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# Evaluation of feeds from tropical origin for *in vitro* methane production potential and rumen fermentation *in vitro*

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## Abstract

Enteric methane arising due to fermentation of feeds in the rumen contributes substantially to the greenhouse gas emissions. Thus, like evaluation of chemical composition and nutritive values of feeds, methane production potential of each feed should be determined. This experiment was conducted to evaluate several feeds for methane production potential and rumen fermentation using *in vitro* gas production technique so that low methane producing feeds could be utilized to feed ruminants. Protein- and energy-rich concentrates (n=11), cereal and grass forages (n=11), and different straws and shrubs (n=12), which are commonly fed to ruminants in India, were collected from a number of locations. Gas production kinetics, methane production, degradability and rumen fermentation greatly varied ($p<0.01$) among feeds depending upon the chemical composition. Methane production (mL/g of degraded organic matter) was lower ($p<0.01$) for concentrate than forages, and straws and shrubs. Among shrubs and straws, methane production was lower ($p<0.01$) for shrubs than straws. Methane production was correlated ($p<0.05$) with concentrations of crude protein (CP), ether extract and non-fibrous carbohydrate (NFC) negatively, and with neutral detergent (NDF) and acid detergent fiber (ADF) positively. Potential gas production was negatively correlated ($p=0.04$) with ADF, but positively ($p<0.01$) with NFC content. Rate of gas production and ammonia concentration were influenced by CP content positively ($p<0.05$), but by NDF and ADF negatively ($p<0.05$). Total volatile fatty acid concentration and organic matter degradability were correlated ($p<0.05$) positively with CP and NFC content, but negatively with NDF and ADF content. The results suggest that incorporation of concentrates and shrubs replacing straws and forages in the diets of ruminants may decrease methane production.

**Additional key words:** degradability; tropical feeds; *in vitro* gas production.

**Abbreviations used:** ADF (acid detergent fiber); ADL (acid detergent lignin); CP (crude protein); DM (dry matter); DORB (deoiled rice bran); EE (ether extract); GNC (groundnut cake); MOC (mustard oil cake); NDF (neutral detergent fiber); NFC (non-fibrous carbohydrate); OM (organic matter); TCA-N (tricloroacetate precipitable N); VFA (volatile fatty acids).

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## Introduction

Enteric methane is normally produced during the fermentation of feeds mostly in the rumen by hydrogenotrophic methanogenic archaea, which results in the inefficient conversion of potential energy of feeds into methane that is not utilized by ruminants. Methane production in the rumen represents 2 to 15% loss of feed energy, decreasing the metabolizable energy content of feeds (Van Nevel & Demeyer, 1996). Besides, contributions of greenhouse gas emissions from livestock production systems to the atmosphere are of great concern in recent years. Methane is the second highest anthropogenic greenhouse gas after carbon dioxide, which contributes to the problems of global warming and climate change (Tubiello *et al.,* 2014). Methane production from enteric fermentation is the largest source of greenhouse gases accounting 40% of agricultural greenhouse gas outputs (Tubiello *et al.,* 2014). In India and developing countries, greenhouse gases from livestock increased due to growing population of livestock and expansion of agricultural outputs in the last few decades (Patra, 2012a, 2014a; Tubiello *et al.,* 2014). Therefore, developing feeding strategies to decrease enteric methane production deserves research attention for long-term mitigation of greenhouse gas emissions into the atmosphere and for short-term economic benefits.

Improvements in digestive efficiency could enhance ruminant production while lowering input costs and undesired environmental impacts. A number of dietary and management mitigation options and policies have been advocated to lowering methane production from livestock production systems (Patra, 2012b; Hristov *et al.,* 2013). However, all these mitigation strategies may not be appropriate for all feeding situations, particularly in developing tropical countries, where livestock production systems are low-input-output enterprises and farmers could not adopt expensive technologies of methane mitigation like other nutritional technologies (Owen *et al.,* 2012).

Chemical composition and nutritive values of feeds are determined to better characterize them and to formulate rations of animals according to the specific feeding standards. Feeds differ in their methane production potentiality depending upon chemical composition and plant metabolites present in them (Benchaar *et al.,* 2001; Patra, 2012b). Because methane production is an important characteristic of feeds accounting loss of energy and contributing to the greenhouse effects, methane production potential per unit of dry matter (DM) or DM degradability should be measured and be tabulated like chemical composition and nutritive values of feeds. Identification of low-methane producing feeds may be practically feasible options to prepare low methane producing rations for ruminants. Therefore, the objective of this study was to determine the methane production potential and fermentation profile of different feedstuffs commonly used to feed ruminants.

# Material and methods

## Experimental procedures

For each feed, we collected from four locations (n=4 for chemical analysis): a) concentrates, *i.e.,* oil cakes, chunies (by-products of pulses), cereal grains and brans (n=11); b) leguminous and non-leguminous forages (n=11); and c) straws and shrubs (n=12). They were pooled for each feed on an equal weight basis for *in vitro* incubations. These feeds are commonly fed to ruminants by rural smallholder farmers in India. They were dried in a hot air oven maintained at 60°C, ground to pass 1 mm screen, and stored in air-tight bags for experimental use. The study was completed in three sets (concentrate, forages, and straws and shrubs). The *in vitro* fermentation of feeds was carried out using 100 mL calibrated glass syringes (Häberle Labortechnik, Lonsee-Ettlenschieß, Germany) in duplicates for each composited feed, standard of wheat straw and blanks in three incubations/run (n=3) conducted at three different weeks for each category of feeds (*i.e.,* 3 runs/set). The anaerobic culture medium consisting of rumen fluid as inoculum and buffer solution (bicarbonate-mineral-distilled water mixture) in the ratio of 1:2 was prepared as described by Menke & Steingass (1988). Rumen fluid was collected from two sheep through stomach tube before morning feeding. The donor sheep were fed a maintenance diet based on cenchrus straw containing 70.6 g crude protein (CP), 714 g neutral detergent fiber (NDF) and 454 g acid detergent fiber (ADF) per kg DM and a concentrate mixture (consisting of maize, barley, mustard oil cake, ground nut cake, mineral mixture and salt, and containing 127 g CP and 252 g NDF/kg DM) in a ratio of 60:40 (cenchrus straw:concentrate). The rumen fluid was taken into a pre-warmed $CO_2$ filled thermos, and immediately carried to the laboratory. Rumen fluid was pooled equally, and filtered through four layers of muslin cloth under continuous flushing of $CO_2$ to maintain the anaerobic conditions.

