Tamarindus indica*	31·1	0·082	8·35	0·08	0·71
Custard apple	*Annona squamosa*	37·9	0·058	11·9	0·09	1·97
Guava	*Psidium guajava*	26·9	0·072	10·2	0·14	1·34
	Mean	29·8	0·072	9·80	0·10	1·34
	S.D.	4·96	0·009	1·36	0·03	0·67
	S.E.M.	2·22	0·004	0·61	0·01	0·30
Cultivated grasses						
Para grass	*Brachiaria mutica*	43·4	0·043	15·9	0·18	3·17
Hybrid Napier	*Pennisetum purpureum*	47·3	0·052	13·3	0·21	3·93
Cowpea leaf	*Vigna sinesis*	34·0	0·084	8·24	0·08	0·49
Black gram leaf	*Vigna mungo*	38·9	0·048	8·20	0·07	0·42
Greengram leaf	*Phaseolus aureus*	33·4	0·060	10·8	0·22	6·81
Lucerne	*Madicago sativa*	29·0	0·054	12·6	0·19	4·43
Gram leaf	*Cicer arietinum*	32·3	0·059	11·7	0·09	1·79
Bajra leaf	*Pennisetum typhoids*	42·9	0·029	23·5	0·12	4·02
Jowar leaf	*Sorghum* spp.	43·6	0·050	13·6	0·09	0·66
Maize green	*Zea mays*	58·9	0·041	16·6	0·13	3·09
Sugarcane leaf	*Saccharum officinarum*	26·4	0·036	19·2	0·12	2·28
	Mean	39·1	0·050	13·9	0·14	2·83
	S.D.	9·37	0·010	4·61	0·05	1·96

Table 2. (*Cont.*)

Common name	Botanical name	PGP production (ml/200 mg DM)	Rate constant (mg/h)	$t^{1/2}$ (h)	Methane proportion in the total gas	IRMO Methane ml/100 mg truly digested substrate
	S.E.M.	2·83	0·004	1·38	0·02	0·59
Cereal by-products						
Wheat bran	*Triticum* spp.	58·4	0·062	10·8	0·17	6·62
Rice bran	*Oryza sativa*	51·2	0·070	9·75	0·14	5·22
Rice polish	*Oryza sativa*	24·5	0·074	9·43	0·13	3·45
	Mean	44·7	0·070	10·0	0·15	5·09
	S.D.	17·8	0·006	0·730	0·02	0·99
	S.E.M.	10·3	0·004	0·421	0·01	0·70
Cereal grains						
Maize grain	*Zea mays*	60·6	0·079	8·69	0·16	3·59
Ragi grain	*Elusine coracana*	52·7	0·054	12·7	0·22	4·10
Bajra grain	*Pennisetum typhhoids*	71·4	0·054	12·8	0·08	1·49
Jowar grain	*Sorghum bicolor*	63·5	0·050	12·2	0·09	1·34
Barley grain	*Hordeum vulgare*	66·1	0·070	9·47	0·09	1·68
Guar grain	*Cyamopsis tetragonolobus*	53·4	0·062	11·1	0·13	2·36
	Mean	61·3	0·06	11·2	0·13	2·43
	S.D.	7·30	0·01	1·73	0·06	1·30
	S.E.M.	2·98	0·005	0·706	0·03	0·58
Oil seed meals						
Groundnut cake	*Arachis hypogaea*	40·6	0·131	6·25	0·21	3·62
Cotton seed cake	*Gossypium* spp.	40·2	0·020	34·1	0·21	4·27
Kum kum cake	*Crocus sativus*	9·75	0·072	9·58	0·20	1·15
Sunflower cake	*Helianthus annus*	34·2	0·090	7·67	0·17	2·87
Subabul seeds	*Leucaena leucocephala*	52·0	0·061	11·2	0·23	6·02
Soybean meal	*Glycine max*	48·8	0·089	7·75	0·21	3·72
Til cake	*Sesamum indicum*	25·7	0·092	7·46	0·10	1·11
Mustard cake	*Brassica* spp.	38·8	0·060	10·9	0·08	0·55
	Mean	36·5	0·080	11·6	0·17	2·91
	S.D.	12·6	0·030	8·57	0·06	1·50
	S.E.M.	4·19	0·010	2·86	0·02	0·57
Compound feed						
Compound pellet-1	–	45·0	0·091	7·55	0·28	5·37
Compound pellet-2	–	44·4	0·092	7·50	0·21	3·67
Mash feed	–	46·4	0·071	9·64	0·26	5·67
Creeper ration	–	24·6	0·039	17·7	0·18	3·53
Home-made feed	–	38·0	0·656	12·3	0·23	6·11
	Mean	39·7	0·190	10·9	0·23	4·87
	S.D.	9·02	0·261	4·24	0·04	1·12
	S.E.M.	4·04	0·117	1·89	0·02	0·56
Total mixed rations						
RS + Feed1 (90 : 10)*		37·2	0·028	24·7	0·20	5·68
RS + Feed1 (80 : 20)		37·8	0·027	25·4	0·19	4·32
RS + Feed1 (70 : 30)		40·8	0·030	22·4	0·20	3·81
RS + Feed1 (60 : 40)		46·2	0·037	18·7	0·18	3·65
RS + Feed1 (50 : 50)		46·1	0·046	14·9	0·19	2·26
RS + Feed1 (40 : 60)		52·9	0·047	14·5	0·18	2·40
RS + Feed1 (30 : 70)		56·9	0·048	14·2	0·17	2·35
	Mean	45·4	0·040	19·3	0·19	3·50

