In vitro methane emission from Indian dry roughages in relation to chemical composition

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Dry roughages, viz. wheat straw (WS), rice straw (RS), barley straw (BS), oat straw (OS), gram straw (GS), lentil straw (LS), sorghum stover (SST), pearl millet stover (PMST), maize stover (MST) and dry mixed grass (DG) fed to livestock were characterized for carbohydrate and protein fractions, energy, *in vitro* dry matter digestibility (IVDMD) and *in vitro* methane (CH_4) emission in buffalo inoculums with the objective to rank dry roughages for CH_4 emission, and to correlate their nutritional constituents with CH_4 production. Crude protein (CP) was more ($P < 0.05$) in LS, whereas MST had higher ($P < 0.05$) CP than SST and PMST. Protein fraction A (P_A) (%CP) was higher in SST and BS, whereas protein fraction C (P_C) (%CP) was highest ($P < 0.05$) in RS and lowest in LS. Carbohydrate fraction C (C_C) (%DM) was higher ($P < 0.05$) in GS, LS and DG, and lowest in MST. Carbohydrate fraction A (C_A) (%DM) was higher ($P < 0.05$) in GS and LS (17.51 and 20.54) and lowest in WS and RS (2.99 and 2.04). Gross energy (GE) of roughages ranged between 16.89 and 18.67 kJ g^{-1}.

CH_4 production (ml g^{-1}) was higher ($P < 0.05$) from LS, BS and MST at 12, 24 and 48 h of incubation. CH_4 production (g kg DDM^{-1} (digestible dry matter)) varied ($P < 0.05$) from 27.46 in MST to 47.37 in WS. CH_4% of GE was higher ($P < 0.05$) from LS and BS, and lowest from SST and MST. NDF, ADF, cellulose and lignin were negatively associated, whereas OM, ether extract (EE) and GE were positively associated with CH_4. Acid detergent insoluble protein (ADIP), protein fraction B3 (P_{B3}) and P_C were negatively associated with CH_4 production. Non-structural carbohydrate (NSC) and C_A were positively correlated with CH_4 ($r = 0.40^*$ and $r = 0.43^{**}$). It is concluded that CH_4 production (g kg DDM^{-1}) was higher from WS followed by LS, BS, OS, GS, PMST, RS, DG, MST and SST respectively. Energy, ADIP, P_C, NSC and C_A are the chemical constituents that significantly affect CH_4 production from dry roughages.

Keywords: Carbohydrate and protein fractions, dry roughage, methane emission, regression equation.

METHANE (CH_4) is one of the important greenhouse gases (GHGs) that affects the earth's energy balance and global climate change due to its radiation forcing properties[1]. CH_4 produced from enteric fermentation of feed/fodder or diet by ruminant animals is one of the important sources. Crop residues as dry roughages from cultivated grain and forage crops constitute the main diet for livestock feeding in India. These feed resources are rich in fibre and low in nitrogen, minerals and vitamins. With forage maturity, there is decreased nitrogen, digestibility and increased fibre and lignin content in the forage[2]. In the process of rumen fermentation its microbes usually convert major fractions of carbohydrate and protein in a feed/fodder or diet to a useful end-product (volatile fatty acids, microbial protein and B-vitamins) and some waste product (mainly CH_4 and CO_2). The pattern and concentration of these end-products depend largely on the chemical make-up (carbohydrate and protein fractions), digestibility and intake. Plant material (roughages) rich in cell content and low in cell wall on fermentation expected to yield low CH_4 on reduction of molar proportion of acetate (60%) and increased molar proportion of propionate (30%)[3]. Fermentation of a feed/fodder containing large cell wall or cellulose fraction is likely to produce a higher molar proportion of acetate (70%) and a lower proportion of propionate (20%). In 12 h fermentation, two times higher CH_4 per unit of organic matter (OM) degradation was observed in grass than legume[4].

CH_4 production resulting from fermentation of feed in the gastrointestinal tract of ruminants represents a loss of dietary energy that is typically about 2–12% of gross energy intake[5]. CH_4 production primarily depends on the quantity and quality of the feed that affects the rate of digestion and the rate of passage in the fermentation process[6]. Reduced forage digestibility is accompanied by decreased forage intake and increased acetate : propionate ratio, which favours increased CH_4 production per unit of forage consumed[7]. A decrease in CH_4 loss (percentage of digestible energy (DE)) with increasing N content in fresh grass was recorded[8]. This reduction was hypothe-

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*For correspondence. (e-mail: singh.sultan@rediffmail.com sized to be linked to either lower fibre content or increased importance of protein fermentation[7]. Protein fermentation *in vitro* has been shown to be associated with lower CH_4 production than fermentation of carbohydrates[9,10]. However, increasing dietary N concentration may also stimulate rumen methanogenesis[11] in situations where feed N is rather low[12]. This was suggested to be due to reduced microbial growth of methanogens, which are less competitive under low N conditions[13].

CH_4 production could be influenced by the nature of the carbohydrate digested, such as cellulose, hemicelluloses and soluble residue[14–16]. Digestible acid detergent fibre (ADF), cellulose and hemicelluloses are important fibre fractions influencing CH_4 production in the rumen[17]. Estermann *et al.*[18] observed a strong relationship between CH_4 production and digestible neutral detergent fibre (NDF) for cows and calves. In contrast, CH_4 production expressed as $\mathrm{m\,mol\,g^{-1}}$ of apparently digested NDF increased with increasing concentration of NDF in feeds[19]. No information is available where carbohydrate and protein fractions of feeds have been linked with CH_4 production through *in vitro* experiments. These *in vitro* experiments could be used to obtain CH_4 production data from diverse feeds/fodder for further use to estimate CH_4 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 Indian dry roughages fed to ruminants and to correlate the chemical make-up of these roughages with *in vitro* CH_4 production to develop CH_4 prediction equations for dry roughages.

Materials and methods

Collection and processing of roughage samples

Samples of dry roughage from common crops residues fed to livestock in India, viz. wheat straw (WS) – *Triticum aestivum*, rice straw (RS) – *Oryza sativa*, barley straw (BS) – *Hordeum vulare*, oat straw (OS) – *Avena sativa*, gram straw (GS) – *Cicer aretinum*, lentil straw (LS) – *Lens culinaris*, sorghum stover (SST) – *Sorghum bicolor*, pearl millet stover (PMST) – *Pennisetum typhoids*, maize stover (MST) – *Zea mays* and dry mixed grass (DG) were collected from the experimental farm of the Indian Grassland and Fodder Research Institute, Jhansi and nearby villages. Samples were dried at 60°C for 48 h and then ground using 1 mm sieve with electrically operated Wiley mill. Grounded samples were stored in Tarson-make plastic containers for chemical and biochemical estimations.

