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Chemical composition, *in-vitro* fermentation and methane production potential of unconventional feed resources in goats

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ABSTRACT

Unconventional feed resources namely *Aloe barbadensis* (ALB), *Musa paradisiaca* (MUP), *Punica granatum* (PUG), *Murraya koenigii* (MUK), *Lawsonia inermis* (LOI) and *Boehravia diffusa* (BOD) were assessed for *in-vitro* methane production potential and fermentation with whole goat rumen flora. Nutrient content of all the bio-resources were different, and the gas production varied from 57.7 to 161.7 ml/ g DM, with the highest gas in ALB and the lowest in BOD. Although, gas production was different among all feed resources, however gas production for each gram DM fermented was similar in MUP, PUG, MUK and LOI leaves. Methane production ranged from 6.7 to 18.9 g and 10.5 to 22.83 g/ kg DM and g/ kg fermented DM respectively. The energy loss in the form of methane also followed the trend of gas production, which ranged from 11.4 to 17.1% of digestible energy. Therefore, feed resources with varying nutrient contents have significant variations in fermentability and methane production potential.

Key words: Feed resources, Fermentation, Goat, Leaves, Methane

Methane is the one of the major greenhouse gas being targeted for reduction under the Kyoto protocol to address global warming issue owing to its 21 times higher warming potential than carbon dioxide. Ruminant animals are significant contributors of methane and India has the largest livestock population in the world. These animals are maintained on tropical pastures and poor quality feed resources (agro-industrial byproducts, straw and strovers) with originating low nitrogen and high fiber contents that result in large amount of methane emission from rumen microbial fermentation. Enteric fermentation is that most important source of methane emission which is responsible for approximately 25% of global methane emission (EPA 2010) whereas ruminant livestock contributes 18% of the global green house gas (GHG) emissions (Chhabra *et al*. 2009). Several strategies have been worked out for reducing methane emission from ruminant farming, however dietary modifications have been recommended as sustainable, economic and eco-friendly way of methane mitigations. The quality of feed and the levels of secondary plant metabolites are helpful in improving the feed value of diet along with reduced methane production in ruminants (Boga *et al*. 2014, Bueno *et al*. 2015). The ruminant diet is composed of cell wall that results in higher methane production per unit organic matter degraded (Widiawali and Thalib 2007), particularly in India where they are maintained on low quality pastures and feed resources. Energy losses in methane emission from rumen apart from global warming, is an energy wasteful process production in rumen which equals to feed energy intake losses ranging from 6 to 12% and up to 14% digestible energy intake loss (Cottle *et al*. 2006) that depends on the type of diet and level of feeding and each gram of methane production causing 55.65kJ energy loss (de Haas *et al.* 2012) in rumen. The methane production potential of feed may be dependent on the nature of feed, fermentation, cell wall content and levels of secondary plant metabolites (Santoso *et al*. 2003). Methane production was less in legume forages than grasses or cereal straws (Singh *et al*. 2012). Incorporation of forages with low methanogenic potential has been recommended in improving the productivity of ruminants with reduced methane production per unit digestible dry matter intake or raw product produced. The *in-vitro* rumen fermentation (IVRF) is presently being exploited to study methane production and mitigation potential from ruminants, and for the formulation of diets with reduced methane production. Therefore, the present study was undertaken to evaluate the chemical constituents, IVRF and methane

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production potential of a few unconventional feed bio-resources for inclusion in ruminant diets for methane mitigation.

MATERIALS AND METHODS

Sample processing and chemical analysis: The clodates of *Aloe barbadensis* (ALB) and leaves of *Musa paradisiaca* (MUP), *Punica granatum* (PUG), *Murraya koenigii* (MUK) and *Lawsonia inermis* (LOI), and whole plant of *Boehravia diffusa* (BOD) were collected from several locations of the ICAR-CIRG Agriculture farm. Samples were dried in hot air oven at 50ºC until constant weight. Dried samples were ground to pass 1 mm screen and, used for chemical analysis and *in-vitro* fermentation. Organic matter content of samples was determined by combustion (AOAC 2000) at 450ºC for 4 h. Nitrogen was determined by the Kjeldahl procedure (AOAC 2000), and the crude protein (CP) was calculated as N×6.25. Total crude fat was estimated by solvent extraction procedure (AOAC 2000) with ethyl ether using a Soxhlet's apparatus. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined following the procedure of Van Soest *et al.* (1991) without sodium sulphite and α-amylase, and expressed with residual ash. Lignin was determined following the procedure of Robertson and Van Soest (1988).