https://doi.org/10.1017/S0021859616000642 Published online by Cambridge University Press

Table 2. (*Cont.*)

Common name	Botanical name	PGP production (ml/200 mg DM)	Rate constant (mg/h)	$t^{1/2}$ (h)	Methane proportion in the total gas	IRMO Methane ml/100 mg truly digested substrate
	S.D.	7·49	0·010	4·89	0·01	1·27
	S.E.M.	2·83	0·003	1·85	0·01	0·48
RS + Feed2 (90 : 10)†		35·2	0·028	24·2	0·20	5·23
RS + Feed2 (80 : 20)		37·2	0·031	21·7	0·19	5·03
RS + Feed2 (70 : 30)		40·0	0·037	18·7	0·19	4·79
RS + Feed2 (60 : 40)		41·6	0·046	14·9	0·18	3·02
RS + Feed2 (50 : 50)		44·2	0·045	15·4	0·12	2·49
RS + Feed2 (40 : 60)		45·1	0·057	12·2	0·11	2·25
RS + Feed2 (30 : 70)		46·6	0·073	9·38	0·11	2·10
	Mean	41·4	0·050	16·6	0·16	3·56
	S.D.	4·19	0·020	5·24	0·04	1·39
	S.E.M.	1·59	0·006	1·98	0·02	0·53
RS + Feed3 (90 : 10)‡		39·6	0·040	29·9	0·20	5·46
RS + Feed3 (80 : 20)		32·4	0·024	28·2	0·19	4·31
RS + Feed3 (70 : 30)		31·2	0·029	23·2	0·16	3·75
RS + Feed3 (60 : 40)		29·2	0·036	21·8	0·16	3·58
RS + Feed3 (50 : 50)		29·9	0·032	19·2	0·20	3·24
RS + Feed3 (40 : 60)		30·9	0·038	18·4	0·13	2·96
RS + Feed3 (30 : 70)		37·1	0·051	13·5	0·15	2·24
	Mean	32·9	0·036	22·0	0·17	3·65
	S.D.	3·98	0·009	5·71	0·03	1·03
	S.E.M.	1·49	0·003	2·16	0·01	0·39

IRMO, *in vitro* rumen methane output; RS, finger millet straw (*E. coracana*).
* Feed 1: crushed maize 45 parts + soybean meal 27 parts + wheat bran 25 parts + mineral mixture 2 parts + salt 1 part.
† Feed 2: crushed maize 45 parts + peanut extract 27 parts + wheat bran 13 parts + de-oiled rice bran 10 parts + mineral mixture 2 parts + salt 1 part.
‡ Feed 3: Commercial concentrate feed.

Oil meals produced comparatively lower methane for two reasons: firstly, fat and other compounds included in the ether extract fraction are mostly not fermented by rumen microbes, and unsaturated fatty acids in particular are known to inhibit the methanogenic microbial system (Czerkawski *et al.* 1966; Demeyer & Van Nevel 1975). Hydrogenation of unsaturated fatty acids increases propionate synthesis, inhibits protozoa and cellulolytic bacterial activity, and thereby affects the methane production (Czerkawski *et al.* 1966). Also, Roger *et al.* (1992) reported that glycerol released from fat hydrolysis suppresses cellulolytic bacterial activity. Secondly, protein is degraded to ammonium (NH_4) in the rumen and it can combine with CO_2 resulting in ammonium bicarbonate (Getachew *et al.* 1998). Therefore, NH_4 produced as a result of rumen incubation of high-protein sources such as oilseed meals can be expected to combine with CO_2, thereby lowering the availability of this substrate for methane production. Among the oil meals, the lowest IRMO were recorded in *Crocus sativus* and *Sesamum indicum* (1·1 ml methane/100 mg truly digested substrate). The lower IRMO of *Gossypium* spp. was due to the presence of high NDF and ADF components.