Source of inoculum

For the estimation of *in vitro* dry matter digestibility (IVDMD) and gas production (total gas and CH_4) from the incubation of dry roughages, rumen liquor was collected from two fistulated adult male buffaloes (Murrah breed) maintained on standard WS–concentrate diet (65 parts WS and 35 parts concentrate). The rumen liquor samples were collected before feeding in pre-warmed steel Thermos and immediately brought to the laboratory. Rumen liquor was filtered through double layer of muslin cloth and bubbled with CO_2 for use as inoculum for IVDMD and gas-production studies.

Analytical techniques

Proximate constituents: Samples of straw, stover and dry grass were analysed with standard methods[20] for dry matter (DM), nitrogen (N), ether extract (EE) and ash content. Crude protein (CP) of samples was determined as Kjeldahl N × 6.25 by digestion in sulphuric acid and digestion mixture (consisting of sodium/potassium sulphate and copper sulphate in 10 : 1 ratio) using semi auto analyser (Kel Plus Classic-DX, Pelican). EE of samples was determined by refluxing in petroleum ether using extraction apparatus.

Cell wall polysaccharides: Cell wall fractions, viz. NDF, ADF, cellulose and lignin were estimated sequentially using the standard procedure[21] (fibre tech, Fibra Plus FES 6, Pelican India). Heat labile alpha amylase and sodium sulphite were not used in NDF estimation. NDF and ADF were expressed inclusive of residual ash. Lignin was determined by solublization of cellulose with $720\ \mathrm{g\,kg^{-1}}$ sulphuric acid.

Protein fractions: Crude protein fractions of dry roughages were determined according to the Cornell net carbohydrate and protein system (CNCPS)[22]. This system partitions the protein into three fractions: protein fraction A (P_A) is non-protein nitrogen (NPN × 6.25), protein fraction B is a true protein and protein fraction C (P_C) is unavailable or lignin-bound protein. Protein fraction B is further divided into three sub-fractions: B_1 (P_{B1}), B_2 (P_{B2}) and B_3 (P_{B3}) of rapid, intermediate and slow rate of rumen degradation respectively. Protein fractions A and B_1 are soluble in borate phosphate buffer, whereas B_2 is insoluble in buffer but soluble in neutral detergent solution. Fraction B_3 is insoluble in neutral detergent solution but soluble in acid detergent, whereas P_C is insoluble in acid detergent (acid detergent insoluble protein (ADIP)) and contains protein bound with lignin, tannin–protein complex and Millard products.

Recommended methods[23] were used for neutral detergent insoluble protein (NDIP), ADIP and NPN estimation. For NDIP and ADIP, samples extracted with neutral detergent and acid detergent respectively, were analysed as Kjeldahl N × 6.25 using semi auto analyser (Kel Plus Classic-DX Pelican India). For NPN estimation, samples were treated with sodium tungastate (0.30 molar), filtered and residual nitrogen was determined by Kjeldahl procedure. NPN of the sample was calculated by subtracting residual nitrogen from total nitrogen.

Soluble protein (SP) was estimated by treating the samples in borate–phosphate buffer, pH 6.7–6.8, consisting of monosodium phosphate ($Na_2PO_4 \cdot H_2O$) 12.20 g l^{-1}; sodium tetraborate ($Na_2B4O_7 \cdot 10H_2O$) 8.91 g l^{-1} and tertiary butyl alcohol 100 ml l^{-1}, and freshly prepared 10% sodium azide solution[24]. The N estimated in the residue gives the insoluble protein fraction. SP was calculated by subtracting insoluble protein from total CP.

P_A was calculated as the difference between the total protein and buffer-precipitated true protein. P_{B1} was calculated as the difference between the true protein and buffer-insoluble protein. Neutral detergent soluble protein (P_{B2}) was estimated as the difference between the buffer-insoluble protein minus NDIP, and B_3 as the protein in NDF (NDIP) minus ADIP. ADIP was classified as P_C.

Carbohydrate fractions: Carbohydrate fractions were estimated according to CNCPS[22], which classifies carbohydrate fractions depending on the degradation rate into four fractions, viz. C_A, rapidly degradable sugars; C_{B1}, intermediately degradable starch and pectins; C_{B2}, slowly degradable cell wall, and C_C, unavailable/lignin-bound cell wall. TCHO (% DM) was determined by subtracting CP, EE and ash content from 100. SC were calculated as the difference between NDF and NDIP and non fibre carbohydrates (NFC) were estimated as the difference between TCHO and SC. Sugar content of samples was extracted with 80% ethyl alcohol. Residue rich in starch was solublized with perchloric acid and the extract was treated with anthrone–sulphuric acid to determine glucose colorimetrically using standard glucose[25].

In vitro dry matter digestibility and energy: IVDMD was determined[26] by incubating 0.5 g sample in 50 ml digestion solution (40 ml of CO_2 saturated phosphate carbonate buffer and 10 ml strained buffalo rumen liquor) for 48 h and then for 24 h with 0.1 g of pepsin (1 : 3000 Sisco Research Laboratories, Mumbai) and 2 ml of 6 N HCl at 39°C. The samples were incubated in duplicate with rumen inoculum from two fistulated buffaloes twice (two periods) using blank (without roughage/sample).

Gross energy (GE) of roughages was estimated with bomb calorimeter (Toshniwal Brothers, CLOI/M2) using benzoic acid as the standard.

In vitro incubation: Total gas production from incubation/fermentation of dry roughages in buffalo inoculum was carried out using pressure transducer technique[27]. The digestion medium used for incubation was prepared by sequential mixing of buffer solution (NH_4HCO_3 and $NaHCO_3$), macro mineral solution, micro mineral solution and resazurin solution, except reducing agent. One gram sample from individual roughage source was put into four serum bottles (150 ml capacity). Four serum bottles without substrate were used as blank (without roughage). Initially sample and control serum bottles were gassed briefly with CO_2 before adding 65 ml of medium. Serum bottles were continued to flux with CO_2 and then 3 ml of reducing agent was added in each bottle. Gassing of bottles with CO_2 continued till the pink colour started fading (pink colour should become colourless, indicating complete reduction). The bottles were sealed with aluminum crimps and put in an incubator at 39°C overnight for inoculation on the following day. Before inoculation the gas pressure transducer was used to adjust the head-space gas pressure in each bottle (to adjust zero reading on the LED display). Serum bottles were inoculated with 8 ml inoculum of two buffaloes using a 10 ml syringe. The inoculated bottles were put in an incubator at 39°C and gas production (ml) was measured at subsequent periods (12, 24 and 48 h) of incubation from two bottles at two periods and thus four observations for individual roughages.