In-vitro *fermentation*: The *in-vitro* fermentation characteristics were determined using the 100 ml glass syringe procedure. In brief, the samples were ground to pass through 1 mm screen. Homogenised sample (200 mg) was mixed with 30 ml microbial inoculums for gas production, whereas 500 mg sample was used for degradability assessment. The samples were incubated at 39°C for 24 h and truly degradable dry matter (TDDM) was assessed by estimating NDF of syringe contents. The truly degradable organic matter (TDOM) was estimated from TDDM by ashing at 450°C for 4 h. Recommended (Menke and Steingass 1988, Mould *et al*. 2005) microbial inocula was used for *in-vitro* fermentation. Rumen fluid was collected using stomach tube before morning feeding from rumen of adult bucks, which were fed gram straw based diet at maintenance having roughage to concentrate ratio (R: C), 60: 40. Metabolizable energy (ME), microbial biomass production (MBP) and fermentation efficiency of feeds were calculated using the mathematical equations (Blummel *et al.* 1997) on basis of total gas production. ME (MJ/ kg DM) = 2.2 + (0.136 × gas, ml/ 200 mg DM) + (0.0057 ×% CP); MBP (mg/ 200 DM) = (Truly degradable OM/ 200 mg DM - (2.2 × actual gas, ml/ 200 mg DM)); Fermentation efficiency = (mg DM digested/ ml gas produced).

Estimation of methane: The methane in fermented gas mixture was estimated by using a gas chromatograph (GC). A dual channel, dual column, microprocessor based gas chromatography was equipped with ECD and FID detector and control module for temperature controls. Stainless steel column suitable for methane analysis was used. The airtight sterilized syringes were used to withdraw the gas from the *in-vitro* syringes at the rubber outlet, which was injected in the GC and area of the graph plotted by output system was recorded for methane estimation. A calibration curve was generated using methane (99% purity) as standard. Temperature values of injector oven, column oven and detector were 100, 50 and 120°C, respectively. Nitrogen was used as a carrier gas.

Statistical analysis: The observations of chemical composition, gas and methane production, digestibility, and fermentation characteristics were analyzed for statistical significance by the analysis of variance procedure using a general linear mathematical model 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 Duncan's multiple range tests (SPSS base 16).

RESULTS AND DISCUSSION

Chemical composition: Chemical constituents were significantly (P<0.001) different among 6 feed resources (Table 1). The clodates of ALB contained lowest DM (2.21%) and organic matter (77.48%), whereas CP content was lowest (7.69%) in BOD leaves and highest (16.03%)

Table 1. Chemical composition (%) of feed resources

Chemical constituents	Feed resources*						SEM
	ALB	MUP	PUG	MUK	LOI	BOD	
Dry matter	2.21^{f}	16.29^{e}	64.26^{a}	37.67^{c}	27.82^{d}	41.76^{b}	4.770
Organic matter	77.48^{f}	88.43^{c}	91.84^{b}	85.26^{d}	93.53^{a}	81.25^{e}	1.372
Crude protein	9.04^{e}	14.29^{c}	13.39^{d}	15.49^{b}	16.03^{a}	7.69^{f}	0.769
Fat	2.06^{e}	6.49^{b}	8.15^{a}	5.50^{c}	4.89^{d}	1.46^{f}	0.571
Neutral detergent fibre	24.06^{e}	65.22^{a}	26.04^{d}	41.22^{b}	16.09^{f}	38.32^{c}	3.861
Acid detergent fibre	16.32^{e}	30.75^{a}	17.90^{d}	21.17^{c}	11.83^{f}	26.34^{b}	1.537
Cellulose	13.25^{d}	25.26^{a}	9.37^{e}	14.39^{c}	8.42^{f}	17.92^{b}	1.374
Hemi-cellulose	7.75^{d}	34.47^{a}	8.14^{d}	20.05^{b}	4.26^{e}	11.98^{c}	2.482
Lignin	3.02^{e}	4.12^{d}	8.82^{a}	6.80^{c}	2.77^{e}	7.98^{b}	0.583