Many studies in the past have shown that methane production could be influenced by the nature of CHO digested, such as cellulose, hemicelluloses and soluble residue (Macheboeuf *et al.* 2014). Santoso *et al.* (2007) observed a positive correlation of methane production with increased NDF digestion. In the present study, methane production tended to be lower than that reported elsewhere for different forages. Many studies have reported correlations between chemical constituents and methane production (Santoso & Hariadi 2009; Singh *et al.* 2011).

https://doi.org/10.1017/S0021859616000642 Published online by Cambridge University Press

Quality of feed/diet has a major effect on methane production, as VFA concentration and their relative proportions are influenced by the nature and fermentation of CHO (Johnson *et al*. 1996). The increment in fibre fractions will have a depressing effect on methane production. The fibre fractions decrease methane production by lowering pH (Bhatta *et al*. 2008). Although an increase in VFA production might be expected as the digestibility of feed increases, this is generally accompanied by a concurrent decrease in *in vivo* methane output (Johnson & Johnson 1995) but an increase in *in vitro* methanogenesis. This difference in methane output between *in vitro* and *in vivo* studies when high VFA concentrations are recorded may reflect the strongly buffered systems used with *in vitro* assays, preventing the pH from declining to a much greater extent than occurs in the *in vivo* rumen. Such a decline in pH has been shown to reduce fibre digestibility and reduce the activity of rumen methanogens.

Several attempts have been made to predict methane production by determining the amount of crude nutrients in cattle and sheep (Holter & Young 1992; Shibata 1994) and it is known that crude fibre is an important component in methane production. Miller (1995) reported that feed ingredients rich in crude fibre stimulated some species of microorganism within the cellulolytic-methanogen consortium, which serve to couple the degradation of CHOs with the use of hydrogen gas (H_2) for the reduction of CO_2 to methane.

CONCLUSIONS

The IRMO of various feeds and diet combinations were investigated. Because a substantial amount of dietary gross energy is lost as methane, knowledge of the methane output from these feedstuffs would help in formulating low methane producing diets for ruminants in tropical regions. The results of the current study established that incorporation of tropical tree leaves in the diet and feeding TMR are potential strategies to reduce enteric methane production in ruminants and thereby help in preventing global warming due to enteric methane.

The financial assistance provided to this work by the Indian Council of Agricultural Research (ICAR), New Delhi, under the 'Outreach Project on Methane' is gratefully acknowledged.

REFERENCES

Aluwong, T., Wuyep, P. A. & Allam, L. (2011). Livestock-environment interactions: methane emissions from ruminants. *African Journal of Biotechnology* **10**, 1265–1269.

AOAC (Association of Official Analytical Chemists) (1995). *Official Methods of Analysis*, 16th edn., Arlington, VA: AOAC International.

AOAC (Association of Official Analytical Chemists) (1997). *Official Methods of Analysis*, 16th edn., 3rd rev, Arlington, VA: AOAC International.

Beauchemin, K. A., Kreuzer, M., O'Mara, F. & McAllister, T. A. (2008). Nutritional management for enteric methane abatement: a review. *Australian Journal of Experimental Agriculture* **48**, 21–27.

Bhatta, R., Enishi, O., Takusari, N., Higuchi, K., Nonaka, I. & Kurihara, M. (2008). Diet effects on methane production by goats and a comparison between measurement methodologies. *Journal of Agricultural Science, Cambridge* **146**, 705–715.

Bhatta, R., Uyeno, Y., Tajima, K., Takenaka, A., Yabumoto, Y., Nonaka, I., Enishi, O. & Kurihara, M. (2009). Difference in the nature of tannins on *in vitro* ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. *Journal of Dairy Science* **92**, 5512–5522.

Bhatta, R., Saravanan, M., Baruah, L. & Sampath, K. T. (2012). Nutrient content, *in vitro* ruminal fermentation characteristics and methane reduction potential of tropical tannin-containing leaves. *Journal of the Science Food and Agriculture* **92**, 2929–2935.

Bhatta, R., Saravanan, M., Baruah, L., Sampath, K. T. & Prasad, C. S. (2013*a*). Effect of plant secondary compounds on *in vitro* methane, ammonia production and ruminal protozoa population. *Journal of Applied Microbiology* **115**, 455–465.

