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Assessment of fodder quality and methane production potential of north-eastern Himalayan forest tree leaves

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ABSTRACT

Nutritional evaluation as tree fodder as well as a rumen manipulator of six multipurpose Himalayan forest tree leaves, viz. Kadam (*Anthocephalus cadamba*), Kaew (*Costus speciosus*), Karoi (*Albizia procera*), Bakful (*Sasbania grandiflora*), Gamar (*Gmelina arborea*), and Barhar (*Artocarpus lakoocha*), were evaluated by *in vitro* gas production test. The mean content of OM, CP, EE, T-CHO, NDF, ADF, cellulose and lignin of these tested tree leaves were 91.4, 14.6, 3.9, 72.8, 42.7, 31.2, 19.8, and 11.1% on DM basis, respectively. The gas production per g digested dry matter varied from 111.1 ml/g DDM/24h in Bakful (*Sasbania grandiflora*) to 612.3 ml/g DDM/24h in Barhar (*Artocarpus lakoocha)* tree leaves while methane production per gram digested dry matter varied from 14.7 ml/g DDM/24h in Kadam (*Anthocephalus cadamba*) to 102.2 ml/g DDM/24h in Bahar (*Artocarpus lakoocha*) tree leaves. TVFA and propionate production were higher due to inclusion of Karoi (*Albizia procera*) tree leaves in the incubation media. However, lowest NH_3-N concentration and rumen protozoal population were observed due to incubation of Kadam (*Anthocephalus cadamba)* tree leave. Similarly, activity of polysaccharide degrading enzyme like carboxymethyl cellulase, xylanase and β-glucoidase enzymes were lower due to incubation of Kadam (*Anthocephalus cadamba)* in comparison to other tested tree leaves. However, activity of amylase enzyme was similar among all the tested tree leaves. Highest IVTDMD (52.3%) was observed for the Karoi (*Albizia procera*) tree leaves followed by Kaew (*Costus speciosus*) (47.9%) and Kadam (*Anthocephalus cadamba*) (43.8%) tree leaves. Similarly, TDN and ME content were also highest for Karoi (*Albizia procera*) tree leaves. The results indicated that among the tested tree leaves, Karoi (*Albizia procera)* was best tree fodder for feeding to the animals and Kadam (*Anthocephalus cadamba)* can be used as rumen manipulator to reduce ruminal methanogenesis and protozoal population for improving animal productivity.

Key words: Fodder quality, *In vitro* digestibility, Methane, Plant secondary metabolites, Rumen protozoa, Tree leaves

Scarcity of feed and fodders, mainly due to ever increasing livestock and human population, is one of the major constraints in ruminant animal productivity in India (Deuri and Wadhwa 2018). Therefore, alternate feed resources have to be explored. Fodder tree and shrub leaves make an important component of ruminant ration particularly in semi-arid region as well as hilly region of India (Bakshi and Wadhwa 2007, Datt *et al*. 2008, Singh *et al*. 2018). Tree leaves are rich in protein and mineral content as compared to grasses and thus can be used as supplement to low quality grasses (Aganga and Tshwenyane 2003). Tree leaves are amply available in North-East region of India. However, information about the nutritive value of such feed resources is very limited.

Manipulating the rumen microbial ecosystem to reduce ruminal methane, ciliate protozoa and ammonia nitrogen production by using feed additives for efficient utilization of dietary energy and protein is a useful strategy to improve production efficiency of ruminant animals (Alexander *et al*. 2008, Bhatta *et al*. 2017). There has been an increase in interest to use natural products containing plant secondary compounds instead of chemical feed additives such as ionophore, antibiotic and anti-methanogenic compound to modify rumen fermentation for improving feed utilization and productive performances of ruminant animals (Bhatta *et al*. 2017, Singh *et al*. 2018). Plant containing high amount of saponins such as *Sapindus saponaria, Yucca schidigear, Enterolobium ciclocarpum* and *Sessbania sesban* had been reported to have a potential to suppress rumen protozoal populations, increase bacterial and fungal populations, propionate production, microbial yield, microbial protein synthesis and decreased ruminal methanogenesis and ammonia production which leads to better productivity of ruminant animals (Kamra *et al.* 2006, Patra and Saxena 2009). Similarly, condensed tannin containing plants reduced dietary protein degradation and methanogenesis in the rumen and increased microbial protein synthesis (Puchala *et al.* 2005, Patra and Saxena 2010). Tree leaves

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also contains plant secondary metabolites (saponins, tannins, essential oils etc) which can reduce protozoal population and methane production in rumen, thus can be use for rumen manipulation for improving animal productivity. However, effectiveness of plant or tree leaves to manipulate rumen fermentation varied depending upon the source and type of secondary metabolites present in these plants or tree leaves. Therefore, the present experiment was conducted to evaluate different tree leaves nutritionally as tree fodder and to find out suitable tree leaves for using as a natural rumen manipulator to reduce ruminal methanogenesis and protozoal population.