Accurately weighed ground substrates (200 mg for gas production kinetics and 400 mg for determination of methane production, degradability of feeds and rumen fermentation) were transferred into the syringes. Anaerobic culture medium (30 mL for gas production kinetics, and 40 mL consisting of 10 mL rumen fluid and 30 mL buffer solution for other variables) was dispensed to each syringe by an automatic dispenser. The sample weights were increased to 400 mg to reduce analytical errors inherent in gravimetric determination of degradability of feeds, and buffer solution were increased to accommodate greater amount of volatile fatty acids (VFA) production with the large sample size (Blummel & Becker, 1997). Syringes were incubated at 39°C and shaken manually at every 2 h for initial 12 h, and then at 6 h intervals. The gas production was recorded at 2, 4, 8, 12, 24, 36, 48, 72 and 96 h of incubation from the markings of the glass syringes, and the fermentation was terminated at 96 h of incubation for determination of gas production kinetics. Net gas production was calculated by subtracting the volume of gas produced in blanks from the volume of gas produced from incubated feeds, and expressed per unit of incubated substrate.

For determination of methane and fermentation characteristics, incubations that were also repeated in three runs for each category of feeds were terminated at 24 h and volumes of gas produced were recorded. Gas samples were collected and immediately injected onto a gas chromatograph to determine methane concentrations. The fermented liquid contents of the syringes were sampled and preserved at -20°C for determination of ammonia nitrogen ($NH_3$-N), trichloroacetic acid precipitable nitrogen (TCA-N) and VFA until analyses. True substrate degradability of diets was measured following the procedure of Blummel *et al.* (1997). Briefly, the contents of the syringes were transferred into the Berzelius beaker by repeated washings with 50 mL of neutral detergent solution (double strength), refluxed for 1 h, filtered through silica crucibles (Grade 1), and then residues were burnt in a muffle furnace at 600°C for 3 h.

## Analytical procedures

The DM, organic matter (OM), CP (N×6.25) and ether extract (EE) concentrations of feed samples were determined following the AOAC (1995) procedures. Concentrations of NDF, ADF and acid detergent lignin (ADL) in feeds were analyzed according to Van Soest *et al.* (1991). Both NDF and ADF contents were expressed exclusive of residual ash, and NDF content was determined without α-amylase and sodium sulfite. The concentration of ADL was determined by solubilisation of cellulose with sulphuric acid in the ADF residue (Van Soest *et al.,* 1991).

For determination of methane concentration, the gas produced during fermentation of feeds in the syringes was collected using Hamilton syringes by piercing the silicon tube fitted with the *in vitro* syringes, and 50 µL of gas sample was immediately injected into the gas chromatograph (GC-1000, Dani, Milan, Italy) equipped with a flame ionization detector and packed column (Chromatopak, Mumbai, India; 2 m in length and 3.2 mm in outer diameter, 10% SP-1000 on 80/100 mess Chromosorb WHP). Concentration of methane in the standard was 99.998% (Sigma-Aldrich, MO, USA). The temperatures of injector oven, column oven and detector were 120, 50 and 120°C, respectively. Nitrogen was used as carrier gas.

Concentration of ammonia nitrogen in fermentation solution was determined according to the modified Wetherburn method (Chaney & Marbach, 1962). The concentration of TCA-N was determined by Kjeldahl procedure (AOAC, 1995) after treating the rumen fluid with 20% TCA. Total VFA concentrations in the fermented incubation media were determined using Markham apparatus (Barnett & Reid, 1956).

## Calculations and statistical analyses

To determine fermentation kinetics the net gas production data were fitted to the following modified exponential model of Ørskov & McDonald (1979):

$$Y = b \times (1 - e^{-k\,t})$$

where $Y$ is the cumulative volume (mL) of gas produced at time $t$ (h); $b$ is the asymptotic gas volume (mL); $k$ the rate constant of gas production ($h^{-1}$). The parameters $b$ and $k$ were determined using the nonlinear procedure of SAS (2001). All data were analyzed using one-way ANOVA using General Linear Model approach of SPSS (1997) in a completely randomized design with location and run as experimental units for chemical composition and *in vitro* fermentation study, respectively. When F-test was significant ($p<0.05$), Tukey test was utilized to compare significant differences ($p<0.05$) among the feeds.

# Results

## Chemical composition

Among the protein concentrate used in this study, CP concentration was highest in soybean meal and groundnut cake (GNC), and lowest in mahua cake (Table 1). Among the energy concentrates, CP content was in the range of 9 to 13%. The concentration of EE was high (8 to 9%) in mustard oil cake (MOC), GNC and til cake, medium in wheat bran (4%), maize grain (3%), and other feeds contained 1-2% EE. Ash content ranged from 2.28% to 12% with the highest concentration in deoiled rice bran (DORB) and low in cereal grains and pulse chunies. The concentrations of fiber were high in brans and pulse chunies.