Bhatta, R., Saravanan, M., Baruah, L., Suresh, K. P. & Sampath, K. T. (2013*b*). Effect of medicinal and aromatic plants on rumen fermentation, protozoa population and methanogenesis *in vitro*. *Journal of Animal Physiology and Animal Nutrition* **97**, 446–456.

Bhatta, R., Enishi, O., Yabumoto, Y., Nonaka, I., Takusari, N., Higuchi, K., Tajima, K., Takenaka, A. & Kurihara, M. (2013*c*). Methane reduction and energy partitioning in goats fed two concentrations of tannin from *Mimosa* spp. *Journal of Agricultural Science, Cambridge* **151**, 119–128.

Boadi, D., Benchaar, C., Chiquette, J. & Massé, D. (2004). Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. *Canadian Journal of Animal Science* **84**, 319–335.

Chaurasia, M., Kundu, S. S., Singh, S. & Mishra, A. K. (2006). Cornell net carbohydrate and protein system for nutritional evaluation of tree leaves, shrub and grasses. *Indian Journal of Animal Science* **76**, 81–87.

Cone, J. W. & Van Gelder, A. H. (1999). Influence of protein fermentation on gas production profiles. *Animal Feed Science and Technology* **76**, 251–264.

Czerkawski, J. W., Blaxter, K. L. & Wainman, F. W. (1966). The metabolism of oleic, linoleic and linolenic acids by sheep with reference to their effects on methane production. *British Journal of Nutrition* **20**, 349–362.

Demeyer, D. I. & Van Nevel, C. J. (1975). Methanogenesis, an integrated part of carbohydrate fermentation, and its control. In *Digestion and Metabolism in the Ruminant* (Eds I. W. McDonald & A. C. I. Warner), pp. 366–382. Armidale, N.S.W., Australia: The University of New England Publishing Unit.

Getachew, G., Blümmel, M., Makkar, H. P. S. & Becker, K. (1998). *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. *Animal Feed Science and Technology* **72**, 261–281.

Getachew, G., Robinson, P. H., DePeters, E. J., Taylor, S. J., Gisi, D. D., Higgginbotham, G. E. & Riordan, T. J. (2005). Methane production from commercial dairy rations estimated using an *in vitro* gas technique. *Animal Feed Science and Technology* **123–124**, 391–402.

Goel, G. & Makkar, H. P. S. (2012). Methane mitigation from ruminants using tannins and saponins. *Tropical Animal Health and Production* **44**, 729–739.

Holter, J. B. & Young, A. J. (1992). Methane prediction in dry and lactating Holstein cows. *Journal of Dairy Science* **75**, 2165–2175.

Jentsch, W., Schweigel, M., Weissbach, F., Scholze, H., Pitroff, W. & Derno, M. (2007). Methane production in cattle calculated by the nutrient composition of the diet. *Archives of Animal Nutrition* **61**, 10–19.

Johnson, D. E., Ward, G. W. & Ramsey, J. J. (1996). Livestock methane: current emissions and mitigation potential. In *Nutrient Management of Food Animals to Enhance and Protect the Environment* (Ed. E. T. Kornegay), pp. 219–234. NY: Lewis Publishers.

Johnson, K. A. & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of Animal Science* **73**, 2483–2492.

Jung, H. G. & Allen, M. S. (1995). Characteristics of plant cell walls affecting intake and digestibility of forages. *Journal of Animal Science* **73**, 2774–2790.

Kajikawa, H., Tajima, K., Mitsumori, M. & Takenaka, A. (2007). Effects of amino nitrogen on fermentation parameters by mixed ruminal microbes when energy or nitrogen is limited. *Animal Science Journal* **78**, 121–128.

Klevenhusen, F., Bernasconi, S. M., Kreuzer, M. & Soliva, C. R. (2008). The methanogenic potential and C-isotope fractionation of different diet types represented by either C_3 or C_4 plants as evaluated *in vitro* and in dairy cows. *Australian Journal of Experimental Agriculture* **48**, 119–123.

Kurihara, M., Magner, T., Hunter, R. A. & McCrabb, G. J. (1999). Methane production and energy partition of cattle in the tropics. *British Journal of Nutrition* **81**, 227–234.

Macheboeuf, D., Coudert, L., Bergeault, R., Lalière, G. & Niderkorn, V. (2014). Screening of plants from diversified natural grasslands for their potential to combine high digestibility, and low methane and ammonia production. *Animal* **8**, 1797–1806.