MATERIALS AND METHODS

Collection and processing of tree leaves: Six tree leaves e.g. Kadam (*Anthocephalus cadamba*), Kaew (*Costus speciosus*), Karoi (*Albizia procera*), Bakful (*Sasbania grandiflora*), Gamar (*Gmelina arborea*) and Barhar (*Artocarpus lakoocha)* were collected from Tripura for experimental studies. Each collected leaves were dried at 50°C for 72 h in a forced hot air oven and ground to pass through 1 mm sieve. These ground tree leaves were analyzed for chemical composition estimation and tested for their effect on ruminal fermentation and methanogenesis by *in vitro* gas production test.

In vitro *gas production test:* For *in vitro* gas production test, 200±5 mg of each ground tree leaves were incubated anaerobically with 30 ml buffered rumen inoculum in 100 ml glass syringe at 39°C for 24 h. Incubation were conducted in triplicate for each tree leaves and it was repeated twice at 10 days interval. The inoculum/incubation medium was prepared by mixing cattle rumen liquor with buffer in the ratio of 1:2 (Menke and Steingass 1988). Rumen liquor was collected just before morning feeding from cattle fed on a diet containing paddy straw and concentrate mixture in 1:1 ratio by using stomach tube. Incubation without sample served as blank. The difference in the composition and activity of rumen inoculum among incubation was controlled by parallel incubation of reference standard feedstuff (maize hay).

Estimation of gas and methane production: After 24 h incubation, gas production was estimated by the displacement of piston during incubation. The gas produced due to fermentation of tree leaves was calculated by subtracting gas produced in blank syringe (containing no substrate/tree leaves, but only the inoculums/rumen liquor and buffer) from total gas produced in the syringe containing tree leaves and buffered rumen inoculums. The gas produced in standard syringe (containing standard maize hay) was used to check day-to-day variation in the quality of inoculums. For methane estimation, 1 ml gas was sampled from the head space of syringe and injected in to Netel Ultima 2100 gas chromatograph equipped with dual flame ionizing detector (FID) and stainless steel column packed with Porapak-Q. The gas flow rates for nitrogen, hydrogen and air were 30, 30 and 320 ml/min, respectively. Temperature of injector, oven and detector were 120, 50 and 120°C, respectively. A mixture of methane and carbon dioxide (31.8:68.2) was used as standard. Methane production was calculated from total gas production and proportion of methane present in total gas.

Staining and counting of rumen protozoa: At the end of 24 h incubation, 1 ml of incubation medium was transferred with a wide orifice pipette into a screw-capped test tube containing 1 ml formalinized physiological saline with brilliant green dye (0.85 % w/v sodium chloride solution containing 20 % w/v formaldehyde and 2 % w/v brilliant green dye). The contents of the test tube were mixed thoroughly and allowed to stand overnight at room temperature before counting. Total and differential counts of rumen protozoa were made in 20 microscopic fields at 100× magnification. Ciliate protozoa were identified by the method of Hungate (1966) and counted as described by Veira *et al*. (1983).

Measurement of volatile fatty acid concentration: Total volatile fatty acids (TVFA) estimation of aliquot (incubation medium) was carried out as described by Barnett and Reid (1957). For estimation of VFA fractions, at the end of 24 h incubation, syringe aliquots were transferred to the 50 ml centrifuge tubes and centrifuged at 2300× g for 20 min at 4ºC (Getachew *et al*. 2000). Supernatant (1 ml) was collected after centrifugation in a micro centrifuge tube containing 0.20 ml metaphosphoric acid (25 ml/100 ml). The mixture was allowed to stand for 4 h at room temperature and centrifuged at 6000× g for 10 min. The clear supernatant was collected and stored at – 20°C until analyzed. Supernatant (1 µl) was injected in a gas chromatograph (Netel Ultima 2100) equipped with dual flame ionizing detector (FID) and chromosorb glass column (4 ft length and 1.8 mm dia) as described by Cottyn and Boueque (1968).

