Indian Journal of Animal Sciences **86** (5): 581–588, May 2016/Article
https://doi.org/10.56093/ijans.v86i5.58504

Nutritive value and methane production potential of energy and protein rich feedstuffs fed to livestock in India

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Received: 24 February 2015; Accepted: 28 September 2015

ABSTRACT

Four protein-rich (groundnut cake-GNC, mustard seed cake-MSC, cotton seed cake-CSC and coconut cake-CNC) and 8 energy-rich (wheat grain-WG, barley grain-BG, oat grain-OG, maize grain-MG, wheat bran A-WBA, wheat bran B-WBB, rice bran-RB, chickpea husk/chuni-GC) feedstuffs were evaluated for their carbohydrate and protein fractions, *in vitro* dry matter degradability, *in vitro* methane production and energy loss as methane. Crude protein (CP) and ether extract contents were higher in protein-rich feedstuffs than in energy feedstuffs. High lignin content was noted in CSC, GNC, MSC and RB. Degradable CP fractions of total CP ranged from 0.61 to 0.97 and were higher for protein-rich than energy-rich feedstuffs. On an average, protein-rich feedstuffs had more undegradable CP fraction than the grains or brans. Starch content was highest ($P< 0.001$) in WBB and least in CSC with values of 369 and 37.3 g/kg DM, respectively. Rapidly degradable carbohydrate fraction (C_A) was highest in WG, OG, MG (all energy-rich feedstuffs) and least in RB (6.7 g/kg DM). Similar to the observation made in the protein fractions, protein-rich feedstuffs had more unavailable CHO. Feedstuffs energy loss as methane was highest ($P<$ 0.001) from GC (1.90 Mj/kg DM) and least from MG (1.19 Mj/kg DM). Methane production of the feedstuffs could be predicted from the chemical composition, CP and CHO fractions. On an average, chemical composition and protein fractions were better predictors of CH_4 production *versus* CHO fractions with mean R^2 values of 0.94 and 0.80, respectively. Data on relative methane emission from energy and protein rich feeds could be utilized to prepare diets that will lead to less methane production from ruminants.

Key words*:* Carbohydrate fractions, Feedstuffs, Methane and gas production, Nutritive value, Protein fractions

India is rich in ruminant livestock biodiversity and population (498 million heads) comprising primarily of cattle, buffaloes, sheep, goats and other ruminants like mithun, yak, camel etc., which constitutes nearly 11% of global livestock population (GOI 2007). Enteric methane (CH_4) emission from Indian livestock was 10.07 Tg in 2007 (MEFGOI 2012). Energy- and protein-rich feedstuffs are mostly fed either as sole or in combination to ruminants by most small-holder livestock farmers with the exception of large-holder farmers and commercial dairy owners. Brans, husks, cereal grains, *chunies* (broken legume seed with husk part) and oil cakes differ in chemical composition, rate and extent of degradability. Crude protein (CP) degradability differ among and within feedstuffs due to source, processing method and particle size (Batajoo and Shaver 1998), composition and activity of rumen microbes, chemical and physical properties of the CP (Van Nevel and Demeyer 1988, NRC 2001) and plant or animal origin (Madsen and Hvelplund 1987).

Once ingested, ruminal microbes usually convert major portions of dietry carbohydrate and protein into useful end-products such as volatile fatty acids and microbial protein, and waste products, primarily CH_4 and CO_2. The pattern and concentrations of these end products mainly depend on the chemical composition of the diets (i.e., CHO and protein fractions), their digestibility and intake. Fermentation of feeds rich in cell content and low in cell wall are expected to yield less CH_4, as well as a decrease in the molar proportion of acetate and an increase in the molar proportion of propionate (Widiawati and Thalib 2009). Methane from enteric fermentation contributes in global greenhouse effect (Rossi *et al*. 2001) and represents a loss of dietary energy in ruminants up to 12% of gross energy (GE) intake (McCrabb and Hunter 1999, Waghorn *et al*. 2007).