Methane measurements: CH_4 in the total gas was measured from four bottles (two bottles each at two periods) incubated for each roughage at different/periods (12, 24 and 48 h) using a gas chromatograph with methanizer (Nucon 5765 microprocessor-controlled gas chromatograph) and equipped with stainless-steel column packed with Porapak-Q and flame ionization detector (FID). The gas chromatograph was calibrated with standard CH_4 (99.995%) and CO_2 (14.52%). Running oven, detector, injector and methanizer temperatures were 100°C, 150°C, 120°C and 320°C respectively. CH_4 was also measured from the bottles kept as blank during the different fermentation periods and used for correction. CH_4 concentration (%) measured in the samples was utilized with total gas to estimate the methane production (ml g^{-1}).

Statistical analysis: Analysis of variance for chemical analysis of forage treatments, protein fractions, carbohydrate fractions, GE, IVDMD and CH_4 production was carried out utilizing the one-way analysis procedure of Statistical Package for Social Sciences (SPSS), version 13.0 using the model $Y_{ij} = \mu + F_i + E_{ij}$, where Y_{ij} represents the individual observations of the variable and F_i is the fixed effect of the ith dry roughage ($i = 1–10$). 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 treatments (dry roughages) were considered at $P < 0.05$ level. Correlation coefficients among the variables and CH_4 production were calculated by the Pearson method. The stepwise multiple regression method was used to develop prediction equations using chemical analysis constituents, protein fractions and carbohydrate fractions as predictors, with 40 observations (10 dry roughages with 4 replications) for each variable/estimate against 24 h CH_4 production (g g DDM^{-1} (digestible dry matter)).

Results

Chemical composition

CP content was higher ($P < 0.05$) in LS than WS, BS, RS, OS and GS, whereas MST had higher ($P < 0.05$) CP than SST and PMST (Table 1). Accumulation of NDF, ADF and cellulose was low ($P < 0.05$) in LS and GS than WS, BS, OS and RS. On the other hand, lignin content was more ($P < 0.05$) in LS and GS than cereal straw and stovers, except DG.

Protein and carbohydrate fractions

NDIP (%CP) and ADIP (%CP) were highest ($P < 0.05$) in RS (55.11 and 46.29) and lowest in LS (17.04 and 9.95; Table 2). SP (%CP) was lower ($P < 0.05$) in DG and higher in WS and PMST. The NPN (%CP) content was lowest ($P < 0.05$) in DG (11.41) and highest in SST (59.50). Concentration of P_A (%CP) was higher ($P < 0.05$) in SST (16.39) than other dry roughages. P_C (%CP) was higher ($P < 0.05$) in RS (46.29) than other cereal straw and legume straw, except DG (56.50) and lowest in LS (9.95).

Non-structural carbohydrates (%TCHO) were more ($P < 0.05$) in GS and LS than cereal straw (WS, OS, RS and BS), stovers (SST, MST and PMST) and grass. C_C (%DM) was significantly higher ($P < 0.05$) in GS, LS and DG (33.28, 27.34 and 31.75; Table 3), and lowest in MST (10.14). C_A (%DM) was highest ($P < 0.05$) in GS and LS (17.51 and 20.54) and lowest in WS and RS (2.99 and 2.04). Contrarily, C_{B2} was low ($P < 0.05$) in GS and LS than cereal straw (WS, OS, RS and BS).

Methane production, CH_4% of gas, energy and CH_4% energy of dry roughages

CH_4 production (ml g DM^{-1}) and its percentage concentration (v/v) in total gas differ ($P < 0.05$) among dry roughages and incubation periods (Table 4). CH_4 production (ml g DM^{-1}) was higher ($P < 0.05$) from LS and GS at 12 h, whereas BS and MST produced more CH_4 at 24 and 48 h of incubation. CH_4% (v/v) of total gas was higher at 48 and 24 h than 12 h of fermentation.

CH_4 production (g kg DDM^{-1}) was higher ($P < 0.05$), from WS, LS, BS and OS than other dry roughages (Table 5), whereas on the g kg DM^{-1} basis CH_4 production was higher ($P < 0.05$) from LS, BS, WS and GS. CH_4% of GE was higher ($P < 0.05$) from LS and BS followed by WS and OS, and it ranged from 6.03% to 8.87% across the roughages. GE (kJ g^{-1} DM) content of LS and GS was relatively more than RS and WS, but at par with BS and OS. IVDMD of legume straw (LS and GS) was higher ($P < 0.05$) than cereal straw (BS, OS and WS) and DG, whereas stovers (SST and MST) exhibited higher IVDMD than cereal straw and DG.

Association between chemical composition and CH_4 production

CP, NDF and ADF of dry roughage were negatively associated with *in vitro* CH_4 production, whereas EE was positively associated with CH_4 production (0.36*, Table 6). ADF, cellulose and lignin contents were inversely

Table 1. Chemical composition (g kg DM^{-1}) of dry roughages ($n = 4$ for each roughage)

Dry roughage	CP	OM	EE	NDF	ADF	Cellulose	Hemicellulose	Lignin
BS	25.9^{b}	871.2^{b}	14.4^{e}	721.3^{e}	462.7^{c}	369.1^{de}	258.7^{c}	47.5^{b}
OS	19.2^{a}	935.5^{g}	12.8^{cde}	785.9^{g}	495.4^{e}	411.8^{g}	290.4^{e}	67.0^{de}
SST	39.5^{d}	937.2^{g}	13.9^{e}	741.4^{f}	466.4^{cd}	388.5^{f}	275.0^{d}	69.9^{e}
WS	35.6^{d}	900.3^{c}	9.4^{b}	753.3^{f}	474.8^{cd}	378.1^{e}	278.5^{de}	63.6^{cd}
GS	27.9^{bc}	907.2^{d}	6.2^{a}	627.5^{b}	479.7^{d}	360.3^{d}	147.8^{a}	121.1^{h}
LS	76.9^{g}	914.1^{e}	13.3^{de}	536.6^{a}	385.6^{a}	282.5^{a}	151.0^{a}	93.8^{f}
PMST	50.47^{e}	924.8^{f}	13.7^{e}	653.2^{c}	405.9^{b}	323.9^{c}	247.3^{c}	61.0^{c}
MST	65.8^{f}	935.3^{g}	9.9^{bc}	688.4^{d}	373.7^{a}	310.0^{b}	314.7^{f}	36.3^{a}
RS	31.52^{c}	830.6^{a}	9.9^{bc}	782.3^{g}	522.2^{f}	376.5^{e}	260.1^{c}	44.8^{b}
DG	49.4^{e}	870.1^{b}	10.5^{bcd}	782.1^{g}	556.6^{g}	374.2^{e}	225.5^{b}	107.1^{g}
Mean	42.2	902.7	11.4	706.7	462.3	357.5	244.9	71.2
SEM	2.81	5.38	0.48	12.36	9.00	6.08	8.56	4.25

Means with letters a, b, c, d, e, f and g in rows within the same column differ significantly at $P < 0.05$.

