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Nutrient composition, *in vitro* true digestibility and methane production potential of feed resources of North Western Himalayan region

A KANNAN[1], R BHAR[2], S RADOTRA[3], G MAL[4], B SINGH[5], R V JADHAV[6],
MANJEET KUMAR[7] and ALOK GAURAV[8]

ICAR-Indian Veterinary Research Institute, Regional Station, Palampur, Himachal Pradesh 176 061 India

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ABSTRACT

The present study assessed the nutrient composition, *in vitro* digestibility and methane production potential of the commonly fed feed resources of North Western Himalayan region. The feed resources were collected from different parts of North Western Himalayan region and dried at 60°C until constant weight and used for further analysis. The *in vitro* digestibility and methane production potential were determined using the *in vitro* gas production technique. The results indicated that in concentrates, cereal grains had significantly high ME value, high digestibility, low methane production potential and low methane energy loss as compared to brans. In roughages, tree fodders, had significantly high digestibility, high ME value, low methane production potential and low methane energy loss as compared to grasses and crop residues. In this study, there was strong negative correlation between OM and methane production; and strong positive correlation between ash, lignin, ADF, cellulose and NDF contents and methane production in concentrates. In contrast to concentrates, in roughages strong positive relationship was observed between OM contents and methane emission. Positive correlations between NDF, HC, cellulose and methane production; and negative relationship between methane production and lignin, ash and EE contents were also observed in roughages. Regression equations developed for predicting methane production showed high level of predictability for concentrates as compared to roughages. The data on nutrient composition, methane production potential reported for various feedstuffs in this study could be utilized judiciously in formulating low methane emission diets for feeding ruminants in the region.

Key words: Chemical composition, Digestibility, Feedstuffs, Methane production potential, North western himalayan region

Globally carbon foot print per kg fat and protein corrected milk is substantially higher in India as compared to Western countries (Opio *et al.* 2013). Methane produced from enteric fermentation of feeds by ruminants is the primary reason and according to a report from Ministry of Environment and Forests, Government of India (MEFGOI 2012), enteric methane emission from Indian livestock was 10.07 Tg in 2007 representing more than 50% of methane emission from India. Therefore, there is an increased pressure on Indian livestock industry to reduce carbon foot print (i.e.) methane emission. Several strategies have been worked out for reducing methane emission, however, only dietary modifications especially feeding balanced ration has been recommended as sustainable long term solution (Kannan and Garg 2009, Kannan *et al.* 2011, FAO 2012). Feeds differ in their methane production potentiality depending upon chemical composition and phytochemical content. Identification of low methane producing feeds and developing rations using them may be one of the feasible options to reduce methane and ultimately the carbon foot print of Indian livestock. Though methane production potential of feed resources of some regions have been carried out by different workers (Singh *et al.* 2011, 2012, Pal *et al.* 2015, Tripathi *et al.* 2016), however, the feed resources of North Western Himalayan region is not yet studied.

In Himalayan region, livestock rearing is the primary occupation of tribal communities and less privileged population especially in rural areas. Traditionally, these animals are reared by grazing or browsing the native pasture and shrubs (Kannan *et al.* 2014, Kumar *et al.* 2017) and however, there is limited information available on the nutritive value of feed resources of North Western Himalayan region (Bhar *et al.* 2015). Therefore, the present study was carried out to evaluate the chemical constituents,

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Present address: [1]Senior Scientist (akanna72@gmail.com), Animal Nutrition Laboratory; [2,4,5]Principal Scientist (rbharplp@gmail.com, gorak14@yahoo.com, bsbpalampur@rediffmail.com). [3]Principal Scientist (sradotra@rediffmail.com), ICAR-IGFRI, Palampur. [6]PhD Scholar (drravindravilas@gmail.com), ICAR-IVRI, Izatnagar. [7]Veterinary Assistant Surgeon (dr.manjeet.13@gmail.com), Himachal Pradesh. [8]Veterinary Assistant Surgeon (alokbuddy@yahoo.com), Uttar Pradesh.

metabolisable energy value, *in vitro* fermentation and methane production potential of feed resources of the region for inclusion in ruminant diets for ration formulation and methane mitigation.

MATERIALS AND METHODS

The commonly available feed resources of concentrates, dry roughages, green fodders and tree leaves (n=4 for each feed) were collected from different parts of North Western Himalayan region. Samples were dried in hot air oven at 60°C until constant weight and ground to pass 1 mm screen and used for chemical analysis and *in vitro* fermentation. The dry matter, nitrogen, ether extract and ash contents of the feed resources were estimated by the AOAC (2000) methods. The neutral detergent fibre and acid detergent fibre were determined as per the methods of Van Soest *et al.* (1991) without sodium sulphite and α-amylase and expressed with residual ash. Lignin was determined by the method of Robertson and Van Soest (1988).