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De Blas *et al*. (2008) reported that most feed ingredients do not have similar effects on methane production. For example, cereal grains having higher proportion of starchy endosperm resulting in less methane production due to faster fermentation and passage rates. Preston *et al.* (2013) showed that *in vitro* methane production was less with fish meal than that of groundnut meal and differences in CH_4 production seems to be related with protein solubility of fish meal (17%) and groundnut meal (70%). Inclusion of starch rich feedstuffs in the ruminant diets favours the propionate production and reduces rumen pH resulting in inhibition of methanogens and protozoa growth (Boadi *et al*. 2004). There is need to evaluate feedstuffs for detailed analyses of protein and carbohydrates, degradability, energy and *in vitro* methane emission potential to formulate diets for ruminants that will result in reduced enteric methane production and to facilitate the accuracy of predicting enteric methane emission of different feedstuffs. The objective of the study was to estimate CH_4 production from some commonly used protein- and energy-rich feedstuffs for their subsequent use to formulate low CH_4 producing diets for ruminants.

MATERIALS AND METHODS

Samples of 12 commonly used feedstuffs consisting of protein (mustard seed cake-MSC, cotton seed cake-CSC, groundnut cake-GNC and coconut cake-CNC) and energy sources (wheat bran A-WBA, wheat bran B-WBB, barley grain-BG, oat grain-OG, maize grain-MG, wheat grain-WG, chickpea husk with broken seed (gram chuni)-GC, rice bran-RB) were collected from the experimental farm of the Institute and a local market. Samples were dried at 60°C for 48 h and then ground using 1 mm sieve in a mill. Samples were stored for subsequent chemical and biochemical analyses.

Chemical analyses: Feedstuff samples were analyzed for dry matter (DM), nitrogen (N), ether extracts (EE) and ash contents (AOAC 2000); crude protein (Kjeldahl N × 6.25); neutral detergent fibre (NDF) and acid detergent fibre (ADF) (Van Soest *et al*. 1991). Heat stable alpha amylase and sodium sulfite were used in NDF estimation. The NDF and ADF were expressed inclusive of residual ash. Lignin (sa) was determined by solublization of cellulose with sulphuric acid in the ADF residue (Van Soest *et al.* 1991). Cellulose was estimated as the difference between ADF and lignin (sa) in the sequential analysis.

Protein fractionation: Protein of feedstuff samples were partitioned into 5 fractions according to the Cornell net carbohydrate and protein system (CNCPS Sniffen *et al*. 1992) as modified by Licitra *et al.* (1996). These are non-protein CP (NPCP), estimated as the difference between total CP and true CP nitrogen precipitated with sodium tungstate (0.30 M) and 0.5 M sulphuric acid; buffer soluble CP (BSP), calculated as the difference between true protein CP and buffer-insoluble CP estimated with borate-phosphate buffer (pH 6.7 to 6.8) and freshly prepared (1g/10 ml) sodium azide solution. Neutral detergent soluble CP (NDSP) was estimated as buffer-insoluble CP minus ND insoluble CP, whereas acid detergent soluble CP (ADSP) was estimated as the difference between ND insoluble CP and acid detergent insoluble CP. Acid detergent insoluble CP (ADICP) is assumed to be indigestible. All fractions, including CP, were determined as Kjeldahl N × 6.25 using semi-auto analyzer in quadruplet.

Carbohydrate fractionation: Carbohydrate fractions of feedstuffs were estimated according to the CNCPS (Sniffen *et al.* 1992), which classifies carbohydrate fractions according to degradation rate into 4 fractions being C_A rapidly degradable CHO including sugars; C_{B1} intermediately degradable starch and pectins; C_{B2} slowly degradable cell wall and C_C unavailable/lignin bound cell wall. Total CHO (g/kg DM) was determined by subtracting CP, EE and ash contents from 1,000. Structural carbohydrates (SC) were calculated as the difference between NDF and neutral detergent insoluble protein (NDIP), and non-fibre carbohydrates (NFC) were estimated as the difference between total CHO and SC (Caballero *et al.* 2001). For starch estimation, feedstuffs samples were extracted with ethyl alcohol to solubilize free sugars, lipids, pigments and waxes. Residue rich in starch was solubilized with perchloric acid and extract was treated with anthrone-sulphuric acid to determine glucose colorimetrically using standard glucose (Sastry *et al.* 1991).