SEM, Standard error of means; BS, Barley straw; OS, Oat straw; SST, Sorghum stover; WS, Wheat straw; GS, Gram straw; LS, Lentil straw; PMST, Pearl millet stover; MST, Maize stover; RS, Rice straw; DG, Dry grass; CP, Crude protein; EE, Ether extract; OM, Organic matter; NDF, Neutral detergent fibre, and ADF, Acid detergent fibre.

Table 2. Protein fraction (%CP) of straw, stovers and dry grass (n = 4 for each roughage)

Dry roughage	NDIP (%CP)	ADIP (%CP)	SP (%CP)	NPN (% CP)	P_A (%CP)	P_{B1} (%CP)	P_{B2} (%CP)	P_{B3} (%CP)	P_C (%CP)
BS	28.68^{b}	15.78^{b}	26.5^{cd}	53.67^{f}	14.27^{ef}	12.17^{b}	44.86^{d}	12.91^{bc}	15.77^{b}
OS	36.38^{cd}	16.72^{bc}	16.84^{b}	21.61^{c}	3.64^{ab}	13.20^{bc}	46.77^{de}	19.65^{d}	16.72^{bc}
SST	35.13^{bcd}	14.23^{ab}	27.51^{cd}	59.50^{g}	16.39^{f}	11.11^{b}	37.35^{cd}	20.90^{de}	14.23^{ab}
WS	27.52^{b}	17.78^{bcd}	31.05^{d}	33.45^{d}	10.47^{cde}	20.57^{d}	41.42^{d}	9.74^{ab}	17.78^{bcd}
GS	34.14^{bc}	15.97^{b}	22.4^{bc}	32.46^{d}	7.282^{bc}	15.11^{bc}	43.46^{d}	18.17^{cd}	15.97^{b}
LS	17.04^{a}	9.95^{a}	25.34^{cd}	48.72^{e}	12.35^{de}	12.99^{b}	57.61^{e}	7.09^{a}	9.95^{a}
PMST	42.50^{d}	22.66^{d}	29.72^{cd}	30.83^{d}	9.18^{cd}	20.54^{d}	27.77^{bc}	19.84^{d}	22.66^{d}
MST	67.30^{f}	22.03^{cd}	25.57^{cd}	46.25^{e}	11.89^{de}	13.67^{b}	7.12^{a}	45.27^{f}	22.03^{cd}
RS	55.11^{e}	46.29^{e}	21.37^{bc}	17.52^{b}	3.74^{ab}	17.58^{cd}	23.55^{b}	8.82^{ab}	46.29^{e}
DG	71.91^{f}	46.50^{e}	4.85^{a}	11.41^{a}	0.55^{a}	4.29^{a}	23.23^{b}	25.41^{e}	46.50^{e}
Mean	41.57	22.79	23.10	35.54	8.97	14.13	35.31	18.78	22.79
SEM	2.79	2.03	1.36	2.46	0.86	0.82	2.48	1.76	2.03

Means with letters a, b, c, d, e, f and g in rows within the same column differ significantly at $P < 0.05$.
NDIP, Neutral detergent insoluble protein; ADIP, Acid detergent insoluble protein; NPN, Non-protein nitrogen; SP, Soluble protein; P_A, Protein fraction A; P_{B1}, Protein fraction B1; P_{B2}, Protein fraction B2; P_{B3}, Protein fraction B3, and P_C, Protein fraction C.

Table 3. Carbohydrate and its fraction (%DM) in dry roughage (n = 4 for each roughage)

Dry roughage	CHO (%DM)	NSC (%TCHO)	SC (%TCHO)	Starch (%NSC)	C_A (%DM)	C_{B1} (%DM)	C_{B2} (%DM)	C_C %DM
BS	83.09^{d}	11.68^{c}	71.40^{d}	42.78^{ab}	8.09^{ab}	5.97^{ab}	72.22^{f}	13.71^{b}
OS	90.34^{h}	12.45^{c}	77.88^{g}	58.82^{b}	5.67^{ab}	8.10^{b}	68.41^{e}	17.79^{cd}
SST	88.38^{g}	15.63^{d}	72.75^{d}	56.56^{b}	7.81^{ab}	9.87^{bc}	63.32^{d}	18.98^{d}
WS	86.00^{e}	11.64^{c}	74.36^{e}	78.39^{c}	2.99^{a}	10.52^{bcd}	68.71^{e}	17.73^{cd}
GS	87.30^{f}	25.51^{f}	61.79^{b}	40.11^{ab}	17.51^{c}	11.70^{bcd}	37.49^{a}	33.28^{g}
LS	82.38^{c}	30.03^{g}	52.35^{a}	43.56^{ab}	20.54^{c}	15.90^{d}	36.20^{a}	27.34^{e}
PMST	86.07^{e}	22.88^{e}	63.18^{bc}	39.27^{ab}	16.13^{c}	10.45^{bcd}	56.39^{b}	17.01^{c}
MST	85.95^{e}	21.78^{e}	64.17^{c}	58.75^{b}	10.54^{b}	14.79^{cd}	64.80^{d}	10.14^{a}
RS	79.16^{a}	3.165^{a}	75.99^{f}	49.06^{ab}	2.04^{a}	1.948^{a}	82.42^{g}	13.588^{b}
DG	81.01^{b}	6.34^{b}	74.67^{ef}	30.86^{a}	5.41^{ab}	2.41^{a}	60.42^{c}	31.75^{f}
Mean	84.97	16.11	68.85	49.82	9.68	9.17	61.04	20.14
SEM	0.53	1.32	1.24	2.66	1.10	0.87	2.21	1.21

Means with letters a, b, c, d, e, f and g in rows within the same column differ significantly at $P < 0.05$.
TCHO, Total carbohydrates; NSC, Non-structural carbohydrates; SC, Structural carbohydrates; C_A, Carbohydrate fraction A; C_{B1}, Carbohydrate fraction B1; C_{B2}, Carbohydrate fraction B2; C_C, Carbohydrate fraction C, and DM, Dry matter.