*Observations with different superscripts in a row differ significantly (P<0.001); clodates of *Aloe barbadensis* (ALB) leaves of *Musa paradisiaca* (MUP), *Punica granatum* (PUG), *Murraya koenigii* (MUK) and *Lawsonia inermis* (LOI), and whole plant of *Boehravia diffusa* (BOD).

in LOI leaves. Crude fat content ranged from 1.46% (BOD) to 8.15% (PUG) among 6 feed resources. Cell wall contents were higher in MUP leaves followed by MUK, BOD, PUG, ALB and LOI leaves. The lignin content varied between 2.77 to 8.82% in different feed resources. The feed resources evaluated had varying levels of nutrients, which could sufficiently support the nutrient requirement of rumen microbes for optimum rumen fermentation. The feed resources with 8% CP are recommended to be appropriate to maintain the desired rumen microbial activity for rumen fiber degradation (Leng 1990). Differences in chemical constituents of plant resources could be due to the genotypic variations and nature of plant (Hanson and Rivera 2010), therefore, observed variations in present study were attributed by the different types of feed resources. Similar to our findings, Singh *et al*. (2012), Bhatt *et al.* (2014) and Ramachandran *et al.* (2015) have reported significant differences in chemical composition of different feed resources used in India for ruminant feeding.

Fermentation and in-vitro *digestibility*: The fermentation attributes, viz. gas production, digestibility and fermentation efficiencies were significantly (P<0.001) different among 6 feed resources (Table 2). The ALB had highest, while BOD had lowest gas production with other feeds resource producing intermediate levels of gas. *In-vitro* dry matter and organic matter digestibility were the highest in LOI followed by ALB, PUG, BOD, MUK and minimum in MUP leaves. Gas production (ml/ g digested organic matter) was similar in MUP, PUG, MUK and LOI but was higher than BOD and lower than ALB. The ME (MJ/kg DM) was higher in ALB (7.11 MJ) and lowest (4.74 MJ) in BOD, whereas other feed resources were having ME between 5.44 to 6.60

Table 2. Gas production, digestibility, fermentation efficiency and microbial biomass production of different feed resources

Attributes	Feed resources*						SEM	P-Value
	ALB	MUP	PUG	MUK	LOI	BOD		
Gas (ml/ g DM)	161.67^{a}	93.33^{c}	125.00^{b}	86.67^{c}	128.33^{b}	77.50^{c}	7.271	<0.001
In-vitro dry matter digestibility (%)	86.48^{b}	63.03^{f}	81.88^{c}	66.18^{e}	90.40^{a}	76.68^{d}	2.446	<0.001
Gas (ml/ g digestible dry matter)	186.98^{a}	148.10^{b}	152.62^{b}	130.98^{b}	141.96^{b}	101.07^{c}	6.701	<0.001
In-vitro organic matter digestibility (%)	90.78^{b}	64.76^{f}	87.14^{c}	69.95^{e}	95.03^{a}	77.05^{d}	0.892	<0.001
Gas (ml/ g digested OM)	178.09^{a}	144.21^{b}	143.42^{b}	124.14^{b}	135.05^{b}	100.60^{c}	6.180	<0.001
ME (MJ/kg DM)	7.11^{a}	5.55^{c}	6.36^{b}	5.44^{c}	6.60^{b}	4.74^{d}	0.199	<0.001
Fermentation efficiency (ml gas /mg DM digested DM)	5.35^{c}	6.75^{bc}	6.55^{bc}	7.85^{b}	7.05^{b}	9.94^{a}	0.375	<0.001
Microbial biomass production (mg /200 mg DM)	62.51^{e}	67.75^{de}	97.06^{b}	75.19^{d}	112.41^{a}	84.84^{c}	4.283	<0.001
Partioning factor (TDOM (mg) /ml gas)	4.13^{c}	5.82^{b}	6.08^{b}	6.73^{ab}	6.59^{ab}	7.65^{a}	0.293	0.001
Efficiency of microbial biomass production (mg MBP/100 mg TDOMR)	71.95^{c}	95.74^{b}	98.20^{b}	101.97^{ab}	102.40^{ab}	109.73^{a}	3.060	<0.001
Truly digestible OM in rumen (truly digestible OM × 0.65)	86.86^{c}	70.73^{e}	98.84^{b}	73.66^{e}	109.77^{a}	77.31^{d}	3.439	<0.001

*Observations with different superscripts in rows differed significantly; clodates of *Aloe barbadensis* (ALB) leaves of *Musa paradisiaca* (MUP), *Punica granatum* (PUG), *Murraya koenigii* (MUK) and *Lawsonia inermis* (LOI), and whole plant of *Boehravia diffusa* (BOD).