In vitro *incubation: In vitro* gas production was determined according to the pressure transducer technique of Theodorou *et al*. (1994). Rumen fluid was collected prior to feeding from 2 fistulated adult male Murrah buffaloes fed a wheat straw-concentrate diet (650:350 DM basis). Rumen fluid was filtered through double layer of cheese cloth and bubbled with CO_2 before commencement of incubation. The incubation medium was prepared by sequential mixing of buffer solution, macro mineral solution, micro mineral solution and resazurin solution (Menke and Steingass 1988). Samples (1 g) of air dry feeds were weighed into 4 serum bottles (150 ml capacity). Four serum bottles without substrate were used as blanks. Bottles were continuously fluxed with CO_2 and then 3 ml of reducing solution was added in each bottle. Gassing of bottles with CO_2 continued till the pink color turned colourless. 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 of buffalo rumen liquor inoculums using a 10 ml syringe. Inoculated bottles were sealed and incubated at 39°C with gas production (ml) measured at 12, 24 and 48 h of incubation. The whole process was repeated to have 8 analytical replicates.

Methane measurements: Methane in total gas was measured from 3 bottles incubated for each of the feedstuffs at 12, 24 and 48 h by gas chromatography equipped with a stainless steel column packed with Porapak-Q and a Flame Ionization Detector. One μl was sampled from gas produced using a Hamilton syringe, which was injected manually (pull and push method of sample injection) into the GC, which was calibrated with standard CH_4 and CO_2. Methane was also measured from blanks at the fermentation times selected and used for correction of CH_4 produced from the inoculum. Methane measured was related to total gas to estimate its concentration (Tavendale *et al*. 2005) and converted to energy and mass values using 39.54 Kj/l CH_4 and 0.716 mg/ml CH_4 factors, respectively (Santoso *et al.* 2007).

In vitro *dry matter digestibility (IVDMD) and energy of feedstuffs:* For determination of IVDMD of the feedstuffs, the method of Tilley and Terry (1963) was followed. Samples were incubated in triplicate with ruminal inoculum from the 2 fistulated buffaloes described previously. Provision was also made for blanks as described for *in vitro* gas production. Digestibility was estimated as the difference between DM incubated and residual DM at the end of the second stage of digestion. A second run was carried out to have six analytical replicates. The GE of the feedstuffs was measured with a bomb calorimeter using benzoic acid as the standard.

Statistical analyses: Data were subjected to analysis of variance using the GLM procedure of SAS (2002) in a completely randomized design. The model used was:

$$Y_{ij}= \mu + F_i + E_{ij}$$

where Y_{ij}, individual observation of the variable; F_i, fixed of the ith feed (i= 1–12); μ, overall mean; E_{ij}, random error associated with Y_{ij} not accounted in the fixed effect. Means were separated using Fisher's LSD and all statistical tests were at α=0.05 level of significance. Differences among means with P<0.05 were accepted as representing statistically significant. Correlation analysis was used to establish relationships between the measured variables and CH_4 production. Forward stepwise multiple regression analysis was used to develop prediction equations for CH_4 production (DDM g/g) using PROC REG of SAS (2002).

RESULTS AND DISCUSSION

Chemical composition of feeds: Crude protein and EE contents of the oil seed cakes (MSC, GNC, CNC and CSC) were higher (P<0.001) than cereal grains or brans (Table 1). High lignin content was noted in CSC, GNC, MSC and rice bran. Chickpea consistently had higher (P < 0.001) NDF, ADF and cellulose contents.

Protein fractions and carbohydrate fractions of feedstuffs: Degradable CP fractions (BSP, NDSP and ADSP) of the feedstuffs total CP ranged from 0.61 to 0.97 (Table 2). Proportion of degradable protein fractions was more in protein-rich than energy-rich feedstuffs. On an average, protein-rich feedstuffs had more undegradable CP fraction than the grains or brans. Total CHO varied from 466 in MSC to 900 g/kg DM in GC (Table 3). Similar trend was observed in the SC content of the feedstuffs. Starch content was highest (P< 0.001) in WBB and least in CSC with values of 369 and 37.3 g/kg DM, respectively. Rapidly degradable carbohydrate fraction (C_A) was highest (P< 0.001) in WG, OG, MG (all energy-rich feedstuffs) and least in RB (6.7 g/kg DM). Similar to the observation made in the protein fractions, protein-rich feedstuffs had more unavailable CHO.