Table 4. Total gas (ml), methane concentration (%) and methane production (ml g^{-1}) from dry roughage incubated for different periods in buffalo inoculum

	12 h			24 h			48 h		
Dry roughage	Total gas	CH_4%	CH_4 (ml g^{-1})	Total gas	CH_4%	CH_4 (ml g^{-1})	Total gas	CH_4%	CH_4 (ml g^{-1})
BS	52.00^{c}	6.37^{b}	5.28^{b}	52.40^{c}	12.81^{e}	10.94^{e}	51.00^{f}	14.87^{ef}	11.35^{f}
OS	55.40^{d}	7.95^{d}	8.08^{c}	49.85^{bc}	8.85^{a}	6.45^{b}	50.40^{e}	10.79^{a}	8.38^{bc}
SST	52.50^{c}	7.26^{c}	5.56^{b}	48.70^{b}	11.36^{c}	7.47^{c}	47.20^{a}	12.83^{bc}	7.34^{a}
WS	55.80^{d}	7.96^{d}	8.28^{c}	50.40^{bc}	10.02^{b}	7.55^{c}	49.30^{d}	11.98^{b}	8.47^{cd}
GS	60.00^{f}	7.32^{c}	9.30^{d}	48.20^{b}	10.43^{b}	6.58^{b}	47.30^{a}	13.10^{cd}	7.70^{a}
LS	62.60^{g}	10.89^{e}	15.12^{e}	49.20^{b}	12.66^{e}	8.63^{d}	47.90^{b}	15.46^{f}	9.55^{e}
PMST	58.60^{e}	6.39^{b}	7.70^{c}	47.90^{b}	8.72^{a}	5.38^{a}	49.60^{d}	10.78^{a}	7.83^{ab}
MST	51.25^{b}	7.81^{cd}	5.01^{b}	48.40^{b}	13.37^{f}	8.55^{d}	48.00^{b}	18.85^{g}	11.53^{f}
RS	50.30^{a}	6.42^{b}	4.73^{b}	49.00^{b}	11.20^{c}	7.59^{c}	47.40^{a}	13.36^{cd}	7.80^{a}
DG	49.86^{a}	5.53^{a}	3.91^{a}	45.00^{a}	12.13^{d}	7.47^{c}	48.70^{c}	13.90^{de}	9.07^{de}
Mean	54.83	7.39	7.30	48.90	11.15	7.70	48.68	13.59	8.90
SEM	0.67	0.23	0.51	0.40	0.25	0.23	0.27	0.38	0.23

Means with letters a, b, c, d, e, f and g in rows within the same column differ significantly at $P < 0.05$.

Table 5. *In vitro* methane production (24 h), energy loss, *in vitro* dry matter digestibility and energy of dry roughages

Dry roughage	CH_4 (g kg DDM^{-1})	CH_4 (g kg DM^{-1})	CH_4 (% GE)	IVDMD (%)	GE (kJ g^{-1})
BS	42.93^{cd}	16.22^{e}	8.57^{e}	39.08^{a}	18.597^{c}
OS	40.20^{c}	14.53^{d}	7.97^{d}	38.06^{a}	18.38^{c}
SST	27.67^{a}	13.03^{bc}	6.26^{a}	45.61^{bc}	17.47^{ab}
WS	47.37^{d}	15.83^{e}	8.16^{d}	41.31^{a}	18.01^{bc}
GS	32.78^{b}	15.88^{e}	6.75^{b}	48.54^{c}	18.59^{c}
LS	43.24^{cd}	23.75^{f}	8.87^{e}	55.20^{e}	18.67^{c}
PMST	32.54^{b}	13.08^{bc}	6.95^{bc}	42.00^{ab}	17.18^{a}
MST	27.46^{a}	13.55^{cd}	6.03^{a}	49.50^{c}	17.43^{ab}
RS	29.38^{ab}	12.32^{ab}	6.66^{b}	42.00^{ab}	16.89^{a}
DG	28.78^{ab}	11.38^{a}	6.40^{ab}	39.81^{a}	17.06^{a}
Mean	35.23	14.96	7.26	44.11	17.80
SEM	1.21	0.54	0.13	0.91	0.12

Means with letters a, b, c, d, e, f and g in rows within the same column differ significantly at $P < 0.05$.
IVDMD, *In vitro* dry matter digestibility and GE, Gross energy.

Table 6. Correlation between *in vitro* methane production and chemical composition of dry roughages

Proximate constituents	CH_4 (g g DDM^{-1})	Protein fraction	CH_4 (g g DDM^{-1})	Carbohydrate fraction	CH_4 (g g DDM^{-1})
CP	–0.26	CP	–0.26	TCHO	0.28
OM	0.18	NDIP	–0.31	NSC	0.40*
EE	0.25	ADIP	–0.37*	SC	–0.24
NDF	–0.29	SP	0.17	Starch % NSC	–0.30
ADF	–0.27	NPN	0.01	C_C	–0.02
Cellulose	–0.01	P_A	0.07	C_{B2}	–0.16
Hemicellulose	–0.15	P_{B1}	0.21	C_{B1}	–0.08
Lignin	–0.02	P_{B2}	0.20	C_A	0.419**
Energy	0.36*	P_{B3}	–0.05		
IVDMD	–0.25	P_C	–0.31*		

*Significant at $P < 0.05$; **Significant at $P < 0.01$.

Table 7. Linear regression equations to predict CH_4 (g g DDM^{-1}) from chemical constituents, protein fractions and carbohydrate fractions of dry roughages

Regression equation	SEM	R^2	P-value
$CH_4 = 0.073 - 0.003 \times CP + 0.003 \times EE - 0.001 \times \text{cellulose}$	0.002	0.81	$P < 0.01$
$CH_4 = -0.029 - 0.007 \times CP - 0.061 \times NDIP + 0.087 \times ADIP - 0.001 \times P_A + 0.001 \times P_{B1} + 0.001 \times P_{B3} + 0.001 \times P_C - 0.008 \times GE$	0.004	0.46	$P < 0.03$
$CH_4 = 0.038 + 0.003 \times TCHO - 0.004 \times C_C - 0.003 \times C_{B2} - 0.003 \times C_{B1} - 0.003 \times C_A + 0.005 \times GE - 0.001 \times IVDMD$	0.002	0.76	$P < 0.01$

related with CH_4 production. NDIP (%CP), ADIP (%CP) and P_C (%CP) fractions of protein were negatively associated with *in vitro* CH_4 production of dry roughage ($r = -0.31$, $r = -0.37$* and $r = -0.31$*). On the other hand, SP, P_{B1} and P_{B2} fractions of protein were positively related with *in vitro* CH_4 production.