Among the forages, the concentrations of CP were in the range of 8 to 12%, except for berseem (20.2%) and cowpea (19.3%) that contained high levels of CP (Table 1). The concentrations of EE and ash varied from 1 to 5% and 12 to 15%, respectively, except in pea pods (5.8%) and sewan grass (6.8%) containing less amount of ash in this category. The concentrations of NDF were high in sewan grass, doob grass and cenchrus grass, and medium in maize hay, rice bean hay and para grass. Pea pods and bajra fodder contained lowest concentrations of ADF.

Among the straws and shrubs, CP content was low in straws (4-7%) and high in shrubs (13-17%) except in kakoda (Table 1). Straws had EE content of 1-2% except for guar straw (4.6% EE), and shrubs contained 3-5% of EE. The ash concentrations ranged from 8 to 13% in different straws and shrubs. The content of NDF was lowest in argemone (28.5%) and arnia (33%) and highest in paddy and cenchrus straws (71%). All straws and kakoda contained high concentrations of ADF. The ADL concentrations were highest in til straw, followed by pala and kakoda, and were low in mustard straw and arnia.

**Table 1.** Chemical composition (% dry matter basis) of different (a) protein and energy concentrates (n=4), (b) forages (n=4) and (c) straws and shrubs (n=4) fed to ruminants

| Feed | | Crude protein | Ether extract | Total ash | NDF[2] | ADF[3] | ADL[4] |
|---|---|---|---|---|---|---|---|
| **Common name[1]** | **Scientific name** | | | | | | |
| **(a) Protein and energy concentrates** | | | | | | | |
| MOC | *Brassica* spp. | 37.5b | 8.40b | 6.04d | 19.1e | 11.7d | 3.71cd |
| Til cake | *Sesamum indicum* | 33.9c | 9.22a | 10.35b | 23.4d | 12.0d | 2.36d |
| Soyabean meal | *Glycine max* | 43.7a | 1.19g | 7.99c | 16.7f | 9.59e | 0.86e |
| Mahua cake | *Madhuca indica* | 21.1d | 1.37f | 6.24d | 14.0g | 8.44f | 3.25cd |
| GNC | *Arachis hypogea* | 42.6a | 8.92ab | 5.35e | 17.4f | 10.3e | 4.13c |
| Maize grain | *Zea mays* | 8.5g | 3.17d | 2.28h | 11.5h | 3.50g | 0.83e |
| Barley grain | *Hordeum vulgare* | 11.3f | 2.41e | 3.92g | 12.8gh | 3.07g | 0.47e |
| DORB | *Oryza sativa* | 11.3f | 0.58g | 12.75a | 46.6a | 22.3a | 8.29a |
| Gram chuni | *Cicer arietinum* | 13.3e | 1.01fg | 4.06fg | 38.9b | 22.3a | 2.91cd |
| Arhar chuni | *Cajanus cajan* | 11.1f | 1.44f | 4.33f | 32.4c | 17.6c | 6.04b |
| Wheat bran | *Triticum aestivum* | 9.56g | 4.11c | 5.62e | 46.3a | 20.0b | 6.94ab |
| SEM | | 0.397 | 0.242 | 0.089 | 0.488 | 0.326 | 0.480 |
| *p-value* | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **(b) Forages** | | | | | | | |
| Rice bean | *Vigna umbellata* | 10.2d | 1.42fg | 15.4a | 57.2d | 40.9b | 12.2a |
| Cowpea | *Vigna unguiculata* | 19.3b | 4.93a | 12.9c | 45.8h | 30.8g | 5.10ef |
| Maize | *Zea mays* | 11.6c | 4.73a | 13.2c | 55.2e | 33.3ef | 5.68e |
| Berseem | *Trifolium alexandrinum* | 20.2a | 5.13a | 13.2c | 47.2g | 35.9d | 6.26d |
| Bajra fodder | *Pennisetum typhoids* | 11.4c | 2.21d | 14.8b | 42.3i | 23.9i | 4.34g |
| Congress grass | *Parthenium hysterophorus* | 11.5c | 3.08c | 15.6.a | 41.3i | 28.8h | 7.45c |
| Para grass | *Brachiaria mutica* | 10.2d | 2.77c | 14.9b | 53.7f | 32.1f | 4.62fg |
| Doob grass | *Cynodon dactylon* | 9.09e | 3.75b | 13.0c | 65.2b | 38.4c | 5.50e |
| Cenchrus grass | *Cenchrus ciliaris* | 7.71f | 1.87de | 12.0d | 63.2c | 32.5f | 4.60fg |
| Sewan grass | *Lasiurus hirsutus* | 9.04fe | 1.47ef | 7.83e | 71.9a | 42.1a | 8.84b |
| Pea pods | *Pisum sativum* | 10.1d | 1.04g | 5.80f | 33.6j | 22.3i | 4.16g |
| SEM | | 0.513 | 0.137 | 0.205 | 0.455 | 0.623 | 0.344 |
| *p-value* | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **(c) Straws and shrubs** | | | | | | | |
| Bajra straw | *Pennisetum typhoids* | 6.28fg | 1.83ef | 13.0a | 54.9d | 32.4f | 4.98fg |
| Guar straw | *Cyamopsis tetragonoloba* | 5.88g | 4.59b | 9.06e | 51.3e | 31.8g | 4.19gh |
| Cenchrus straw | *Cenchrus ciliaris* | 7.08cde | 2.19e | 11.1c | 71.0a | 47.2b | 7.76e |
| Mustard straw | *Brassica* spp. | 3.65h | 0.95f | 8.93e | 62.4b | 46.1c | 2.13j |
| Paddy straw | *Oryza sativa* | 4.04h | 0.98f | 11.6c | 71.2a | 44.3d | 5.70f |
| Til straw | *Cicer arietinum* | 6.64ef | 1.29f | 12.1b | 60.1c | 49.5a | 13.2a |
| Pala | *Ziziphus racemosa* | 17.0a | 3.85c | 7.80f | 39.2h | 25.6i | 12.3b |
| Dhawasi | *Tephrosia apollinea* | 12.3c | 4.95ab | 11.4c | 47.4f | 23.8j | 3.09i |
| Kakoda | *Mimordica* spp. | 7.95d | 4.80ab | 13.6a | 47.1f | 37.7e | 11.4c |
| Argemone | *Argemone mexicana* | 12.87c | 5.25a | 11.6c | 28.5j | 12.3l | 3.92h |
| Peelwani | *Cocculus hirsutus* | 13.4b | 2.97d | 8.99e | 45.1g | 27.8h | 9.16d |
| Arnia | *Clerodendrum multiflorum* | 13.4b | 2.88d | 10.0d | 33.0i | 21.5k | 3.02i |
| SEM | | 0.579 | 0.211 | 0.160 | 0.370 | 0.333 | 0.259 |
| *p-value* | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