Total gas and methane production: Total gas production was higher (P<0.001) for cereal grains than oil cakes at 12, 24 and 48 h of incubation (Table 4). Methane either as proportion of total gas (not shown) or expressed as ml/g DM was consistently higher for WG and MSC and lowest for RB and GC. The IVDMD of the feedstuffs ranged from 345 g/kg DM in GC to 897 g/ kg in WG (Table 5). Methane production (g/kg DM) at 24 h of incubation was highest (P<0.001) for WG and least for RB. Gross energy of feedstuffs differ significantly (P<0.001) being highest in

Table 1. Chemical composition (g/kg DM*) of energy and protein feedstuffs

Feedstuffs	Class	CP	OM	EE	NDF	ADF	Cellulose	Lignin(sa)
Barley grain	Energy	103	943	15.2	414	157	113	23.0
Chickpea husk	Energy	45.0	951	5.88	672	648	592	55.5
Maize grain	Energy	99.1	979	47.7	364	61.8	47.6	15.5
Oat grain	Energy	96.4	963	52.3	351	156	134	12.5
Rice bran	Energy	70.1	803	45.6	668	523	249	128
Wheat bran A	Energy	132	929	27.1	560	174	121	39.8
Wheat bran B	Energy	154	940	34.9	399	121	86.0	31.4
Wheat grain	Energy	117	973	14.2	319	43.6	30.8	7.55
Coconut cake	Protein	254	937	61.3	596	228	195	38.9
Cotton seed cake	Protein	240	951	72.0	517	365	261	100
Groundnut cake	Protein	332	924	106	322	247	152	81.6
Mustard seed cake	Protein	348	895	82.0	264	223	123	72.2
SEM		2.43	1.9	1.583	5.2	5.27	2.69	1.766
LSD at P<0.05		23.9	14	10.16	111	112.6	29.3	71.5
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

* DM, dry matter; CP, crude protein; OM, organic matter; EE, ether extract; NDF, neutral detergent fibre expressed inclusive of residual ash; ADF, acid detergent fibre expressed inclusive of residual ash; Lignin(sa), lignin solubilized with sulphuric acid.

Table 2. Protein fractions of energy and protein feedstuffs (g/kg DM*)

Feedstuffs	Class	NPCP	BSP	NDSP	ADSP	ADIP
Barley grain	Energy	3.36	13.8	67.1	16.4	2.50
Chickpea husk	Energy	3.31	6.44	17.9	3.44	13.9
Maize grain	Energy	3.67	20.3	64.5	8.44	2.19
Oat grain	Energy	3.14	34.4	51.2	6.25	1.41
Rice bran	Energy	1.41	8.12	43.5	2.04	15.6
Wheat bran A	Energy	22.4	32.8	48.0	24.5	4.22
Wheat bran B	Energy	15.2	38.3	66.2	32.2	2.04
Wheat grain	Energy	1.70	28.6	61.7	23.4	1.26
Coconut cake	Protein	1.20	20.7	54.9	164	12.8
Cotton seed cake	Protein	23.3	76.3	126	1.72	12.7
Groundnut cake	Protein	29.6	173	115	6.56	7.50
Mustard seed cake	Protein	130	76.4	123	7.81	10.5
SEM		1.384	3.934	5.54	1.398	0.504
LSD at P< 0.05		7.77	62.78	69.4	7.92	1.03
P value		<0.001	<0.001	<0.001	<0.001	<0.001

* DM, dry matter; NPCP, non-protein CP; BSP, buffer soluble CP; NDSP, neutral detergent soluble CP; ADSP, acid detergent soluble CP; ADIP, acid detergent insoluble CP.

Table 3. Carbohydrate and its fractions of the energy and protein feedstuffs (g/kg DM*)