TCHO (%DM), NSC (%TCHO) and carbohydrate C_A (%DM) fractions of dry roughage were positively associated with the *in vitro* CH_4 production in buffalo inoculum with r values of 0.28, 0.40* and 0.42* respectively, whereas the SC (%TCHO) and starch (%NSC) were negatively correlated with CH_4 production for dry roughage.

Regression equations and CH_4 production

Results of linear regression of dry roughages derived from 40 observations indicated that the equations developed with proximate constituents (CP, EE and cellulose) of dry roughage were better predictors of CH_4 production (g g DDM^{-1}) followed by carbohydrate fractions (TCHO, C_C, C_{B1}, C_{B2} and C_A) along with GE and IVDMD, and protein fractions. Proximate constituents and carbohydrate fractions had a positive and significant ($P < 0.01$) relationship for daily CH_4 production (g g DM^{-1}) with R^2 value of 0.81 and 0.76 respectively (Table 7). Nitrogen fractions were not a good predictor of CH_4 production as $R^2 = 0.46$ was low, though the relationship was significant ($P < 0.03$).

Discussion

Chemical composition

Dry roughages mainly from the cereal straw and stover particularly from tropical countries are usually low in CP and high in cell-wall constituents. CP content of the tested dry roughages, except LS, was below the ruminant maintenance requirement[28]. The present results of cereal and legume straw on chemical composition are identical to those of Lopez *et al.*[29] who observed more CP, lignin and low NDF, ADF and cellulose in legume (GS and LS) than cereal straw (BS, WS and OS) and MST. CP, EE, OM, NDF, ADF and lignin in the range 26–33, 7–12, 933–962, 716–795, 423–544 and 46–68 g kg^{-1} respectively, for cereal straw, and 56–111, 6–24, 877–943, 454–669, 280–500 and 54–115 g kg^{-1} respectively, for legume straw are at par with the present observations. Further the values of cellulose, hemicellulose and lignin for barley straw (413–445, 270–328 and 63–98 g kg^{-1}) and wheat straw (362–406, 218–360 and 49–103 g kg^{-1}) compiled earlier[30] also substantiate the present results. Data on CP, EE and cell-wall polysaccharides of SST and MST are consistent with the reported observations[29,30]. Chemical composition values reported for cereal and legume straw[31,32] are consistent and within the range of the observed values.

Carbohydrate and protein fractions

Dry roughages used in present study represent the chemical composition and nutritive value of major crop residues used as dry forage to livestock feeding in India. NDIP, ADIP, SP and NPN protein fractions of dry roughage are found to differ ($P < 0.05$). Differences in concentration of these fractions may be partly attributed to variability in cell content and cell-wall contents. NDIP, ADIP and SP values for sorghum stover reported earlier[33] are consistent with our results; however NPN content is higher (59.10) in the present study. Lower NDIP, ADIP, P_C and C_C values for mixed grass in an earlier study by Singh *et al.*[33] than our findings may be due to more NDF, ADF and lignin content recorded by us for DG. Total carbohydrate, and starch (%NSC) of SST and DG of Singh *et al.*[33] corroborate well with our results. NSC, NDIP and ADIP of urad legume straw recorded by these workers[33] are consistent with the values of GS and LS of the present study. Protein fractions P_A, P_{B2} and P_C of mung straw and P_{B1}, P_{B3} and P_C of sorghum stover reported by Singh *et al.*[33] are consistent with our LS and SST values. Further, carbohydrate fractions C_{B2} and C_C of LS were similar to the reported values of legume urad straw. NDIP, ADIP, carbohydrate fractions C_A, C_{B2} and C_C along with protein fractions P_{B1}, P_{B2} and P_{B3} for wheat straw reported by Bovera *et al.*[34] were consistent with our estimates of WS.

Methane production, energy and percentage of energy loss as CH_4 from dry roughages

Methane is produced as a result of anaerobic fermentation of cell contents and cell-wall contents of feeds/fodder by rumen microbes in ruminant animals. Methane production (ml g^{-1}) and its concentration (%) from tested dry roughages differs significantly ($P < 0.05$) at different periods of incubation. Such variation in *in vitro* CH_4 has been reported from feed stuffs (mainly straw) from agricultural and food industry by-products[35]. Variation in CH_4 production from dry roughages may be attributed to significant difference in the NDF, ADF, carbohydrate fractions and protein fractions as recorded in the present study. Getachew *et al.*[36] reported 16% proportion of CH_4 in total gas which seems to be comparable with LS, BS and MST, and higher than other roughages of the present study at 48 h of incubation. CH_4 production (ml/g) was higher ($P < 0.05$) from LS and GS, and lower from DG, RS and MST at 12 h of fermentation; however, at 24 and 48 h of incubation, CH_4 production was higher from MST and BS. Higher CH_4 from LS and GS at the early hour of fermentation may be due to low NDF and SC, ADIP and higher NSC, C_A and IVDMD. Many studies in the past have shown that CH_4 production could be influenced by the nature of CHO digested such as cellulose, hemicellulose and soluble residue[14–16]. Santoso *et al.*[37] observed positive correlation of CH_4 production with increased NDF digestion. In the present study, CH_4 production (ml g^{-1} and g g DDM^{-1}) tended to be lower than that reported for different forages[38].

Our values of CH_4% of GE (6.40–8.87) from tested dry roughages are more or less comparable to those of Bhatta *et al.*[39] who reported CH_4 as proportion of GE between 4.4% and 7.8% from 19 diets at 24 h of incubation. Observations from the present study on CH_4 as proportion of GE were within the range reported by Pelchen and Peters[40], but relatively higher than 5.5–6.5% observed losses for cattle, sheep and goats on tropical forage[41]. Higher values in the present study may be presumably attributed to relatively higher level of fibre and lignin[42] recorded for dry roughages (Table 1), and low digestibility[2].

Jung and Allen[43] have described the plant cell wall characteristics affecting intake and digestibility of forages in ruminants. IVDMD of cereal straw, legume straw and stovers ranged from 38.06% to 42.00%, 48.54% to 55.20% and 42.00% to 49.50% respectively. Higher digestibility of legume straw than cereal straw and stovers may be attributed to their lower NDF, ADF, cellulose and lignin contents respectively (Table 1). Higher DM digestibility of legume straw by 10% than cereals straw reported earlier[44] is in conformity 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[45]. Low IVDMD of cereal straw and DG in the present study may be attributed to low microbial activity due to inadequate protein to meet their requirement during incubation.