Estimation of in vitro *true dry matter digestibility (IVTDMD):* For the estimation of IVTDMD, the content of syringe was transferred quantitatively to spoutless beaker by repeated washing with 100 ml neutral detergent solution. The content was refluxed for 1 h and filtered through pre-weighed gooch crucible (Grade G1). The DM of the residue was weighed and IVTDMD of feed/tree leaves was calculated as follows:

$$\textit{In vitro}\text{ true dry matter degradability (IVTDMD)} = \frac{\text{(DM of tree leave taken for incubation} - \text{NDF residue)}}{\text{DM of tree leave taken for incubation}} \times 100$$

Chemical analysis of sample: The samples were evaluated for dry matter (DM) after drying for 24 h at 100°C in hot air oven. Organic matter (OM) was done by ashing at 550°C for 4 h, and for crude protein (CP) estimation, Kjeldahl technique (AOAC 2005) was followed. The NDF and ADF were estimated as per Van Soet *et al.* (1991). Microbial biomass was calculated according to Blumel *et al*. (1997). Ammonia nitrogen concentration in the incubation media was estimated by the method of Weatherburn (1967). For estimation of enzyme activity, the content of each syringe was processed as described by Patra

et al. (2006a). The reducing sugars produced were estimated by the method of Miller (1959). The metabolizable energy (ME) content of tree leaves was computed from the equation given by Krishnamoorthy *et al*. (1995) and total digestible nutrient (TDN) was calculated from ME as per (NRC 1989).

Statistical analysis: Data were analyzed by the methods described by Snedecor and Cochran (1994). The data were subjected to analysis of variance (ANOVA) and significant treatment effect was determined by comparing the means with Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

Chemical composition of tree leaves: Collected tree leaves contained 22.8 to 48.3% DM on fresh basis. Datt *et al*. (2007) also reported that DM content of tree leaves from Tripura varied from 23.5 to 48.8% on fresh basis. The Gamar (*Gamelina arborea*) leaves had the highest CP (15.9%) content and the lowest was found in Kadam (*Anthocephalus cadamba*) (11.6%), among the tested tree leaves (Table 1). All the tested tree leaves had more than 9% CP and CP level below this adversely affects the fermentation in rumen if given to ruminant animal exclusively (Alam and Djajanigara 1994). Lowest EE content (2.4%) was observed in Barhar (*Artocarpus lookacha*) and it was highest (6.7%) in Bakful (*Sasbania grandiflora*) tree leaves. There was large variation between the lignin content of different tree leaves. The NDF and ADF content of the tree leaves were between 34.2 to 49.7% and 21.4 to 43.8% on DM basis, respectively. Highest lignin content (17.8%) was observed in Barhar (*Artocarpus lookacha*) and it was lowest (8.9%) in Kadam (*Anthocephalus cadamba*) tree leaves. The chemical composition of different tree leaves in present study was in the range as reported by other investigator in different parts of the country (Singh 1999, Sharma *et al*. 2000, Bakshi and Wadhwa 2007, Datt *et al*. 2007).

Effect of different tree leaves on in vitro *gas and methane production:* Highest (P<0.01) gas production (120.6 ml/g DM/24h) was observed due to incubation of Barhar (*Artocarpus lakoocha)* tree leaves followed by Karoi (*Albebizia procera)* and Kaew (*Costus speciosus)* tree leaves (Table 2). However, in terms of digested dry matter, it was highest (P<0.01) for Barhar (*Artocarpus lakoocha)* tree leaves (612.3 ml/g DDM/24h) while it was lowest (111.1 ml/g DDM/24h) for Bakful (*Sasbania grandiflora)* followed by Kaew (*Costus speciosus)* and Kadam (*Anthocephalus cadamba)* tree leaves. Lower gas production (ml/g DDM/24h) due to incubation of Bakful (*Sasbania grandiflora),* Kaew (*Costus speciosus)* and Kadam (*Anthocephalus cadamba)* tree leaves might be due to presence of high concentration of tannic acid, phenolic compounds and saponins. Earlier it was reported that tannic acid (Makkar *et al.* 1995, Patra *et al*. 2006b), phenolic compounds and saponins (Goel *et al*. 2008) reduce gas production significantly under *in vitro* gas production test.