Feedstuffs	Class	TCHO	NSC	SC	Starch	C_A	C_{B1}	C_{B2}	C_c
Barley grain	Energy	824	429	395	347	82.6	347	339	55.2
Chickpea husk	Energy	900	245	655	64.5	181	64.5	522	133
Maize grain	Energy	832	479	354	272	207	272	316	37.1
Oat grain	Energy	814	471	343	224	247	224	313	29.9
Rice bran	Energy	686	36.1	650	183	6.70	29.4	342	308
Wheat bran A	Energy	770	239	531	307	84.7	154	436	95.5
Wheat bran B	Energy	751	386	365	369	74.0	312	290	75.3
Wheat grain	Energy	842	548	294	270	278	270	276	18.1
Coconut cake	Protein	622	203	419	81.8	121	81.8	325	93.4
Cotton seed cake	Protein	639	136	502	37.3	98.8	37.3	262	241
Groundnut cake	Protein	486	179	308	55.6	123	55.6	112	196
Mustard seed cake	Protein	466	220	245	58.9	161	58.9	72.3	173
SEM		3.4	6.19	5.8	12.01	10.431	9.38	5.42	4.24
LSD at P< 0.05		46	155.3	135	58.5	44.13	35.7	119.3	72.9
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

* DM, dry matter; TCHO, total carbohydrates; NSC, non-structural carbohydrates; SC, structural carbohydrates; C_A, rapidly degradable sugars; C_{B1}, intermediately degradable starch and pectins; C_{B2}, slowly degradable cell wall; C_C, unavailable/lignin bound cell wall.

GNC (21.8 Mj/kg DM) and lowest in GC (15.8 Mj/kg DM). Feedstuffs energy loss as methane was highest (P<0.001) from GC (1.90 Mj/kg DM) and least from MG (1.19 Mj/kg DM).

Regression equations for methane production: Methane production of the feedstuffs could be predicted from the chemical composition, CP and CHO fractions (Table 6). On the average, chemical composition and protein fractions were better predictors of CH_4 production *versus* CHO fractions with mean R^2 values of 0.94 and 0.80, respectively.

Chemical composition of feeds: Evaluated energy- and protein-rich feedstuffs are adequate to meet the nutritional requirement of animals when supplemented to a basal diet of poor quality forages during the winter season. The CP, NDF, ADF, cellulose and lignin contents of feedstuffs recorded in our study are consistent with earlier reports (Sniffen *et al*. 1992, Kumar *et al.* 2007, Mondal *et al.* 2008, Gupta *et al.* 2011, Kim *et al*. 2012). Despite the high energy content of the feedstuffs, most of the grains and their by-products had relatively low protein contents ranging from 70.1 to 117 g/kg DM. Low protein content of chickpea husk; a legume by-product could be attributed to its high fibre content. High lignin content of RB may have led to the observed low CP content. High gross energy values in the cereal grains and their by-products indicate that these feedstuffs are good sources of digestible and metabolizable energy (McDonald *et al.* 2002).

Protein fractions and carbohydrate fractions of feedstuffs: Higher proportion of NPCP in MSC *versus* other feedstuffs makes it suitable in diets that have high CHO C_A

Table 4. Total gas (ml/g DM*) and methane (CH_4) production (ml/g DM) of the energy and protein feedstuffs

		12 h		24 h		48 h	
Feedstuffs	Class	Gas	CH_4	Gas	CH_4	Gas	CH_4
Barley grain	Energy	67.8	18.6	121	35.9	169	53.1
Chickpea husk	Energy	60.5	6.69	114	17.3	173	38.7
Maize grain	Energy	71.0	13.2	125	26.2	174	39.1
Oat grain	Energy	74.0	19.0	126	32.0	174	45.6
Rice bran	Energy	61.5	9.08	106	15.7	151	24.2
Wheat bran A	Energy	67.8	18.1	120	36.0	164	50.1
Wheat bran B	Energy	72.0	21.1	122	38.1	166	52.3
Wheat grain	Energy	75.0	23.2	131	44.3	179	62.3
Coconut cake	Protein	64.0	15.6	115	30.4	166	51.7
Cotton seed cake	Protein	62.5	12.5	109	22.0	155	34.7
Groundnut cake	Protein	65.5	15.4	114	27.7	158	40.6
Mustard seed cake	Protein	64.5	19.5	115	37.9	160	53.7
SEM		0.63	0.502	0.7	0.46	0.8	0.64
LSD at P< 0.05		1.6	1.02	2	0.9	3	1.7
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*DM, dry matter; Expressed as a proportion of total gas production.