Association between chemical composition and methane production

Like in the present study, many workers in the past[15,16,35] have explained the relation between chemical constituents and CH_4 production. However, information on the association between CH_4 production and carbohydrate fractions and or/protein fractions is scanty. Quality of feed/diet has a major effect on CH_4 production as VFA concentration and its relative proportion are influenced by the nature and fermentation of carbohydrate[41]. Moss[46] reported that CH_4 production has a positive relation with NDF content ($R^2 = 79\%$) and negative correlation with CP content ($R^2 = -76.8\%$). Yan *et al.*[47] observed a positive relationship ($P < 0.001$) between gross energy and CH_4 output. Similarly, Ellis *et al.*[48] recorded positive relation between EE and CH_4 production. A negative correlation between cell wall (NDF, ADF, cellulose and lignin) and CH_4 production observed in the present study is substantiated by earlier findings[47], where negative correlation was recorded among ADF, cellulose, lignin and CH_4 production.

Regression equations and CH_4 production

Enteric CH_4 emission estimated using equations by different workers was reviewed by Wilkerson *et al.*[49], and the factors taken into account differ widely as they include either dry matter digestibility, dry matter intake, energy, carbohydrate, non-fibrous carbohydrate, ADF, cellulose, hemicellulose, CP or EE. No information is available on the use of carbohydrate fractions and protein fractions in the prediction equations for CH_4 production. In the present study, equation using CP, EE and cellulose has $R^2 = 0.81$ ($P < 0.01$), whereas equations using protein fractions and carbohydrate fractions have $R^2 = 0.46$ ($P < 0.03$) and 0.76 ($P < 0.01$) respectively. This shows that carbohydrate and its fractions are a better estimate of *in vitro* CH_4 production from dry roughages. Our observation is substantiated by an earlier study[5] which identified that carbohydrate fed to livestock has a major effect on CH_4 production most likely due to the effect on rumen pH and its microbial population. Santoso *et al.*[37] indicated that digested NDF is a better CH_4 predictor than digested ADF, cellulose and hemicelluloses.

Conclusion

The results of the present study revealed that CH_4 production was higher from WS followed by LS, BS, OS, GS, PMST, RS, DG, MST and SST. Energy, NSC and C_A ($P < 0.01$) were positively related with CH_4 production, and ADIP and P_C were negatively ($P < 0.01$) associated with CH_4 production for dry roughages. Percentage of CH_4 energy was more for cereal straw than other dry roughages. Proximate constituents (CP, cellulose and EE) and carbohydrate fractions (TCHO, C_C, C_{B2}, C_{B1} and C_A along with energy and digestibility) were a better predictor of CH_4 production with $R^2 = 0.81$ and 0.76 respectively, than nitrogen fractions.

1. Harper, L. A., Danmead, O. T., Freney, J. R. and Byers, F. M., Direct measurement of methane emission from grazing and feedlot cattle. *J. Anim. Sci.*, 1999, **77**, 1392–1401.
2. Minson, D. J., *Forage in Ruminant Nutrition*, Academic Press, London, 1990.
3. Dougherty, R. W., Physiology of the ruminant digestive tract. In *Duke' Physiology of Domestic Animals* (ed. Swenson, M.), Cornell University Press, New York, 1984, pp. 51–358.
4. Widiawati, Y. and Thalib, A., Comparison fermentation kinetics (*in vitro*) of grass and shrub legume leaves. The pattern of VFA concentration, estimated CH_4 and microbial biomass production. *J. Indones. Trop. Anim. Agric*., 2007, **12**(2), 96–104.
5. Johnson, K. A. and Johnson, D. E., Methane emission from cattle. *J. Anim. Sci.*, 1995, **73**, 2483–2492.
6. Van Soest, P. J., *Nutritional Ecology of the Ruminant*, Cornell University Press, Ithaca, NY, 1982, pp. 325–343.
7. McAllister, T. A., Okine, E. K., Mathison, G. W. and Cheng, K. J., Dietary, environmental and microbiological aspects of methane production in ruminants. *Can. J. Anim. Sci.*, 1996, **76**, 231–243.
8. Tamminga, S., Gaseous pollutants produced by farm animal enterprises. In *Farm Animals and the Environment* (eds Phillips, C. and Piggins, D.), CAB International, Wallingford, UK, 1992, pp. 345–357.
9. Demeyer, D. and Van Nevel, C., Protein fermentation and growth by rumen microbes. *Annu. Res. Vet.*, 1979, **10**, 277–279.
10. Cone, J. W. and Van Gelder, A. H., Influence of protein fermentation on gas production profiles. *Anim. Feed Sci. Technol.*, 1999, **76**, 251–264.
11. Kurihara, M., Magner, T., Hunter, R. A. and McCrabb, G. J., Methane production and energy partitioning of cattle in the tropics. *Br. J. Nutr.*, 1999, **81**, 227–234.
12. Kirchgeβner, M., Windisch, W., Müller, H. L. and Kreuzer, M., Release of methane and of carbon dioxide by dairy cattle. *Agribiol. Res.*, 1991, **44**, 91–102.
13. Moss, A. R., *Methane Global Warming and Production by Animals*, Chalcombe Publications, Canterbury, UK, 1993.
14. Moe, P. W. and Tyrrell, H. F., Methane production in dairy cows. *J. Dairy Sci.*, 1979, **62**, 1583–1586.
15. Takahashi, J., Nutritional manipulation of methanogenesis in ruminants. *J. Anim. Sci.*, 2001, **14**, 131–135.
16. Santoso, B., Kume, S., Nonaka, K., Kimura, K., Mizokoshi, H., Gamo, Y. and Takahashi, J., Methane emission, nutrient digestibility, energy metabolism and blood metabolites in dairy cows fed silages with and without galacto-oligosaccharides supplementation. *Asian Aust. J. Anim. Sci.*, 2003, **16**, 534–540.
17. Moss, A. R., Methane production by ruminants – literature review of: I. Dietary manipulation to reduce methane production and II. Laboratory procedures for estimating methane of diets. *Nutr. Abstr. Rev.* (*Ser. B*), 1994, **64**, 785–806.