[1] MOC, mustard oil cake; GNC, ground nut cake; DORB, deoiled rice bran; SEM, standard error of mean. [2] NDF, neutral detergent fiber. [3] ADF, acid detergent fiber. [4] ADL, acid detergent lignin. Values followed by different letters within columns and groups differ significantly.

## Gas production

The quantity and rate of gas produced varied markedly among the feeds (Table 2). In general, greater amount of gas was produced by cereal grains than oil cakes. Cereal grains produced the highest amount of gas, whereas til cake and mahua cake produced the lowest amount of gas at 24 h. Thus, potential gas production ranged from 28.4 to 75.2 mL/200 mg with the highest for gram chuni, and the lowest for til cake, mahua cake and GNC. However, rate of gas production was highest for GNC, followed by mahua cake and barley grains, and was lowest for gram chuni followed by arhar chuni and til cake.

Among forages, potential gas production was estimated to be greatest for pea pods, followed by maize and sewan grass hays, and lowest for congress grass hays (Table 2). However, rate of gas production was highest for cow pea and berseem hays, and lowest for sewan grass and doob grass hays.

Among the straws and shrubs, the highest gas production was noted for dhawasi and peelwani, and the lowest gas production for til straw, followed by kakoda and pala at 24 h (Table 2). Potential maximum gas production was estimated to be highest in dhawasi and paddy straws, and lowest in til straw. Rate of gas production was greatest for arnia and argemone, and lowest for paddy straw and pala. Overall, concentrates (33.4 mL/200 mg) produced higher ($p$<0.001) amount of gas than straws, shrubs (24.1 mL/200 mg) and forages (26.8 mL/200 mg).

## Methane production

Among concentrates, methane production varied considerably among the feeds. Methane production (mL/g DM) was significantly higher ($p$<0.05) for DORB and soybean meal, followed by mahua seed cake, and was lower for maize grain than for other feeds (Table 2). Among the oil cakes, methane was lower for MOC, til cake and GNC than soybean meal and mahua seed cake. When methane production was expressed to mL/g degraded OM, methane production was highest for DORB and lowest for maize grain. Methane production increased for barley grain *vs.* maize grain, DORB *vs.* wheat bran, gram chuni *vs.* arhar chuni.

Methane production in terms of mL/g feeds was highest for pea pods, berseem and cowpea hay, followed by maize hay and congress grass hay, and lowest for cenchrus gass hay (Table 2). However, methane production expressed as mL/g degradable OM was greatest for sewan grass hay, followed by berseem hay, cow pea hay, and lowest for cenchrus grass hay. Among the grass hay, sewan grass produced the greatest amount of methane followed by doob grass, and then para grass and maize hay. Among the leguminous hay, methane production was greater for berseem *vs.* cowpea, and cowpea *vs.* rice bean hay.

Among the straws and shrubs, methane production (mL/g DM) was highest for peelwani and argemone, followed by guar straw and arnia, and was lowest for paddy straw, til straw and mustard straw (Table 2). However, methane production expressed in term of mL/g degraded OM was highest for cenchrus straw and peelwani, followed by mustard straw, til straw, and was lowest for pala, followed by dhawasi. In general, straws produced higher ($p$<0.01) amount of methane than shrubs (30.9 *vs.* 24.8 mL/g degraded OM).

Among the categories of feeds, methane production expressed as mL/g feed did not differ ($p$=0.17) among the three categories. However, methane production in mL/g of degraded OM was lower ($p$<0.01) for concentrate feeds (21.0 mL) than straws-shrubs (27.0 mL) and forages (27.7 mL). Again among the forages, grass and cereal forages produced higher ($p$=0.02) amount of methane compared with legume forages (29.6 mL *vs.* 25.5 mL/g of degraded OM).

## Concentrations of total VFA and N, and degradability

Total VFA concentrations (4.9 to 7.6 mmol/100 mL) were highest for mahua cake, MOC and maize grain; whereas it was lowest for til cake, gram and arhar chuni. (Table 3). Concentrations of ammonia in the fermentation media were generally greater for oil cake than grains and brans (29.1 *vs.* 15.9 mg/100 mL). The concentration of TCA-N was greatest for GNC, followed by til cake and DROB, and lowest for arhar chuni, gram chuni and MOC. Degradability of DM and OM was higher for MOC, til cake, soybean meal and maize grain than other feeds. Among the chunies and brans, wheat bran had lowest degradability, followed by DORB, gram chuni and then arhar chuni.

Among forages, total VFA concentration was highest for pea pod skin, followed by cenchrus gass hay, bajra, congress grass, para grass, rice bean cow pea and maize hay, and was lowest for sewan grass hay. Concentration of ammonia-N was highest for bajra, congress grass hay and berseem, followed by para grass and rice bran, and was lowest for pea pod skin (Table 3). The concentration of TCA-N was greatest for sewan grass, followed by maize hay, and lowest in bajra and pea pods. Degradability of DM and OM was greatest for pea pods, followed by maize hay, congress grass hay, and was lowest for sewan grass hay.