In vitro *fermentation:* The *in vitro* fermentation characteristics were determined using the *in vitro* gas production technique as per the procedure of Menke and Steingass (1988). Rumen liquor was collected from two rumen cannulated adult male cattle before feeding in pre-warmed thermos flask, strained through a four layered muslin cloth and pooled together under continuous flushing of CO_2 to maintain anaerobic condition which was used as inoculum. The donor animals were fed with 60% wheat straw and 40% concentrate ration according to the requirements. About 200 mg of feed sample was taken in a glass syringe and 30 ml of buffered rumen liquor was added and incubated for 24 h in a water bath at 39°C. Gas measurements were carried out at 0, 2, 4, 6, 8, 10, 12 and 24 h after incubation. Incubations were stopped at 24 h by dipping the syringes in cold water. The feedstuffs were incubated in triplicate on two different days yielding six parallel measurements. The methane in fermented gas mixture was estimated by gas liquid chromatography as described in detail earlier (Jadhav *et al.* 2016). Methane was converted to energy and mass values using 39.54 kJ/l CH_4 and 0.716 mg/ml CH_4 factors, respectively. The ME value of concentrates and roughages were calculated by using the prediction equations of Menke and Steingass (1988) whereas TDN was calculated from ME value as per the equations of NRC (1989).

The prediction equations are

For concentrate feeds

ME (MJ/kg DM) = 1.06 + 0.1570 × gas produced (ml/200 mg DM) + 0.0084 × CP (g/kg DM) + 0.022 × EE (g/kg DM) – 0.0081 × ash (g/kg DM)

For roughage feeds

ME (MJ/kg DM) = 2.20 +0.13576 × gas produced (ml/200 mg DM) + 0.0057× CP (g/kg DM) + 0.0002859 × EE^2 (g/kg DM)

For estimation of *in vitro* true digestibility, about 400 mg feed sample was taken in a glass syringe and 40 ml of buffered rumen liquor was added and incubated in water bath at 39°C, for 24 h. After the end of 24 h of incubation, contents in the syringes were transferred into spoutless beakers by repeated washing with neutral detergent solution (NDS). The contents were refluxed for 1 h and filtered through pre-weighed Gooch crucible (Grade 1). The residues were dried in hot air oven and weighed. The truly degraded organic matter was estimated from TDDM by ashing at 450°C for 4 h.

$$\text{IVTDMD}(\%) = \frac{\text{Weight of DM of sample incubated} - \text{weight of NDF residue}}{\text{Weight of DM of sample incubated}} \times 100$$

$$\text{IVTOMD}(\%) = \frac{\text{Weight of OM of sample incubated} - (\text{weight of NDF residue-ash})}{\text{Weight of OM of sample incubated}} \times 100$$

Statistical analysis: The observations of chemical composition, gas, methane production and digestibility were analyzed for statistical significance by the analysis of variance procedure using a general linear model approach of SPSS (2000) in a completely randomized design as;

$$Y_{ijk} = \mu + T_i + e_{ij},$$

where Y_{ijk}, observation mean; μ, general mean; T_i , effect of i^{th} feed (I,6); e_{ij} random error.

The significant means were separated, when f was <0.05, using Tukey test. Correlation coefficients among the variables and methane production and digestibilities were calculated by the Pearson method. The stepwise multiple regression method was used to develop prediction equations to predict methane and *in vitro* digestibilities from chemical composition by using SPSS (2000) software.

RESULTS AND DISCUSSION

Chemical composition: The chemical composition of concentrate feed and roughages are given in Table 1 and 2 respectively. Among the concentrates, the chemical composition showed significantly (P<0.001) wide variations. Crude protein and EE contents of oil seed cakes were significantly (P<0.001) higher than cereal grains and brans. The CP concentration ranged from 8.80 to 45.23% and was the highest in soybean meal and lowest in broken rice. The EE concentrate ranged from 0.65 to 13.76 and was highest in rice polish. The concentration of EE was high in oil cakes, medium in brans and low in deoiled cake/meals/brans and other feeds. Ash content was high in deoiled rice bran and rice polish and low in cereal grains. High fibre and lignin contents were observed in brans. The CP, EE, NDF, ADF and lignin contents of concentrates observed in our study were consistent with earlier reports (Gupta *et al.* 2011, Garg *et al.* 2012).

Roughages also showed significant (P<0.001) differences in chemical composition (Table 2). The organic matter content was higher (P<0.001) in green forages and grasses as compared to crop residues and tree fodders. The concentrations of CP were in the range of 3.02 to 24.53%.

Table 1. Chemical composition of concentrate feed stuffs (on % DM basis)