The lowest (P<0.01) methane production (14.7 ml/g DDM/24h) was observed due to incubation of Kadam (*Anthocephalus cadamba*) tree leaves followed by Kaew (*Costus speciosus)* and Karoi (*Albizia procera)* leaves. Per cent of methane produced in total gas was also lowest for Kadam (*Anthocephalus cadamba)* tree leaves followed by *Costus speciosus, Albizia procera, Gamelina arborea, Artocarpus lakoocha* and *Sasbania grandiflora*. This reduction in methane production might be due to the presence of tannin and phenolic compound in Kadam (*Anthocephalus cadamba*), Kaew (*Costus speciosus)* and Karoi (*Albizia procera)* tree leaves. Min *et al*. (2005) and Bhatta *et al*. (2009), reported that tannins (condensed tannins) inhabited methanogenesis *in vitro*. Phenolic acids such as p-coumaric acid, ferulic acid, cinnamic acid are found to decrease methane production (Asiegbu *et al.* 1995, Patra *et al*. 2006b). It should be noted that not all types of tannins produce beneficial nutritional response. Generally tannin with low molecular weight showed greater inhibitory effect on rumen microbes, because of their higher protein precipitating capacities than high molecular weight polymeric tannins. Recently, it was also confirmed that samples containing both hydrolysable plus condensed tannins were more effective in reducing *in vitro* total gas and methane production than those containing only hydrolysable tannins (Bhatta *et al*. 2015). Jeyalalitha *et al*. (2015) reported that Kadam (*Anthocephalus cadamba*) tree leaves contained different phytochemicals like terpenoids, tannins, steroids, flavonoids and alkaloids.

Effect of different tree leaves on rumen protozoal population and ammonia nitrogen concentration: Ciliate protozoa present in the collected rumen liquor and incubation medium was B type population due to presence of *Epidinium* sp. and the absence of *Polyplastron*

Table 1. Chemical composition (on % DM basis) of different tree leaves

Tree leaves (Local /Scientific name)	Chemical composition (on % DM)							
	OM	CP	EE	T-CHO	NDF	ADF	Cellulose	ADL
Kadam (*Anthocephalus cadamba*)	93.3	11.6	3.6	78.1	34.2	21.4	12.2	8.9
Kaew (*Costus speciosus*)	85.3	13.8	4.7	66.8	39.4	27.9	18.4	9.3
Karoi (*Albizia procera*)	95.1	15.5	2.5	77.1	47.6	32.4	22.9	9.2
Bakful (*Sasbania grandiflora*)	88.7	15.4	6.7	66.6	46.4	39.2	27.2	11.8
Gamar (*Gmelina arborea*)	96.9	15.9	4.1	76.9	39.2	22.2	12.6	9.3
Barhar (*Artocarpus lookacha*)	89.3	15.8	2.4	71.1	49.7	43.8	25.7	17.8

OM, Organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre. T-CHO, total carbohydrates.

Table 2. Effect of different tree leaves on ruminal gas and methane production

Tree leaves (Local /Scientific name)	Gas production (ml/g DM/24h)	Gas production (ml/g DM/24h)	CH_4 production (ml/g DM/24h)	CH_4 production (ml/g DM/24h)
Kadam (*Anthocephalus cadamba*)	56.1^{B}	127.7^{AB}	6.4^{A}	14.7^{A}
Kaew (*Costus speciosus*)	57.8^{B}	120.9^{AB}	13.8^{B}	28.1^{B}
Karoi (*Albizia procera)*	75.4^{C}	144.5^{B}	17.1^{BC}	32.6^{B}
Bakful (*Sasbania grandiflora)*	21.2^{A}	111.1^{A}	25.1^{CD}	83.3^{C}
Gamar (*Gmelina arborea)*	24.8^{A}	130.9^{AB}	28.2^{D}	85.8^{C}
Barhar (*Artocarpus lakoocha)*	120.6^{D}	612.3^{C}	31.1^{D}	102.2^{D}
SEM	3.95	15.2	3.95	3.89
Level of significance	P<0.01	P<0.01	P<0.01	P<0.01

^{ABCD}Values with different superscripts in a column differ significantly (P<0.01). DM, Dry matter; DDM, Digested dry matter; SEM, Standard error of mean.