**Table 2.** Gas production kinetics, total gas and methane production *in vitro* of different (a) protein and energy concentrates, (b) forages and (c) straws and shrubs

| Feed common name | *b* | *k* | $TGP_{24}$ | Methane | |
|---|---|---|---|---|---|
| | | | | (mL/g DM) | (mL/g TDOM) |
| **(a) Protein and energy concentrates** | | | | | |
| MOC | 39.4e | 0.056d | 30.1f | 15.4ef | 17.8de |
| Til cake | 29.1f | 0.044e | 20.9i | 14.1f | 15.9ef |
| Soybean meal | 46.7d | 0.056d | 34.0e | 20.1ab | 22.7bc |
| Mahua cake | 28.4f | 0.071b | 23.5h | 18.2c | 24.2b |
| GNC | 29.6f | 0.127a | 27.5g | 14.53ef | 19.1d |
| Maize grain | 67.9b | 0.049e | 51.4a | 10.90g | 13.8f |
| Barley grain | 59.4c | 0.061c | 44.4b | 15.9de | 21.9c |
| DORB | 37.4e | 0.051d | 26.6g | 21.4a | 30.5a |
| Gram chuni | 75.4a | 0.022g | 36.8d | 18.7bc | 24.6b |
| Arhar chuni | 68.5b | 0.034f | 41.5c | 17.5cd | 21.6c |
| Wheat bran | 43.0d | 0.055d | 31.2f | 11.0g | 18.9d |
| SEM | 1.962 | 0.00275 | 0.444 | 0.640 | 0.741 |
| *p-value* | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **(b) Forages** | | | | | |
| Rice bean | 24.8f | 0.052cd | 19.6e | 15.5ef | 26.6d |
| Cowpea | 40.1c | 0.087a | 34.0b | 21.2b | 30.0c |
| Maize | 45.1b | 0.0464d | 34.2b | 18.6c | 25.9d |
| Berseem | 33.8e | 0.0833a | 29.7c | 22.2a | 32.9b |
| Bajra | 24.5f | 0.037e | 17.1f | 16.0e | 22.7g |
| Congress grass | 21.6g | 0.057c | 15.8f | 18.0cd | 24.8ef |
| Para grass | 38.7c | 0.038e | 26.0d | 17.5d | 25.8de |
| Doob grass | 36.8d | 0.030f | 20.0e | 14.5g | 29.2c |
| Cenchrus grass | 42.0c | 0.040de | 27.8cd | 12.4h | 20.8h |
| Sewan grass | 44.3b | 0.025g | 21.3e | 15.0fg | 37.1a |
| Pea pods | 62.4a | 0.073b | 49.0a | 22.2a | 24.4f |
| SEM | 1.063 | 0.0035 | 0.703 | 0.626 | 0.934 |
| *p-value* | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **(c) Straws and shrubs** | | | | | |
| Bajra straw | 31.8c | 0.081ab | 25.3d | 16.1de | 28.3d |
| Guar straw | 37.1bc | 0.05±c | 29.2c | 19.4abc | 31.5cd |
| Cenchrus straw | 33.8c | 0.033c | 20.8e | 15.4ef | 35.5a |
| Mustard straw | 40.5b | 0.026c | 20.3e | 13.4fg | 32.5bc |
| Paddy straw | 49.1a | 0.015d | 15.7f | 10.8g | 27.9d |
| Til Straw | 23.8d | 0.032c | 13.8g | 12.9g | 29.8c |
| Pala | 36.0c | 0.022c | 15.6f | 14.8ef | 18.7h |
| Dhawasi | 50.9a | 0.061b | 38.1a | 16.0de | 20.6g |
| Kakoda | 32.6c | 0.032c | 17.4f | 16.6de | 23.4f |
| Argemone | 38.7bc | 0.094ab | 25.8d | 19.8ab | 26.7e |
| Peelwani | 36.8bc | 0.080ab | 33.4b | 21.6a | 33.6ab |
| Arnia | 33.4c | 0.103a | 29.9c | 19.0bc | 25.5ef |
| SEM | 1.460 | 0.0187 | 0.562 | 0.793 | 1.215 |
| *p-value* | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

MOC, mustard oil cake; GNC, ground nut cake; DORB, deoiled rice bran; SEM, standard error of mean. DM, dry matter; *b,* potential gas production (mL/200 mg DM); *k,* rate of gas production ($h^{-1}$); $TGP_{24}$, total gas production at 24 h (mL/200 mg DM); TDOM, truly degraded organic matter. Values followed by different letters within columns and groups differ significantly.

**Table 3.** Concentration of total volatile fatty acids (TVFA), $NH_3$-N, and trichloroacetic acid precipitable N (TCA-N), and degradability of different (a) protein and energy concentrates, (b) forages and (c) straws and shrubs