Local name	Class	OM	CP	EE	NDF	ADF	Lignin
Maize	Energy	97.86^{ab}	9.29^{g}	4.07^{c}	18.19^{c}	5.25^{f}	1.33^{de}
Wheat	Energy	97.81^{ab}	11.14^{f}	2.17^{def}	21.48^{c}	6.10^{f}	1.53^{de}
Jowar	Energy	97.37^{b}	8.81^{g}	2.72^{cde}	18.42^{c}	6.33^{ef}	1.53^{de}
Broken rice	Energy	98.90^{a}	8.80^{g}	1.98^{def}	23.20^{c}	11.14^{cde}	0.98^{f}
Wheat bran	Energy	94.19^{c}	15.29^{d}	3.24^{cd}	53.14^{a}	14.54^{bc}	3.16^{c}
Deoiled rice bran	Energy	87.26^{e}	14.84^{d}	0.65^{f}	50.65^{a}	21.60^{a}	5.77^{b}
Rice polish	Energy	87.11^{e}	13.11^{e}	13.76^{a}	39.40^{b}	21.67^{a}	6.94^{a}
Groundnut meal	Protein	93.30^{c}	42.67^{b}	1.00^{f}	15.71^{c}	7.50^{def}	3.43^{c}
Mustard cake	Protein	94.55^{c}	37.10^{c}	7.88^{b}	24.52c	15.73^{bc}	3.33^{c}
Deoiled mustard cake	Protein	91.67^{d}	37.31^{c}	0.83f	38.75^{b}	20.82^{ab}	3.22^{c}
Soybean meal	Protein	91.05^{d}	45.23^{a}	1.12^{ef}	21.50^{c}	11.91^{cd}	1.75^{d}
Groundnut cake	Protein	93.86^{c}	42.11^{b}	7.95^{b}	25.20^{c}	17.57^{ab}	3.41^{c}
SEM	-	0.18	0.21	0.30	17.71	2.9	0.06
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different superscripts within the same column differ significantly.

Table 2. Chemical composition of dry and green roughages (on % DM basis)

Local name	Class	OM	CP	EE	NDF	ADF	Lignin
Wheat straw	Dry	89.98^{cde}	3.02^{j}	1.16^{k}	77.60^{cd}	52.69^{a}	8.91^{bc}
Paddy straw	Dry	83.90^{j}	3.37^{j}	1.44^{j}	72.47^{efg}	45.10^{bc}	10.18^{a}
Jowar straw	Dry	86.95^{gh}	3.88^{j}	1.79^{ij}	76.59^{cde}	46.46^{bc}	9.29^{ab}
Rice husk	Dry	84.15^{j}	3.17^{j}	1.71^{jk}	68.51^{gh}	41.10^{efg}	9.15^{b}
Oat fodder	Green fodder	89.05^{def}	12.74^{d}	2.59^{ef}	64.10^{ij}	36.78^{hi}	3.42^{h}
Chrysopogon	Grass	92.16^{a}	7.96^{fg}	2.02^{ghij}	84.04^{a}	41.75^{def}	3.78^{gh}
Festuca	Grass	91.77^{ab}	8.68^{f}	3.57^{bc}	80.74^{abc}	38.76^{fgh}	4.90^{ef}
Haathi grass	Grass	91.16^{abc}	6.72^{hi}	3.21^{cd}	70.27^{fgh}	37.66^{gh}	4.23^{fgh}
Para grass	Grass	92.22^{a}	7.32^{ab}	2.40^{fgh}	78.21^{bcd}	39.41^{fgh}	4.32^{fg}
Cango signal	Grass	91.86^{ab}	6.70^{hi}	2.36^{fghi}	73.31^{ef}	36.71^{jk}	4.05^{gh}
Chij	Grass	92.12^{a}	5.78^{i}	2.75^{def}	82.20^{ab}	40.97^{efg}	5.43^{e}
Setaria	Grass	90.51^{bcd}	6.62^{hi}	2.83^{def}	74.81^{de}	31.78^{hi}	3.57^{gh}
Bokna	Grass	85.60^{hij}	11.15^{e}	3.02^{cde}	54.84^{k}	41.56^{def}	8.16^{cd}
Rhodes grass	Grass	90.02^{cde}	9.02^{f}	1.97^{hij}	62.42^{j}	33.37^{ij}	3.70^{gh}
Doob grass	Grass	88.76^{ef}	12.29^{de}	3.21^{bcd}	66.73^{hi}	43.53^{cde}	8.56^{bcd}
Bamboo	Tree fodder	85.39^{ij}	19.68^{c}	2.34^{fghi}	79.66^{bc}	47.36^{b}	8.41^{bcd}
Biul	Tree fodder	88.36^{fg}	24.53^{a}	3.80^{b}	48.41^{l}	24.59^{l}	4.13^{fgh}
Subabul	Tree fodder	86.95^{hi}	21.34^{b}	6.11^{a}	45.27^{lm}	20.72^{m}	8.77^{bc}
Mulberry	Tree fodder	79.52^{k}	18.61^{c}	6.34^{a}	41.80^{m}	28.22^{kl}	7.72^{d}
SEM		0.23	0.14	0.04	1.87	1.49	0.08
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different superscripts within the same column differ significantly.

The CP concentration was highest (P<0.001) in tree fodders followed by green forages and grasses and lowest in crop residues. The CP contents of the crop residues were well below the maintenance requirement for feeding ruminants. The NDF and ADF concentrations were higher for most of the roughages. Lignin content was higher (P<0.001) in crop residues as compared to green fodder or grasses. The higher lignin content in tree fodders is mainly due to the presence of plant secondary metabolites, especially tannins. Similar to our findings, Singh *et al.* (2011), Garg *et al.* (2012), Singh *et al.* (2012), Ramachandran *et al.* (2015) and Tripathi *et al.* (2016) also reported significant variation in chemical composition of different roughages. Difference in the values in roughages could be due to the differences in plant variety, harvesting stage, soil type and environmental conditions.