Table 5. *In vitro* dry matter digestibility (IVDMD, g/kg DM*), methane (CH_4) production, gross energy (GE, Mj/kg DM) and energy loss as CH_4 (Mj/kg DM) from energy and protein feedstuffs at 24 h of incubation

Feedstuffs	Class	IVDMD	CH_4 g kg DM	CH_4 g/kg DDM	GE	CH_4
Barley grain	Energy	743	25.8	34.7	19.0	1.86
Chickpea husk	Energy	345	12.4	36.0	15.8	1.93
Maize grain	Energy	847	18.8	22.2	19.9	1.19
Oat grain	Energy	698	23.0	32.9	18.0	1.76
Rice bran	Energy	386	11.2	29.4	17.7	1.57
Wheat bran A	Energy	734	25.8	35.1	18.6	1.88
Wheat bran B	Energy	784	27.3	34.9	19.1	1.87
Wheat grain	Energy	897	31.8	35.4	18.0	1.90
Coconut cake	Protein	866	21.8	25.1	18.5	1.35
Cotton seed cake	Protein	503	15.7	31.3	21.2	1.68
Groundnut cake	Protein	724	19.9	27.4	21.8	1.47
Mustard seed cake	Protein	794	27.2	34.3	21.2	1.84
SEM		13.1	0.33	0.86	0.16	0.046
LSD at *P*< 0.05		69	0.5	3.0	0.2	0.06
P value		<0.001	<0.001	<0.001	<0.001	<0.001

* DM, dry matter; DDM, degraded dry matter; Expressed as a proportion of gross energy.

fraction to maximize efficiency of microbial protein synthesis. Protein-rich feedstuffs have significant amount of NDSP, some of which will escape digestion in the rumen to the lower gut (Sniffen *et al.* 1992). The proportion of NDSP utilized in the rumen *versus* lower gut depends on rate of ruminal passage and digestion. Due to ADSP's association with plant cell wall, it contains a high proportion of rumen undegradable protein with a degradation rate of less than 0.015/h (Sniffen *et al*. 1992). As it was highest in CNC, it has the possibility to supply more protein to the duodenum than the other feedstuffs. Higher ADIP observed in RB *versus* the other feedstuffs could be attributed to its high lignin content.

Total carbohydrate contents were more in cereal grains and brans than oilseed cakes. This was expected as they are classified as energy-rich feedstuffs. Values for TCHO, NSC, SC and starch of different feedstuffs are consistent with values reported earlier for these feeds (Singh *et al.* 2002, Gupta *et al*. 2011, Mirzaii *et al*. 2005, Kamble *et al*. 2010). Slowly degradable carbohydrate fraction (C_{B2}) was more in brans and GC hulls than oilseed cakes, which implies that these feedstuffs will deliver adequate amounts of rumen undegradable carbohydrate fraction to the small intestine. The highest and lowest contents of C_C fraction (carbohydrate bound to lignin) in RB (308 g/kg DM) and WG (18.1 g/kg DM) may be attributed to their lignin contents. Higher C_C and low C_{B1} in oilseed cakes *versus* grains reported earlier (Kamble *et al.* 2010, Gupta *et al.* 2011) is consistent with the present study.

Total gas and methane production, dry matter

Table 6. Linear regression equations to predict CH_4* (g/g DDM) from chemical constituents, protein and carbohydrate fractions of energy and protein feedstuffs

	SEM	R^2	*P*
EnergyCH_4 = 36.146 + 0.147 * CP – 0.222 * EE – 0.035 * ADF – 0.023 * Hemicellulose	0.0802	0.92	<0.001
CH_4 = 28.347 – 0.267 * NPCP + 0.599 * ADSP – 0.852 * ADIP	0.0450	0.90	<0.001
CH_4 = –10.868 + 0.014 * SC + 0.101 * Starch + 0.066 * C_A	0.0689	0.77	<0.001
ProteinCH_4 = 392.031 – 0.404 * OM+ 0.027 * NDF	0.0327	0.96	<0.001
CH_4 = 8.969 + 0.143 * NPCP + 0.049 * BSP+ 0.046 * NDSP + 0.107 * ADSP	0.0497	0.97	<0.001
CH_4 = 18.169 + 0.104 * NSC – 0.021 * SC	0.0398	0.83	<0.001

*CH_4, methane; DDM, degraded dry matter; CP, crude protein; EE, ether extract; ADF, acid detergent fibre; NPCP, non-protein CP; ADSP, acid detergent soluble CP; ADIP, acid detergent insoluble CP; SC, structural carbohydrates; C_A, rapidly degradable sugars; OM, organic matter; NDF, neutral detergent fibre; BSP, buffer soluble CP; NDSP, neutral detergent soluble CP; NSC, non-structural carbohydrates.