| Feed common name | TVFA (mmol/100 mL) | $NH_3$-N (mg/100 mL) | TCA-N (mg/100 mL) | TDDM (%) | TDOM (%) |
|---|---|---|---|---|---|
| **(a) Protein and energy concentrates** | | | | | |
| MOC | 7.2ab | 30.2b | 17.4de | 84.2b | 86.2b |
| Til cake | 5.3fg | 26.5c | 36.1b | 88.4a | 89.2a |
| Soybean meal | 6.8bc | 35.1a | 24.5c | 87.8a | 88.4ab |
| Mahua cake | 7.6a | 24.7c | 26.7c | 59.8f | 65.2f |
| GNC | 5.6ef | 28.8bc | 42.0a | 75.4d | 76.1d |
| Maize grain | 7.2ab | 17.5e | 25.3c | 88.8a | 89.4a |
| Barley grain | 6.2cde | 20.5d | 26.9c | 81.0c | 81.5c |
| DORB | 6.5bcd | 13.6f | 35.6b | 71.0e | 70.1e |
| Gram chunni | 5.2fg | 12.8f | 17.7de | 74.2d | 76.1d |
| Arhar chunni | 4.9g | 13.4f | 14.9e | 79.8c | 81.0c |
| Wheat bran | 6.0de | 17.8e | 20.4d | 59.4f | 58.3g |
| SEM | 0.236 | 0.650 | 1.027 | 0.690 | 0.788 |
| *p-value* | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **(b) Forages** | | | | | |
| Rice bean | 5.4b | 14.9cd | 33.4c | 56.8f | 58.3f |
| Cowpea | 5.4b | 13.6de | 29.7d | 66.8d | 70.6c |
| Maize | 5.3bc | 9.7±gh | 37.9b | 70.8b | 71.9bc |
| Berseem | 4.5de | 17.24ab | 28.8d | 69.2c | 67.3d |
| Bajra | 5.3bc | 17.6a | 17.8g | 70.0c | 70.6c |
| Congress grass | 5.3bc | 17.6a | 23.8ef | 70.1bc | 72.5b |
| Para grass | 5.4b | 15.7bc | 24.6e | 69.8c | 67.8d |
| Doob grass | 4.8cd | 11.0fg | 21.7ef | 50.1g | 49.7g |
| Cenchrus grass | 5.5b | 12.1ef | 36.8bc | 61.6e | 59.7e |
| Sewan grass | 4.3e | 12.9e | 42.3a | 42.3h | 40.5h |
| Pea pods | 6.3a | 8.8h | 20.6fg | 80.8a | 81.0a |
| SEM | 0.177 | 0.566 | 1.213 | 0.657 | 0.857 |
| *p-value* | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **(c) Straws and shrubs** | | | | | |
| Bajra straw | 5.3bcd | 11.1ef | 19.2i | 58.3f | 56.9f |
| Guar straw | 6.6a | 16.8a | 28.7de | 63.1d | 61.6e |
| Cenchrus straw | 5.9bc | 11.4ef | 27.4ef | 47.9g | 43.3g |
| Mustard straw | 4.5e | 11.7ef | 31.8cd | 42.7h | 41.4h |
| Paddy straw | 4.5e | 11.6ef | 45.0a | 36.7i | 38.7i |
| Til straw | 4.4e | 14.2b | 40.5b | 42.3h | 43.5g |
| Pala | 5.2cd | 13.6bc | 26.8efg | 78.3a | 78.6a |
| Dhawasi | 5.8bc | 13.4bd | 33.9c | 77.9a | 77.7a |
| Kakoda | 4.8de | 11.6ef | 41.5b | 72.5c | 70.7c |
| Argemone | 6.0bc | 18.1a | 26.0efg | 73.3b | 74.1b |
| Peelwani | 5.7bc | 13.5bc | 20.1i | 60.7e | 65.4d |
| Arnia | 5.5bc | 17.9a | 24.6fh | 72.0c | 74.5b |
| SEM | 0.228 | 0.559 | 1.152 | 0.509 | 1.187 |
| *p-value* | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

MOC, mustard oil cake; GNC, ground nut cake; DORB, deoiled rice bran; SEM, standard error of mean. TDDM, truly degraded dry matter; TDOM, truly degraded organic matter. Values followed by different letters within columns and groups differ significantly.

For straws and shrubs, concentration of ammonia did not vary much for different feeds except for guar straw, argemone and arnia, which increased ammonia concentrations compared with other feeds (Table 3). Guar straw produced highest amount of VFA, but mustard straw, paddy straw and til straw produced lowest amount of VFA in the incubation media. Concentrations of TCA-N were highest for paddy straw, followed by til straw and kakoda and lowest for peelwani and bajra straw. Degradability of DM was greatest for pala and dhawasi, followed by argemone. The true OM degradability was also higher for pala and dhawasi, followed by argemone and arnia, and was lowest for paddy straw, and then mustard straw. True OM degradability was higher ($p$<0.001) for concentrate (79.2%) than for straws-shrubs (60.5%) and forages (64.5%). Similarly, ammonia concentration was greater ($p$<0.001) for concentrate (20.1 mg/100 mL) than for straws (13.7 mg/100 mL) and forages (13.8 mg/100 mL).

### Correlations between chemical composition and response variables

Total gas production at 24 h was negatively correlated with NDF and ADF concentrations, but positively correlated with NFC concentrations (Table 4). Methane production was negatively correlated with CP, EE and non-fibrous carbohydrate contents, and positively with NDF and ADF contents. Potential gas production was negatively correlated with ADF, but positively with NFC content. Rates of gas production and ammonia concentration were positively influenced by CP and EE concentrations, but negatively by NDF and ADF contents. Concentration of TCA-N was positively related with NDF and ADF contents, and negatively with NFC content. Total VFA concentration and TDOM were positively correlated with CP, EE and NFC content, and negatively with NDF and ADF content.

## Discussion

### Chemical composition

Chemical composition of straws, oil cakes, grains, grasses and shrubs were within range of reported values in the tropical regions of the world though variations prevailed compared with other studies (Singh *et al.,* 2012; Feedipedia, 2014). Chemical composition of some shrubs (*i.e*., dhawasi, kakoda, argemone, peelwani and arnia) has not been reported yet.