Table 3. Methane production and energy loss as methane in different feed resources

Attributes	Feed resources*						SEM	P-Value
	ALB	MUP	PUG	MUK	LOI	BOD		
Gas (ml/200 mg DM)	32.33^{a}	18.67^{c}	25.00^{b}	17.33^{c}	25.67^{b}	15.67^{c}	1.447	<0.001
Methane (%)	17.22^{b}	16.43^{b}	17.33^{b}	16.52^{b}	14.70^{b}	21.20^{a}	0.567	<0.001
Methane (ml/g DM)	26.44^{a}	13.85^{c}	20.29^{b}	12.75^{c}	17.04^{b}	15.62^{c}	1.212	<0.001
Methane (ml/g digestible DM)	30.48^{a}	21.79^{b}	24.68^{ab}	19.20^{b}	18.80^{b}	20.40^{b}	1.183	<0.001
Methane (g/kg DM)	18.93^{a}	9.92^{c}	14.53^{b}	9.13^{c}	12.20^{bc}	11.19^{c}	0.868	<0.001
Methane (g/kg digestible DM)	21.83^{a}	15.60^{b}	17.67^{ab}	13.74^{b}	13.46^{b}	14.61^{b}	0.847	<0.001
Energy loss (MJ/kg feed DM)	1.05^{a}	0.55^{c}	0.81^{b}	0.51^{c}	0.68^{bc}	0.62^{c}	0.048	<0.001
Energy loss (MJ/kg feed digestible DM)	1.21^{a}	0.87^{b}	0.98^{ab}	0.76^{b}	0.74^{b}	0.81^{b}	0.047	0.009
% Energy loss (MJ/kg feed DM)	14.80^{a}	9.92^{c}	12.69^{ab}	9.28^{c}	10.29^{bc}	13.07^{a}	0.556	0.003
% Energy loss (MJ/kg feed digestible DM)	17.07^{a}	15.61^{a}	15.43^{a}	13.97^{ab}	11.35^{b}	17.07^{a}	0.624	0.037

*Observations with different superscripts in rows differed significantly; clodates of *Aloe barbadensis* (ALB) leaves of *Musa paradisiaca* (MUP), *Punica granatum* (PUG), *Murraya koenigii* (MUK) and *Lawsonia inermis* (LOI), and whole plant of *Boehravia diffusa* (BOD).

MJ. Fermentation efficiency was lowest (5.35) in ALB and highest (9.94) in BOD, whereas MUP, PUG, MUK and LOI were having similar fermentation efficiency. Microbial biomass production (MBP) was not different between ALB and MUP, MUP and MUK, whereas other feed, were different to each other in MBP. Partitioning factor followed the trend of fermentation efficiency. Efficiency of MBP was lowest in ALB and highest in BOD, whereas MUP, PUG, MUK and LOI, and MUK, LOI and BOD had similar efficiency of MBP. Truly digestible organic matter in rumen (TDOMR) was the highest in LOI followed by PUG, ALB, BOD, MUK and MUP, where MUK and MUP had similar TDOMR.

In-vitro gas production has positive relationship with digestibility of feeds, and variations in crude protein and NDF concentrations have the major influence on digestibility and thus on gas production. Significant differences among feed resources in fermentation and digestibility were due to variations in chemical constituents of feeds (Singh *et al.* 2012). Digestibility of dry matter and organic matter in present study were higher than that reported by Bhatt *et al*. (2014) and Ramachandran *et al.* (2015) and can be attributed to the adequate or higher levels of CP in the feeds. Crude protein content of the feeds was above the critical level of 70g/ kg DM and below which restricts microbial activity due to inadequate availability of nitrogen for microbial growth (Leng 1990). Higher ME, low fermentation efficiency and partitioning factor of ALB might be due to the higher digestibility and ME, and adequate CP content of the ALB, which provided synchrony between energy and protein availability for microbial growth and therefore, better fermentation. Low ME content along with higher CP content of MUP, whereas low ME (due to higher lignin content) of BOD could have reduced the digestibility due to restricted microbial growth because of restricted availability of energy in relation to nitrogen availability (Blummel *et al.* 2005). Differences in other fermentation attributes could be due to the assigned variations in digestibility and ME content of the feeds. The observations of fermentation efficiency, microbial biomass production and partitioning factor were within the reported range of variations (Blummel *et al.* 2005) in Indian feed resources (Bhatt *et al.* 2014). Therefore, it may be remarked that feed resources with varying chemical composition have the different levels of digestibility, fermentation, efficiency and microbial biomass production, which are affected chiefly by the crude protein and soluble carbohydrate levels.