degradability and CH_4 % gross energy: Lower gas production noted for CSC, GNC, RB, GC and CNC could be a result of their lower non-structural carbohydrates and starch contents. The amount of gas produced from a feedstuff relies mainly on its chemical composition and rate and extent of digestibility. Higher NDF and ADF contents of a feed inhibit starch degrading bacteria through reduced availability of rapidly fermented carbohydrates (Wilson and Hatfield 1997), which in turn suppresses gas production (Njidda and Nasiru 2010). Feedstuffs which have higher gas production and IVDMD usually tend to have higher CH_4 production per gram DM incubated (Durmic *et al.* 2010, Njidda and Nasiru 2010, Jayanegara *et al*. 2011). This was consistent with our observations on gas production, IVDMD and methane production for all the feedstuffs except MG and CNC, where methane production was less in spite of higher IVDMD and total gas production. Consistent with this observation, Machmüller *et al.* (2003) reported that refined oils that are high in medium-chain fatty acids (*i.e.* C_{12} and C_{14}), such as coconut oil, palm kernel oil, high-laurate canola oil, or pure myristic acid are particularly effective in reducing CH_4.

Bonhomme (1990) reported that grains rich in soluble carbohydrates increase the population of ciliate protozoa and stimulate their hydrogen transfer to methanogens resulting in more methane production. Higher methane production from grains can be attributed to their higher NSC and IVDMD, while lower methane from MG suggests that this feedstuff favorably modulates the rumen fermentation to produce less methane than expected considering their higher total gas production and IVDMD.

Generally, the type of CHO present in feedstuff is thought to dictate CH_4 production via shifts in the ruminal microbial population (Johnson and Johnson 1995). High soluble CHO content is suggested to promote the production of propionate in the rumen, lower ruminal pH and inhibit methanogen growth thereby reducing CH_4 production per unit of OM fermented (Van Kessel and Russell 1996). Boadi and Wittenberg (2002) found CH_4 production (L CH_4/kg digestible OM intake) was 25% higher for low *versus* medium or high nutritional quality diets in a feeding study where intake was restricted to 2% of body weight. Lower CH_4 losses with the high quality diet were expected as lower fibre content shifted fermentation towards propionate production.

Regression between methane and chemical composition: Chemical composition, intake and other degradability characteristics of feedstuffs have been used to develop equations to predict CH_4 production. Recently, Ramin and Huhtanen (2013) utilized respiratory studies data from 52 research papers involving 298 treatments with 207 cattle and 91 sheep diets and found that intake, organic matter degradability and dietary concentrations of NDF, NFC and EE as variables are best fit to predict CH_4 energy (CH_4-E) as a proportion of gross energy (GE) intake. In the present study, chemical composition, dry matter digestibility, CHO and protein fractions were used to predict CH_4 production. Results showed that CH_4 production could be predicted from both energy- and protein-rich feedstuffs, but protein-rich feedstuffs had better coefficients than energy-rich feedstuffs (0.97, 0.96 and 0.83 *versus* 0.92, 0.90 and 0.77, respectively). With the advances in feed and fodder evaluation, efforts are being made to improve the precision of methane production prediction by considering variables that are more related to methane emission.

Overall, rice bran and gram *chuni* were low in CP, high in structural carbohydrate which resulted in low *in vitro* dry matter degradability. Both coconut cake and maize grain produced less CH_4 despite high gas production and *in vitro* dry matter degradability. Their CHO and protein components may have modulated the *in vitro* rumen fermentation for more propionate production.Feedstuffs chemical composition and protein fractions were better predictors of CH_4 production *versus* carbohydrate fractions with mean R^2 values of 0.94 and 0.80, respectively. Feedstuffs data on methane production and their energy loss as methane could be utilized judiciously in formulating low CH_4 emission diets for ruminants.

ACKNOWLEDGEMENT

Authors thank Ministry of Environment, Forests and Climate change, Government of India, New Delhi for providing financial support to carry out this work. We also thank the Head, PAR Division and Director, IGFRI, Jhansi for providing the necessary facilities.

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