Gas production, digestibility and metabolisable energy value: The quantity of gas produced varied markedly (P<0.001) among the feeds (Tables 3, 4). Among concentrates, cereal grains produced more gas (P<0.001) than other feed resources. Jowar produced the highest amount of gas and deoiled rice bran and groundnut meal produced the lowest amount of gas. Singh *et al.* (2016) also observed more gas from cereal grains than other feeds. The amount of gas produced from feeds depends upon chemical composition and feed degradability and the gas is produced mainly when substrate is fermented to acetate and butyrate.

Table 3. Total gas production, *in vitro* digestibility and metabolisable energy values in concentrates

Local name	Total gas (ml/200mg DM)	ME (MJ/kg DM)	ME (MCal/kg DM)	TDN (%)	TDMD (%)	TOMD (%)
Maize	57.38^{ab}	13.49^{abc}	3.22^{abc}	82.49^{abc}	85.73^{a}	87.85^{a}
Wheat	58.38^{ab}	13.39^{bc}	3.20^{bc}	81.96^{bc}	85.03^{a}	86.47^{a}
Jowar	60.74^{a}	13.69^{ab}	3.27^{ab}	83.60^{ab}	83.60^{a}	84.91^{a}
Broken rice	55.39^{b}	12.59^{d}	3.01^{d}	77.68^{d}	82.13^{ab}	83.24^{abc}
Wheat bran	50.33^{c}	11.43^{e}	2.73^{e}	71.45^{e}	73.06^{abc}	75.22^{abc}
Deoiled rice bran	40.29^{e}	9.81^{f}	2.34^{f}	62.75^{f}	66.87^{bc}	68.65^{bc}
Rice polish	42.21^{e}	12.86^{cd}	3.07^{cd}	79.13^{cd}	65.95^{c}	68.09^{c}
Groundnut meal	40.58^{e}	11.78^{e}	2.82^{e}	73.32^{e}	81.08^{abc}	83.38^{abc}
Mustard cake	43.88^{de}	13.24^{bcd}	3.16^{bcd}	81.16^{bcd}	80.36^{abc}	82.17^{abc}
Deoiled mustard cake	43.65^{de}	11.91^{e}	2.85^{e}	74.00^{e}	77.07^{abc}	78.88^{abc}
Soybean meal	49.10^{c}	13.54^{abc}	3.24^{abc}	82.77^{abc}	84.74^{a}	86.30^{a}
Groundnut cake	46.59^{cd}	14.16^{a}	3.38^{a}	86.10^{a}	80.27^{abc}	82.99^{abc}
SEM	2.2	0.05	0.00	1.54	29.48	26.50
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different superscripts within the same column differ significantly.

Table 4. Total gas production, *in vitro* digestibility and metabolisable energy values in roughages

Local name	Total gas (ml/200mg DM)	ME (MJ/kg DM)	ME (MCal/kg DM)	TDN (%)	TDMD (%)	TOMD (%)
Wheat straw	26.46^{fg}	6.00^{gh}	1.43^{gh}	42.31^{gh}	40.67^{ij}	41.52^{i}
Paddy straw	26.34^{fg}	6.03^{g}	1.44^{g}	42.45^{g}	40.94^{ij}	41.53^{i}
Jowar straw	30.39^{e}	6.64^{f}	1.59^{f}	45.73^{f}	42.98h^{i}	43.47^{hi}
Rice husk	23.63^{g}	5.68^{gh}	1.36^{gh}	40.57^{gh}	36.33^{j}	37.95^{i}
Oat fodder	42.40^{a}	8.87^{a}	2.12^{a}	57.73^{a}	75.27^{b}	76.53^{b}
Chrysopogon	35.02^{d}	7.52^{e}	1.80^{e}	50.48^{e}	48.07^{hi}	48.98^{gh}
Festuca	36.67^{cd}	8.03^{cd}	1.92^{cd}	53.23^{cd}	52.42^{gh}	52.99^{fg}
Haathi grass	39.00^{bc}	8.17^{bcd}	1.95^{bcd}	53.95^{bcd}	63.10^{de}	63.81^{cd}
Para grass	41.76^{ab}	8.45^{b}	2.02^{b}	55.46^{b}	58.36^{ef}	59.33^{de}
Cango signal	41.92^{ab}	8.43^{bc}	2.01^{bc}	55.35^{bc}	66.52^{c}	66.97^{c}
Chij	40.96^{ab}	8.30^{bc}	1.98^{bc}	54.67^{bc}	68.15^{c}	68.82^{c}
Setaria	40.13^{ab}	8.25^{bcd}	1.97^{bcd}	54.39^{bcd}	49.02^{gh}	49.70^{fg}
Bokna	35.12^{d}	7.86^{de}	1.88^{de}	52.30^{de}	51.87^{gh}	52.39^{fg}
Rhodes grass	39.21^{bc}	8.15^{bcd}	1.95^{bcd}	53.83^{bcd}	53.21^{fgh}	53.76^{efg}
Doob grass	35.82^{d}	8.06^{bcd}	1.93^{bcd}	53.35^{bcd}	54.14^{fg}	54.86^{ef}
Bamboo	15.69^{h}	5.61^{h}	1.34^{h}	40.21^{h}	51.47^{gh}	52.75^{fg}
Biul	30.61^{e}	8.17^{bcd}	1.95^{bcd}	53.94^{bcd}	81.30^{a}	82.14^{a}
Subabul	27.32^{f}	8.19^{bcd}	1.96^{bcd}	54.08^{bcd}	80.31^{a}	81.39^{ab}
Mulberry	28.14^{ef}	8.23^{bcd}	1.97^{bcd}	54.27^{bcd}	84.38^{a}	85.61^{a}
SEM	2.1	0.018	0.001	0.512	3.37	3.31
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different superscripts within the same column differ significantly.