18. Estermann, B. L., Sutter, F., Schlegel, P. O., Erdin, D., Wettsten, H. R. and Kreuzer, M., Effect of calf age and dam breed on intake, energy expenditure and excretion of nitrogen, phosphorus and methane of beef cows with calves. *J. Anim. Sci.*, 2002, **80**, 1124–1134.
19. Hindrichsen, I. K., Kreuzer, M., Machmuller, A., Bach Knudsen, K. E., Madsen, J. and Wettstein, H. R., Methane release and energy expenditure of dairy cows fed concentrates characterized by different carbohydrates. In *Energy and Protein Metabolism and Nutrition* (eds Souffrant, W. M. and Metges, C.), Wageningen, Academic Publishers, The Netherlands, 2003, pp. 413–416.
20. AOAC, *Official Methods of Analysis*, Association of Official Analytical Chemists, Arlington, USA, 1995.
21. Van Soest, P. J., Robertson, J. B. and Lewis, B. A., Methods for dietary fibre, neutral detergent fibre and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 1991, **74**, 3583–3597.
22. Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G. and Russel, J. B., A net carbohydrate and protein system for evaluating cattle diets II carbohydrate and protein availability. *J. Anim. Sci.*, 1992, **70**, 3562–3577.
23. Licitra, G., Harnandez, T. M. and Van Soest, P. J., Standardizations of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.*, 1996, **57**, 347–358.
24. Krishnamoorthy, U., Muscato, T. V., Sniffen, C. J. and Van Soest, P. J., Nitrogen fractions in selected feedstuffs. *J. Dairy Sci.*, 1982, **65**, 217–225.
25. Sastry, V. R. B., Kamra, D. N. and Pathak, N. N., *Laboratory Manual of Animal Nutrition*, Indian Veterinary Research Institute (ICAR), Izatnaar, 1991.
26. Tilley, J. M. A. and Terry, R. A., A two-stage technique for the *in vitro* digestion of forage crops. *J. Br. Grass. Soc.*, 1963, **18**, 104–111.
27. Theodorou, M. K., Williams, A. B., Dhanoa, M. S., McAllan, A. B. and France, J., A simple gas production method using pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed Sci. Technol*., 1994, **48**, 185–197.
28. National Research Council, *Nutrient Requirements of Dairy Cattle*, National Academy Press, Washington DC, 2001.
29. Lopez, S., Davies, D. R., Giraldez, F. J., Dhanoa, M. S., Dijkstra, J. and France, J., Assessment of nutritive value of cereal and legume straws based on chemical composition and *in vitro* digestibility. *J. Sci. Food Agric*., 2005, **85**, 1550–1557.
30. Antongiovanni, M. and Sargentini, C., Variability in chemical composition of straws. CIHEAM–Options Mediterranean's Series Seminars, 1991, vol. 16, pp. 49–53.
31. Theander, O. and Aman, P., Anatomical and chemical characteristics. In *Straws and other Fibrous Byproducts as Feed* (eds Sundstol, F. and Owen, E.), Elsevier, Amsterdam, 1984, pp. 45–78.
32. Hadjipanayiotou, M., Chemical composition, digestibility and *in situ* degradability of narbon vetch grain and straw grown in a Mediterranean region. *Ann. Zootech.,* 2000, **49**, 475–478.
33. Singh, K. K., Samanta, A. K., Kundu, S. S. and Sharma, D., Evaluation of certain feed resources for carbohydrate and protein fractions and *in situ* digestion characteristics. *Indian J. Anim. Sci*., 2002, **72**(9), 794–797.
34. Bovera, F., Spanghero, M., Galassi, G., Masoero, F. and Buccioni, A., Repeatability and reproducibility of the Cornell net carbohydrate and protein system analytical determinations. *Ital. J. Anim. Sci.*, 2003, **2**, 41–50.
35. Santoso, B. and Hariadi, B. T., Evaluation of nutritive value and *in vitro* methane production of feed stuffs from agricultural and food industry byproducts. *J. Indones. Trop. Anim. Agric.*, 2009, **34**(3), 189–195.
36. Getachew, G., Robinson, P. H., DePeters, E. J., Taylor, S. J., Gisi, D. D., Higgginbotham, G. E. and Riodan, T. J., Methane production from commercial dairy ration estimated using *in vitro* gas technique. *Anim. Feed Sci. Technol*., 2005, **23**, 391–402.
37. Santoso, B., Mwenya, B., Sar, C. and Takahashi, J., Methane production and energy partition in sheep fed timothy silage or hay-based diets. *J. Anim. Sci. Vet*., 2007, **12**(1), 27–33.
38. Filippo, R., Vecchia, P. and Masoero, F., Estimate of methane production from rumen fermentation. *Nutr. Cycl. Agroecosyst.*, 2001, **60**, 89–92.
39. Bhatta, R., Enishi, O., Takusari, N., Higuchi, K., Nonaka, I. and Kurihara, M., Diet effects on methane production by goats and a comparison between measurement methodologies. *J. Agric. Sci.*, 2008, **146**, 705–715.
40. Pelchen, A. and Peters, K. J., Methane emission from sheep. *Small Ruminant Res.*, 1998, **27**, 137–150.
41. Johnson, D. E., Ward, G. W. and Ramsey, J. J., Livestock methane: Current emissions and mitigation potential. In *Nutrient Management of Food Animals to Enhance and Protect the Environment* (ed. Kornegay, E. T.), Lewis Publishers, New York, 1996, pp. 219–234.
42. Van Soest, P. J., *Nutritional Ecology of the Ruminant*, Cornell University Press, Ithaca, NY, 1994, 2nd edn.
43. Jung, H. G. and Allen, M. S., Characteristics of plant cell walls affecting intake and digestibility of forages. *J. Anim. Sci.*, 1995, **73**, 2774–2790.
44. Haddad, S. G. and Hussain, M. Q., Nutritive value of lentil and vetch straws as compared with alfalfa hay and wheat straw for replacement ewe lambs. *Small Ruminant Res.*, 2001, **40**, 255–260.
45. Chesson, A., Mechanistic models of cell wall degradation. In *Forage Cell Wall Structure and Digestibility* (eds Jung, H. H. *et al.*), ASA–CSSA–SSAA, Madison, WI, USA, 1993, pp. 347–376.
46. Moss, A. R., Environment control of methane production by ruminants. In *Green House Gases and Animal Agriculture* (eds Takahashi, J. and Young, B. A.), Elsevier, Amsterdam, The Netherlands, 2002, pp. 67–76.
47. Yan, T., Porter, M. G. and Mayne, C. S., Prediction of methane emission from beef cattle using data measured in indirect open-circuit respiration calorimeters. *Animal*, 2009, **3**(10), 1455–1462.
48. Ellis, J. L., Kebreab, E., Odongo, N. E., McBride, B. W., Okine, E. K. and France, J., Prediction of methane production from dairy and beef cattle. *J. Dairy Sci.*, 2007, **90**, 3456–3467.
49. Wilkerson, V. A., Casper, D. P. and Mertens, D. R., The prediction of methane production of Holstein cows by several equations. *J. Dairy Sci*., 1995, **78**, 2402–2414.

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