**Table 4.** Pearson correlations ($r$) observed between chemical composition (% dry matter basis) of feeds and response variables

| | Correlation | CP | EE | NDF | ADF | NFC |
|---|---|---|---|---|---|---|
| $TGP_{24}$ | $r$ | 0.09 | -0.01 | -0.50 | -0.57 | 0.63 |
| | $p$-value | 0.61 | 0.98 | <0.01 | <0.01 | <0.01 |
| Methane (mL/g TDOM) | $r$ | -0.41 | -0.40 | 0.67 | 0.70 | -0.50 |
| | $p$-value | 0.02 | 0.02 | <0.01 | <0.01 | <0.01 |
| $k$ ($h^{-1}$) | $r$ | 0.43 | 0.40 | -0.48 | -0.44 | 0.21 |
| | $p$-value | 0.01 | 0.02 | <0.01 | 0.01 | 0.23 |
| $b$ (mL) | $r$ | -0.13 | -0.25 | -0.22 | -0.33 | 0.51 |
| | $p$-value | 0.47 | 0.16 | 0.20 | 0.04 | <0.01 |
| Ammonia-N (mg/100 mL) | $r$ | 0.87 | 0.45 | -0.72 | -0.66 | 0.24 |
| | $p$-value | <0.01 | 0.01 | <0.01 | <0.01 | 0.17 |
| TCA-N (mg/100 mL) | $r$ | -0.03 | 0.12 | 0.34 | 0.38 | -0.43 |
| | $p$-value | 0.85 | 0.51 | 0.05 | 0.03 | 0.01 |
| Total VFA (mmol/100 mL) | $r$ | 0.35 | 0.11 | -0.65 | -0.70 | 0.58 |
| | $p$-value | 0.04 | 0.55 | <0.01 | <0.01 | <0.01 |
| TDOM (%) | $r$ | 0.57 | 0.41 | -0.84 | -0.83 | 0.58 |
| | $p$-value | <0.01 | 0.01 | <0.01 | <0.01 | <0.01 |

CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; NFC, non-fiber carbohydrate calculated by subtracting CP, EE and NDF from OM content. $TGP_{24}$, total gas production at 24 h (mL/200 mg DM); $k$, rate of gas production; $b$, potential gas production; TCA-N, tricholoacetic acid precipitable nitrogen; VFA, volatile fatty acids; TDOM, truly degraded organic matter.

## Gas and methane production

Potential gas production and rate of gas production varied to a great extent among the feeds. The amount of gas produced from feeds depends largely upon chemical composition and rate and extent of degradability of feeds (Blummel *et al.,* 1999). Gas production is mostly the result of fermentation of carbohydrates to acetate, propionate and butyrate (Menke & Steingas, 1988), and substantial differences in carbohydrates fractions in feeds mainly influence total gas production (Deaville & Givens, 2001). Gas production from protein fermentation is comparatively small as compared to carbohydrate fermentation while contribution of fat to gas production is negligible (Menke & Steingas, 1988; Cone & van Gelder, 1999). Thus, oil cakes produced less gas compared with cereal grains and chunnies. There were no correlations noted between CP concentration and potential gas production, but gas production was correlated negatively with NDF content, and positively with non-fibrous carbohydrate (NFC) content in this study. Doane *et al.* (1997) also noted that gas production during *in vitro* fermentation of six forages was linearly related to NDF degradation. Energy rich-concentrates and pulse by-products produced higher ($p<0.001$) amounts of gas compared with straws and forages because concentrates had high degradability of OM that fermented to VFA and gas. The rates of gas production differed among the feeds. The slowest rate of gas production could possibly be influenced by the fiber contents of feeds as confirmed from the negative correlations between NDF and ADF content in this study. Slower rates of gas production observed for some substrates might be attributed to high concentrations of structural carbohydrates that are fermented at a slower rate by the rumen micro-organisms. The negative correlation between fibre content and gas production is consistent with other studies (Nsahlai *et al.,* 1994; Larbi *et al.,* 1998; Getachew *et al.,* 2004). Though the positive correlation between EE content and rate constant was significant, partial correlation between these two factors after eliminating the effect of NDF content did not show any relationship ($r=0.28$; $p=0.12$) suggesting fiber content of feeds influenced the correlation.

Variations in methane production among the feeds may be due to variations in their chemical components. In this study, concentrations of CP, EE and NFC were negatively associated, but fiber concentrations were positively associated with methane production. Chemical composition of diet has also been earlier shown to have association with *in vitro* methane output (Singh *et al.,* 2012). However in few *in vivo* studies, chemical components of the diets had little effect to methane yield with a wide range of chemical composition (Hammond *et al.,* 2013; Patra, 2014b). Usually, the type and amounts of carbohydrate present in feeds influences methane production via changes in microbial populations in the rumen (Johnson & Johnson, 1995). Oil cakes produced lesser amount of gas than other concentrates, but methane production from oil cakes and other concentrates was generally in the similar range. High amounts of soluble carbohydrates in energy concentrates promote the production of propionate in the rumen and inhibit the growth of methanogens thereby reducing methane production per unit of OM fermented (Van Kessel & Russell, 1996). Propionate acts as hydrogen sink decreasing availability of hydrogen for methane production (McAllister & Newbold, 2008). Methane production was negatively related with NFC content of feeds in this study. Oil cakes (*i.e*., MOC, GNC and til cake) containing higher concentrations of EE resulted in lower methane production than the oil cakes (*i.e*., soybean and mahua cake) containing low levels of EE. High concentration of EE might lower methane yield from these feeds. Inclusion of EE in the diets causes a decrease in methane production depending upon the levels of EE supplementation, EE sources, forms of EE supplementation and types of diets (Patra, 2014c). Overall, EE concentration was also negatively correlated with methane production in the present study. A decrease in methane production by EE supplementation may be mediated through combined influences on the inhibition of growth of methanogens, and reduction of ruminal OM fermentation, and hydrogenation of unsaturated fatty acids (acting as an alternative $H_2$ sink) in the rumen (Beauchemin *et al*., 2008; Patra & Yu, 2015).