The gas that is released with the generation of propionate is only the indirect gas produced from buffering. Carbohydrates produce maximum gas followed by proteins with negligible amount of gas from fat (Menke and Steingass 1988). Since cereal grains contain more highly degradable carbohydrates, their gas production was also higher. Brans due to their higher lignin and fibre contents, the gas production was significantly lower (P <0.001) than cereal grains.

The gas production was lower (P <0.001) in tree fodders and crop residues as compared to grasses (Table 4). The higher CP content and presence of plant secondary metabolites could be responsible for lower gas production in tree leaves observed in the study. Crop residues due to their low digestibility, high lignin content and very low CP content, the gas production was lower than grass.

The true DM and OM digestibilities were significantly (P<0.001) higher for cereal grains and cakes in comparison with brans (Table 3). *In vitro* true DM digestibility varied from 82.13 to 85.73 for cereal grains; 77.07 to 84.74 for

Table 5. Methane production and methane energy loss from concentrates

Local name	Methane (ml/g DM)	Methane (ml/g DDM)	Methane (ml/g OM)	Methane (ml/g DOM)	Methane (g/kg DM)	Methane (g/kg DDM)	Energy loss (MJ/kg Feed DM)	Energy loss (MJ/kg Feed DDM)
Maize	18.99^{e}	22.23^{f}	19.41^{f}	22.12^{h}	13.60^{f}	15.92^{f}	0.75^{f}	0.88^{f}
Wheat	19.23^{e}	22.77^{f}	19.66^{f}	22.85^{gh}	13.77^{f}	16.30^{f}	0.76^{f}	0.90^{f}
Jowar	19.77^{e}	23.72^{ef}	20.30^{f}	23.96^{fgh}	14.16^{f}	16.98^{f}	0.78^{f}	0.9^{ef}
Broken rice	19.36^{e}	23.62^{ef}	19.58^{f}	23.54^{gh}	13.86^{f}	16.91^{f}	0.77^{f}	0.93^{ef}
Wheat bran	19.73^{e}	27.07^{def}	20.95^{f}	27.89^{efgh}	14.13^{f}	19.38^{def}	0.78^{f}	1.07^{def}
Deoiled rice bran	28.96^{a}	43.55^{a}	33.19^{a}	48.61^{a}	20.74a	31.19^{a}	1.15^{a}	1.72^{a}
Rice polish	25.20^{c}	38.37^{ab}	28.93^{c}	42.74^{ab}	18.04^{c}	27.47^{ab}	1.0^{c}	1.52^{ab}
Groundnut meal	25.05^{c}	31.03^{cd}	26.84^{d}	32.34^{cde}	17.93^{cd}	22.22^{cd}	0.99^{cd}	1.2^{cd}
Mustard cake	23.88^{d}	29.80^{cde}	25.26^{e}	30.83^{cdef}	17.10^{de}	21.33^{cde}	0.94^{de}	1.18^{cde}
Deoiled mustard cake	26.84^{b}	34.89^{bc}	29.28^{c}	37.20^{bc}	19.22^{b}	24.99^{bc}	1.06^{b}	1.38^{bc}
Soybean meal	28.90^{a}	34.14^{bc}	31.74^{b}	36.80^{bc}	20.69^{a}	24.44^{bc}	1.14^{a}	1.35^{bc}
Ground nut cake	23.09^{d}	28.84^{cdef}	24.60^{e}	29.71^{defg}	16.53^{e}	20.64^{cdef}	0.91^{e}	1.14^{cdef}
SEM	0.20	5.35	0.22	6.08	0.10	2.74	0.01	0.01
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different superscripts within the same column differ significantly.