McAllister, T. A., Okine, E. K., Mathison, G. W. & Cheng, K.-J. (1996). Dietary, environmental and microbiological aspects of methane production in ruminants. *Canadian Journal of Animal Science* **76**, 231–243.

McCrabb, C. J. & Hunter, R. A. (1999). Prediction of methane emissions from beef cattle in tropical production systems. *Australian Journal of Agricultural Research* **50**, 1335–1340.

Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D. & Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *Journal of Agricultural Science, Cambridge* **93**, 217–222.

Miller, T. L. (1995). Ecology of methane production and hydrogen sinks in the rumen. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. Proceedings of the Eighth International Symposium on Ruminant Physiology* (Eds W. V. Engelhardt, S. Leonhard-Marek, G. Breves & D. Giesecke), pp. 317–331. Stuttgart, Germany: Ferdinand Enke Verlag.

Moss, A. R. (1994). Methane production by ruminants – literature review of I. Dietary manipulation to reduce methane production and II. Laboratory procedures for estimating methane of diets. *Nutrition Abstracts and Reviews* (*Series B*) **64**, 785–806.

Navarro-Villa, A., O'Brien, M., López, S., Boland, T. M. & O'Kiely, P. (2011). *In vitro* rumen methane output of red clover and perennial ryegrass assayed using the gas production technique (GPT). *Animal Feed Science and Technology* **168**, 152–164.

Roger, V., Fonty, G., Andre, C. & Gouet, P. (1992). Effects of glycerol on the growth, adhesion, and cellulolytic activity of rumen cellulolytic bacteria and anaerobic fungi. *Current Microbiology* **25**, 197–201.

Santoso, B. & Hariadi, B. T. (2009). Evaluation of nutritive value and *in vitro* methane production of feedstuffs from agricultural and food industry by-products. *Journal of the Indonesian Tropical Animal Agriculture* **34**, 189–195.

Santoso, B., Kume, S., Nonaka, K., Kimura, K., Mizukoshi, H., Gamo, Y. & Takahashi, J. (2003). Methane emission, nutrient digestibility, energy metabolism and blood metabolites in dairy cows fed silages with and without galacto-oligosaccharides supplementation. *Asian-Australasian Journal of Animal Sciences* **16**, 534–540.

Santoso, B., Mwenya, B., Sar, C. & Takahashi, J. (2007). Methane production and energy partition in sheep fed timothy silage- or hay-based diets. *Indonesian Journal of Animal and Veterinary Sciences* **12**, 27–33.

SAS Institute® (2002). *User's Guide: Statistics, Version 9. 1*. Cary, NC: SAS Institute Inc.

Shibata, M. (1994). Methane production in ruminants. In *CH_4 and NO_2: Global Emissions and Controls from Rice Fields and Other Agricultural and Industrial Sources* (Eds K. Minami, A. Mosier & R. Sass), pp. 105–115. Yokendo, Tokyo, Japan: NIAES.

Singh, K. K., Das, M. M., Samanta, A. K., Kundu, S. S. & Sharma, D. (2002). Evaluation of certain feed resources for carbohydrate and protein fractions and *in situ* digestion characteristics. *Indian Journal of Animal Sciences* **72**, 794–797.

Singh, S., Kushwaha, B. P., Nag, S. K., Mishra, A. K., Bhattacharya, S., Gupta, P. K. & Singh, A. (2011). *In vitro* methane emission from Indian dry roughages in relation to chemical composition. *Current Science* **101**, 57–65.

Takahashi, J. (2001). Nutritional manipulation of methanogenesis in ruminants. *Asian-Australasian Journal of Animal Science* **14**, 131–135.

Tamminga, S. (1992). Gaseous pollutants produced by farm animal enterprises. In *Effect of Farm Animals on the Human Environment* (Eds C. Phillips & D. Piggins), pp. 345–357. Wallingford, UK: CAB International.

Van Soest, P. J. (1994). *Nutritional Ecology of the Ruminant*, 2nd edn, Ithaca, NY: Comstock Publishing Associates/ Cornell University Press.

Van Soest, P. J., Robertson, J. B. & Lewis, B. A. (1991). Symposium: carbohydrate methodology, metabolism and nutritional implications in dairy cattle. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597.

Widiawati, Y. & Thalib, A. (2007). Comparison fermentation kinetics (*in vitro*) of grass and shrub legume leaves: the pattern of VFA concentration, estimated CH_4 and microbial biomass production. *Indonesian Journal of Animal and Veterinary Sciences* **12**, 96–104.