Overall, straws produced relatively more methane compared with forages. Boadi & Wittenberg (2002) also found that methane production (L/kg digestible OM intake) was 25% higher for low quality forages than medium or high nutritional quality diets. Again, increasing the levels of green fodder such as berseem, oat and sorghum in straw- and stover-based diets may lower methane production. For instance, methane production in crossbred cows decreased by 33% when green sorghum replaced the wheat straw by 30% (Haque *et al.,* 2001). Correlation between methane production and NDF or ADF was highly negative in this study as well. Lower methane losses with the high quality diet were expected as lower fibre or high concentrate diet shifts fermentation towards propionate production (Patra & Yu, 2015).

Methane production expressed as mL/g degraded OM was lower for some shrubs (pala, dhawasi and kakoda) than straws and forages, which may be attributed to the presence of various phytochemicals, mainly tannins in shrubs (Patra & Saxena, 2010; Pal *et al.,* 2014). Different sources of tannin extracts or tannin containing forages have been shown to decrease methane production both *in vitro* and *in vivo* depending upon doses (Patra *et al.,* 2006; Puchala *et al.,* 2012). It has been suggested that the action of tannins on methanogenesis may be attributed to the direct inhibitory effects on methanogens depending upon the chemical structure of tannins, and also indirectly by decreasing fiber degradation (Beauchemin *et al.*, 2008; Patra & Saxena, 2010).

Some legume forages have been shown to decrease methane production in ruminants, which are often explained by the presence of low fiber content, high DM intake and faster rate of passage from the rumen (Beauchemin *et al.,* 2008). Based on meta-analysis, Archimède *et al.* (2011) concluded that legumes produced less methane than grasses when methane production was expressed relative to DM intake. A lower methane production expressed as mL/g DM for red clover compared with perennial ryegrass was also noted, but methane production expressed relative to feed degradability was reversed (Navarro-Villa *et al.,* 2011), which has been explained due to the greater degradability of ryegrass. Methane production expressed in terms of feed intake was also higher with alfalfa than with grass (Chaves *et al*., 2006). However in this study, methane production (per unit of DM incubated or degraded OM) potential was generally lower for cereal and grass forages than legume forages though fiber content was lower in berseem and cowpea than in grass forages, and degradability of legume forages was high. It appears that legume forages favoured the growth of methanogens in this study. This result agrees with Murray *et al.* (2001) who reported that sheep grazing high proportion of legume pasture produced greater amounts of methane compared with sheep grazing grass-alone pasture. Overall, Benchaar *et al.* (2001) using a modeling approach predicted that methane production could be lowered by increasing concentrate proportions of diets (-40%), replacing fibrous concentrates with starchy concentrates (-22%), with the utilization of less ruminally degradable starch (-7%), increasing the degradability of forage (-15%), with legumes compared to grass forages (-28%) and with silages compared to hay (-20%). In the present *in vitro* study, it was noted that methane (mL/g degraded OM) could be decreased by 23% using concentrates, by 20% replacing straws with shrubs (30.8 mL *vs.* 24.6 mL for straws and shrubs, respectively; $p<0.001$), by 19% replacing fiber-rich concentrates (*i.e.*, brans and chunies) with oil cakes and cereal grains (19.3 mL *vs.* 23.9 mL for oil cakes and cereal grains, and fiber-rich concentrates, respectively; $p=0.004$).

## Degradability of feeds and rumen fermentation

In the present study, degradability of feeds, particularly of energy feeds was directly related to VFA concentrations in the rumen ($r=0.51$; $p=0.002$). Degradability of straws was generally lower than other types of feeds, because fiber components in straws are of recalcitrant type, which are not easily attacked by the rumen microbes (Varga & Kolver, 1997). Legumes have generally greater degradability compared with grass and cereal forages. Legume, cereal and some grass forages in the present study had, in general, comparable concentrations of NDF, which might have contributed to the similar degradability values. Degradability of feeds had strong negative associations with NDF ($r=-0.84$; $p<0.01$) and ADF ($r=-0.83$; $p<0.01$) content in this study. The negative effect of NDF and ADF on degradability is in close agreement with Iantcheva *et al.* (1999) and Getachew *et al.* (2004), although the extent of the negative effect of NDF on degradability was much higher for grass hay *vs.* alfalfa in the study of Iantcheva *et al.* (1999). There was a significant correlation ($r=0.35$; $p=0.04$) between CP content and VFA concentration indicating that CP fermentation may contribute to VFA production, but no correlation was noted between EE content and VFA concentration in this study. The highly significant correlation between CP level and valerate and isovalerate production was noted earlier (Getachew *et al.,* 2004). Ammonia-N concentrations differed among the feeds due to variations in CP contents. As expected, ammonia-N had a strong positive correlation with CP content in feeds (Cone & van Gelder, 1999). A negative correlation was noted between NDF content and ammonia concentration in this study. Partial correlation analysis showed that a negative correlation existed between NDF content and ammonia concentration after eliminating the effect of CP concentration ($r=-0.43$; $p=0.012$) and EE concentration ($r=-0.67$; $p<0.001$) in feeds. Thus, increasing NDF contents in feeds perhaps enhanced utilization of ammonia for microbial growth.

In conclusion, methane production potential (mL/unit of degradable OM) of different feeds varied greatly depending upon chemical composition, which was positively correlated with fiber content, and negatively with CP, EE and NFC contents of feeds. Utilization of low methane producing feeds that are available at livestock farms could be strategically considered to feed ruminants decreasing environmental impacts. The results suggest that incorporation of concentrates and shrubs replacing straws and forages in the diets of ruminant may decrease methane production, which could be more feasible and practical for mitigating enteric methane emissions while improving production of livestock. Along with chemical composition and nutritive values, data of methane production potential of different feedstuffs should be tabulated. This information on methane production potential of feeds could be useful to prepare low methane producing rations for ruminants.

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