Table 6. Pearson correlations observed between chemical composition (% DM basis) of concentrate feeds and response variables

	Correlation	OM	CP	EE	Ash	NDF	ADF	HC	Cell	Lignin
Methane	r	-0.93	0.30	0.12	0.93	0.56	0.76	0.31	0.67	0.80
(ml/g DDM)	p-value	<0.01	0.07	0.49	<0.01	<0.01	<0.01	0.07	<0.01	<0.01
Methane	r	-0.94	0.27	0.13	0.94	0.57	0.78	0.31	0.67	0.81
(ml/g DOM)	p-value	<0.01	0.11	0.45	<0.01	<0.01	<0.01	0.06	<0.01	<0.01
Methane	r	-0.85	0.61	-0.05	0.85	0.33	0.62	0.07	0.57	0.57
(g/kg DM)	p-value	<0.01	<0.01	0.76	<0.01	0.05	<0.01	0.67	<0.01	<0.01
Methane	r	-0.93	0.30	0.12	0.93	0.56	0.76	0.31	0.67	0.80
(g/kg DDM)	p-value	<0.01	0.07	0.49	<0.01	<0.01	<0.01	0.07	<0.01	<0.01
TDMD	r	0.67	0.13	-0.32	-0.65	-0.67	-0.67	-0.52	-0.57	-0.76
(%)	p-value	<0.01	0.44	0.06	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
TOMD	r	0.66	0.16	-0.30	-0.67	-0.69	-0.68	-0.53	-0.58	-0.76
(%)	p-value	<0.01	0.34	0.08	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

protein concentrates; 66.87 to 73.06 for cereal by products. The OM digestibility also showed similar trend. In the study, strong positive correlations were observed for DM digestibility and OM and strong negative associations were observed with lignin, NDF, ADF, ash, and cellulose contents (Table 6).

In roughages, the DM and OM digestibilities of tree leaves were the highest, followed by grasses and least in crop residues (Table 4). Low amount of CP and high content of lignocelluloses could be responsible for the lower digestibility in crop residues. In the present study, strong positive association was found for CP, EE and digestibility values and strong negative correlation was found for ADF, cellulose and NDF contents (Table 8).

In general, the predicted ME values were very low in the feedstuffs having high fibre and low protein contents. The ME values were highest in cereal grains and protein concentrates and ranged from 12.59 to 14.16 (Table 3). Among the concentrates, the lowest ME value (MJ/kg DM) was observed in deoiled rice bran. In roughages, the lowest ME values (MJ/kg DM) were observed in crop residues (Table 4) which ranged from 5.68 to 6.64. Most of the grasses and tree fodder had comparable ME values.

Methane production and methane energy loss from concentrates: Significant (P<0.001) variations in methane production among the feeds were observed which could be due to their chemical composition (Table 5). In concentrates, deolied rice bran produced the highest amount and the maize grain lowest amount. Methane produced (ml/g DOM) varied from 22.12 to 48.61 and these values were higher than reported by Pal *et al.* (2015), and their methane production (ml/g TDOM) values ranged 13.8 to 30.50. In the study, methane produced (g/kg DDM) varied from 15.92 to 31.19 which were comparable to the values reported by Singh *et al.* (2016) for energy and protein feedstuffs. Energy loss from methane (MJ/kg feed DDM) also showed similar trend and varied from 0.88 to 1.72. Variation in methane production could be attributed to significant difference in the fibre fractions, protein and EE contents.

In this study, in concentrates there was strong negative correlation between OM and methane production and strong positive correlation between ash, lignin, ADF, cellulose and NDF contents (Table 6). Oil cakes containing higher concentration of EE (i.e. mustard oil cake, groundnut cake) resulted in lower methane production than the oil cakes containing low levels of EE (i.e. deoiled mustard cake, groundnut meal). The type of carbohydrates present in feedstuffs, affect methane production via shift in the rumen microbial population. Less fibre and more soluble carbohydrate in a feed stuff promote production of propionate which reduce the availability of substrates for methane production by ciliate protozoa. This could result in less methane per unit of organic matter digested as noticed for maize grain as compared to rice bran. Methane energy loss also showed similar trend, significantly higher ($P<0.001$) for de-oiled rice bran as compared to cereal grains.

Methane production and methane energy loss from roughages: In roughages, methane produced (ml/g DOM) varied from 29.78 to 76.30. Setaria grass produced the highest amount and the mulberry produced the lowest amount. Highest amount of methane was produced (ml/g DOM) in grasses followed by crop residues and lowest in tree leaves (Table 7). The lower amount of methane (ml/g DOM) produced in tree leaves may be due to the presence of appreciable quantity of plant secondary metabolites especially tannins which are known to reduce methane emission (Patra and Saxena 2010). Different sources of