Gas and methane production: Gas production (ml/200 mg DM), proportion (%) of methane in total gas, methane production (ml or g per unit of dry matter and digested dry matter), and methane energy loss (MJ/ kg DM or % of feed ME) were different ($P<0.001$) among six feed resources (Table 3). MUK and MUP produced similar but lowest gas, whereas LOI and PUG produced similar but medium level; however, ALB produced highest gas during 24 h fermentation. Proportion of methane was higher in BOD compared to other feed resources, which were having similar proportion of methane in gas. Based on DM incubated, methane production (ml or g/ kg DM) was lowest ($P<0.001$) in MUK, which was not significantly different with the MUP, BOD and LOI feeds, the highest ($P<0.001$) methane production was observed with ALB followed by PUG. Methane production on unit digested dry matter was higher ($P<0.05$) in ALB compared to other feeds. Feed energy loss in terms of MJ/ kg feed DM or digested DM and percent energy loss of feed energy followed the trend of methane production. Methane energy loss in terms of MJ/kg feed DM and MJ/ kg feed digested DM, and percent energy loss (MJ/kg feed DM and MJ/ kg feed digested DM) were higher in ALB and BOD feeds. During the fermentation of feed in rumen, a mixture of gases including methane and volatile fatty acids (VFAs) are produces by the consortia of anaerobes. Host animal as source of energy uses these VFAs. Hydrogen is also formed during fermentation in rumen. With the accumulation of H_2, there is decline in pH of rumen that results into decreased fermentation and re-oxidation of NADH is also inhibited that, in turn, inhibits microbial growth, decreases forage digestion, and thus reduces the associated production of acetate, propionate, and butyrate (Joblin 1999). The methanogenic archaea play an important role in maintaining the proper microbial activity of rumen by converting CO_2 and H_2 to form CH_4, thus reducing the metabolic H_2 produced during microbial metabolism (McAllister and Newbold 2008). The methane gas thus produced is exhaled into the atmosphere by the ruminants, contributing to global methane pool. With an energy content of 55.22 MJ/kg (de Haas *et al*. 2012), CH_4 represents a significant loss of dietary energy consumed from the production system because a significant portion of the energy consumed by ruminant is converted to CH_4 and expelled through respiratory gases. Enteric CH_4 production in livestock represents a loss of nearly 6–15% of the animal's gross energy (GE) intake (Johnson and Johnson 1995, Cottle *et al*. 2006). Therefore, reducing enteric CH_4 production may also lead to production benefits. The degree of fermentation of feed determines the level of production of gas production and ultimately, methane and the nutrient composition of feed is detrimental for the fermentation of feed. Since feed resources evaluated in present study had different nutrient composition and digestibility, they showed varying levels of methane production and energy loss. The methane production observed in the present study was within the reported range of variation of methane production in Indian feed resources (Singh *et al*. 2012, Bhatt *et al*. 2014, Ramachandran *et al*. 2015). High fiber and lignin contents of feeds have known to produce more methane (Morgavi *et al*. 2010). Feed energy losses as methane in the present study were in agreement with the reported energy losses in ruminant feeds (Cottle *et al*. 2006). Thus, variations in methane production among feeds may attribute to varying levels of crude protein, fiber and lignin contents.

It may be concluded that the clodates of *Aloe barbadensis* and leaves of *Musa paradisiaca*, *Punica granatum*, *Murraya*

koenigii and *Lawsonia inermis*, and whole plant of *Boehravia diffusa* contain crude protein between 7.7 to 16.0% and lignin 2.8 to 8.8%. The methane production potential ranged from 13.46 to 21.83 g/kg digestible dry matter, which accounted feed energy loss of 9.3 to 14.8%. Among six feed resources evaluated in present study, *Aloe barbadensis* produced highest methane on unit digestible dry matter, while other feeds produced lower but similar methane.

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