Table 7. Methane production and methane energy loss in roughages

Local name	Methane (ml/g DM)	Methane (ml/g DDM)	Methane (ml/g OM)	Methane (ml/g DOM)	Methane (g/kg DM)	Methane (g/kg DDM)	Energy loss (MJ/kg Feed DM)	Energy loss (MJ/kg Feed DDM)
Wheat straw	14.80^{h}	36.90^{hi}	18.67^{i}	40.16^{gh}	10.74^{h}	26.43^{hi}	0.59^{h}	1.46^{hi}
Paddy straw	15.00^{h}	38.92^{ighi}	18.88^{i}	45.64^{fgh}	11.34^{h}	27.87^{ghi}	0.63^{h}	1.54^{ghi}
Jowar straw	15.84^{h}	34.53^{i}	17.02^{i}	39.25^{h}	10.60^{h}	24.72^{i}	0.59^{h}	1.37^{i}
Rice husk	16.07^{h}	44.31^{g}	19.09^{i}	50.39^{ef}	11.50^{h}	31.73^{g}	0.64^{h}	1.75^{g}
Oat fodder	32.05^{de}	42.59^{g}	35.99^{de}	47.04^{f}	22.95^{de}	30.49^{gh}	1.27^{de}	1.68^{gh}
Chrysopogon	29.39^{f}	61.16^{cd}	31.90^{g}	65.12^{bc}	21.04^{f}	43.79^{cd}	1.16^{f}	2.42^{cd}
Festuca	30.06^{ef}	57.42^{ef}	32.76^{fg}	61.89^{cd}	21.53^{ef}	41.11^{de}	1.19^{ef}	2.27^{de}
Haathi grass	31.99^{de}	50.72^{f}	35.10^{ef}	55.02^{e}	22.91^{de}	36.32^{f}	1.27^{de}	2.00^{f}
Para grass	36.13^{ab}	61.93^{bcd}	39.18^{abc}	66.07^{bc}	25.87^{ab}	44.34^{bcd}	1.43^{ab}	2.45^{bcd}
Cango signal	37.91^{a}	57.00^{de}	41.27^{a}	61.64^{cde}	27.15^{a}	40.81^{def}	1.50^{a}	2.25^{def}
Chij	36.09^{ab}	52.97^{ef}	39.18^{abc}	56.94^{de}	25.84^{ab}	37.93^{ef}	1.43^{ab}	2.09^{ef}
Setaria	34.31^{bcd}	70.02^{a}	37.91^{bcd}	76.30^{a}	24.57^{bc}	50.14^{a}	1.36^{bcd}	2.77^{a}
Bokna	33.69^{cd}	64.95^{abc}	39.36^{abc}	75.13^{a}	24.12^{cd}	46.51^{abc}	1.33 cd	2.57^{abc}
Rhodes grass	34.27^{bcd}	64.41^{bc}	38.07^{bcd}	70.83^{ab}	24.54^{bcd}	46.12^{abc}	1.36^{bcd}	2.55^{abc}
Doob grass	32.56^{cd}	60.17^{de}	36.69^{cde}	66.93^{bc}	23.32^{cd}	43.08^{cd}	1.29^{cd}	2.38^{cd}
Bamboo	34.68^{bc}	67.49^{ab}	40.61^{ab}	77.15^{a}	24.82^{bc}	48.32^{ab}	1.37^{bc}	2.67^{ab}
Biul	33.65^{cd}	41.41^{g}	38.08^{bcd}	46.39^{f}	24.09^{cd}	29.65^{gh}	1.33^{cd}	1.64^{gh}
Subabul	22.29^{g}	27.80^{h}	25.88^{h}	31.86^{g}	15.96^{g}	19.90^{i}	0.88^{g}	1.10^{i}
Mulberry	20.27^{g}	24.02^{hi}	25.49^{h}	29.78^{g}	14.91^{g}	17.20^{j}	0.80^{g}	0.95^{j}
SEM	0.59	3.27	0.77	4.7	0.30	2.16	0.01	0.01
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different superscripts within the same column differ significantly.

Table 8. Pearson correlations observed between chemical composition (% dry matter basis) of roughage feeds and response variables

	Correlation	OM	CP	EE	Ash	NDF	ADF	HC	Cell	Lignin
Methane	r	0.54	-0.18	-0.39	-0.53	0.49	0.26	0.46	0.43	-0.45
(ml/g DDM)	p-value	<0.01	0.18	<0.01	<0.01	<0.01	0.05	<0.01	<0.01	<0.01
Methane	r	0.44	-0.14	-0.38	-0.44	0.44	0.27	0.38	0.41	-0.38
(ml/g DOM)	p-value	<0.01	0.29	<0.01	<0.01	<0.01	0.04	<0.01	<0.01	<0.01
Methane	r	0.56	0.25	0.06	-0.56	0.11	-0.25	0.38	-0.02	-0.72
(g/kg DM)	p-value	<0.01	0.06	0.68	<0.01	0.42	0.06	<0.01	0.87	<0.01
Methane	r	0.53	-0.181	-0.39	-0.53	0.49	0.26	0.46	0.43	-0.45
(g/kg DDM)	p-value	<0.01	0.18	<0.01	<0.01	<0.01	0.05	<0.01	<0.01	<0.01
TDMD	r	-0.08	0.72	0.77	0.08	-0.63	-0.78	-0.19	-0.73	-0.35
(%)	p-value	0.55	<0.01	<0.01	0.55	<0.01	<0.01	0.15	<0.01	<0.01
TOMD	r	-0.09	0.72	0.77	0.09	-0.64	-0.78	-0.20	-0.73	-0.34
(%)	p-value	0.50	<0.01	<0.01	0.50	<0.01	<0.01	0.15	<0.01	<0.01

Table 9. Linear regression equations to predict methane and digestibility from chemical constituents in concentrates

Regression equation	SEM	R^2	P-value
CH_4 (ml/g DDM) = 164.73 - 1.455 × OM - 0.298 × EE + 0.217 × ADF	2.30	0.90	$P<0.01$
CH_4 (ml/g DOM) = 18.096 + 1.872 × total ash - 0.338 × EE + 0.231 × ADF	2.52	0.92	$P<0.01$
CH_4 (g/kg DM) = 72.624 - 0.592 × OM + 0.034 × CP - 0.259 × EE - 0.079 × HC + 0.104 × cellulose	0.62	0.95	$P<0.01$
CH_4 (g/kg DDM) = 117.945 - 1.042 × OM - 0.214 × EE + 0.155 × ADF	1.65	0.89	$P<0.01$
TDMD (%) = 91.931 - 2.982 × lignin - 0.244 × HC	4.78	0.66	$P<0.01$
TOMD (%) = 93.571 - 2.861 × lignin - 0.253 × HC	4.58	0.67	$P<0.01$

Table 10. Linear regression equations to predict methane and digestibility from chemical constituents in roughages

Regression equation	SEM	R^2	P value
CH_4 (ml/g DDM) = -4.071 + 0.492 × OM + 0.772 × cellulose - 2.161 × lignin	10.81	0.49	$P < 0.01$
CH_4 (ml/g DOM) = 43.922 - 0.075 × ash + 0.866 × cellulose - 2.334 × lignin	16.4	0.51	$P < 0.01$
CH_4 (g/kg DM) = 23.253 – 1.408 × lignin + 0.44 × CP - 0.477 × ash + 0.85 × ADF	3.48	0.66	$P < 0.01$
CH_4 (g/kg DDM) = 28.40 + 0.618 × cellulose - 1.903 × lignin	7.70	0.41	$P < 0.01$
TDMD (%) = 60.083 - 0.312 × ADF + 0.640 × CP + 4.730 × EE - 1.645 × lignin	7.10	0.78	$P < 0.01$
TDMD (%) = 46.565 + 0.701 × CP + 5.822 × EE - 2.019 × lignin	7.10	0.77	$P < 0.01$
TOMD (%) = 60.499 - 0.311 × ADF + 0.659 × CP + 4.726 × EE - 1.616 × lignin	7.10	0.78	$P < 0.01$
TOMD (%) = 47.051 + 0.720 × CP + 5.872 × EE - 1.989 × lignin	7.12	0.77	$P < 0.01$

tannins or tannin extracts have been shown to decrease methane production both *in vitro* and *in vivo* (Patra and Saxena 2010, Bhat *et al.* 2013). Methane (g/kg DDM) also showed similar trend and varied from 17.20 to 50.14. Singh *et al.* (2012) also reported methane (g/kg DDM) production of 20.3 to 35.2 and 27.46 to 47.37 in green fodders and crop residues, respectively. Energy loss from methane (MJ/kg feed DDM) also showed similar trend and it varied from 0.95 to 2.77.

In contrast to concentrates, in roughages strong positive relationship was observed between OM contents and methane emission (Table 8). Positive correlation between NDF, HC, and cellulose and methane production was also observed. In this study, in roughages there was negative relationship between methane production and lignin, ash and EE contents. Similar to our findings, Singh *et al.* (2012) also observed positive correlation between methane production and OM, NDF, ADF, cellulose and lignin contents. Since tannins also forms part of lignin especially in tree leaves there was negative correlation between them. Poor degradability and lower degradation at 24 h of incubation might have been responsible for lower methane emission per unit DM or OM observed in crop residues as compared to grasses. Methane energy loss also showed similar trend with higher energy loss (MJ/kg feed DDM) in grasses ($P<0.01$), followed by crop residues and lowest in tree fodder.

Regression between methane and chemical composition: Regression equations developed for predicting methane production (ml/g DDM or ml/g DOM) using chemical composition (Table 9 and Table 10) showed high level of predictability for concentrates, $R^2 = 0.90$ and 0.92 ($P<0.01$) as compared to roughages, $R^2 = 0.49$ and 0.51 ($P<0.01$). The regression equation using OM or ash, EE and ADF were better predictors of methane production (ml/g DDM or ml/g DOM) in concentrates; whereas, OM or ash, cellulose and lignin were better predictors in roughages. Similarly, high level of predictability observed for predicting methane (g/kg DM or g/kg DDM) with $R^2 = 0.95$ and 0.89 ($P<0.01$) in concentrates, as compared to roughages, $R^2 = 0.95$ and 0.89 ($P<0.01$).

Overall, cereal grains had high ME, high digestibility, low methane production potential and low methane energy loss as compared to bran. In roughages, tree fodders had high digestibility, high ME value, low methane production potential and low methane energy loss as compared to grasses and crop residues. There was strong negative correlation between OM and methane production and strong positive correlation between ash, lignin, ADF, cellulose and NDF contents in concentrates. In contrast to concentrates, in roughages strong positive relationship was observed between OM contents and methane emission. Positive correlation between NDF, HC, and cellulose and methane production was also observed. In roughages, there was negative relationship between methane production and lignin, ash and EE contents. Regression equations developed for predicting methane production using chemical composition showed high level of predictability for concentrates, as compared to roughages. Feedstuffs data on nutrient composition, methane production potential could be utilized judiciously in formulating low methane emission diets for feeding ruminants in the region.

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