multivesiculatum (Coleman 1980). Numerically spirotrich protozoa comprised more than 80% of total protozoal population in the present experiment is also similar to the earlier finding (Santra and Karim 2002). In present experiment, spirotrich protozoa comprised 98% of total rumen protozoal population and Holotrich protozoa comprised only 2% of the total rumen protozoal population. Overall rumen total protozoal number varied from 46.8 to 53.8 × 10^3/ml incubation media where as holotrich and spirotrich protozoal number varied from 0.4 to 1.6 × 10^3 and 46.4 to 52.2 × 10^3/ml incubation media, respectively. Lowest (P<0.01) number of spirotrich, holotrich and total rumen protozoal count was observed due to incubation of Kadam (*Anthocephalus cadamba)* tree leaves. A reduction in numbers of total rumen protozoa as well as holotrich and spirotrich protozoa due to incubation of Kadam (*Anthocephalus cadamba*) leaves may be attributed to the presence of essential oils or tannins in that tree leave as this plant secondary metabolites had anti protozoal activity (Patra and Saxena 2009). Hristov *et al.* (2003) reported that tannic acid reduced the rumen protozoal population drastically. Further, the difference in the response of rumen protozoa to tannins or essential oils might be due to different chemical and physical structure of tannins or essential oils in different leaves as also reported by Waghorn and McNabb (2003).

Table 3. Effect of different tree leaves on rumen ammonia concentration and protozoal number

Tree leaves (Local/Scientific name)	NH_3-N (mg/dl)	Holotrich protozoa (×10^3/ml)	Spirotrich protozoa (×10^3/ml)	Total protozoa (×10^3/ml)
Kadam (*Anthocephalus cadamba)*	7.6^{A}	0.4^{A}	46.4^{A}	46.8^{A}
Kaew (*Costus speciosus)*	8.2^{B}	0.5^{AB}	47.2^{AB}	47.7^{AB}
Karoi (*Albizia procera)*	8.6^{B}	0.6^{B}	48.2^{B}	48.8^{B}
Bakful (*Sasbania grandiflora)*	10.1^{D}	1.5^{CD}	52.1^{C}	53.6^{C}
Gamar (*Gmelina arborea)*	9.4^{C}	1.4^{C}	51.8^{C}	53.2^{C}
Barhar (*Artocarpus lakoocha)*	9.6^{C}	1.6^{D}	52.2^{C}	53.8^{C}
SEM	0.12	0.07	0.38	0.42
Level of significance	P<0.01	P<0.01	P<0.01	P<0.01

^{ABCD}Values with different superscript in a column differ significantly (P<0.01). SEM, Standard error means.

Ammonia nitrogen concentration reduced (P<0.01) due to incubation of Kadam (*Anthocephalus cadamba)* (7.6 mg/dl) tree leaves in comparison to other leaves, which might be due to reduced rumen protozoal population. The presence of protozoa in the rumen ecosystem is associated with increased recycling of microbial nitrogen in the rumen and therefore, decreased protozoal population in the rumen are usually associated with lowered ammonia concentrations, primarily as a result of a decrease in proteolysis of bacterial protein by ruminal protozoa (Hristov *et al*. 2005, Santra *et al*. 2013).

Effect of different tree leaves on TVFA production: TVFA and propionic acid production was higher (P<0.01) due to incubation of Karoi (*Albizia procera)* tree leaves while it was lowest due to incubation of Barhar (*Artocarpus lakoocha)* tree leaves (Table 4). The higher TVFA and propionic acid production due to incubation of Karoi (*Albizia procera)* tree leaves might be due to their higher IVTDMD. Although rumen protozoal number become decreased due to addition of Kadam (*Anthocephalus cadamba)* tree leaves in the incubation media, TVFA and propionate production was not increased in comparison to Karoi (*Albizia procera)* tree leaves in present experiment. The reduced rumen protozoal number is associated with increase in TVFA and propionate production (Hess *et al*. 2003). However, according to Jouany *et al*. (1988) changes in TVFA and VFA pattern due to reduction in rumen protozoa population is not always consistent because nature of diet also plays an important role in TVFA production and VFA pattern. Acetate:propionate ratio was also lower (P<0.01) for the incubation of Karoi (*Albizia procera)* tree leaves.

Lower TVFA production for incubation of Barhar (*Artocarpus lakoocha)* tree leaves might be due to poor ruminal digestibility of that leaves due to content of lignin and tannin. The variability in the TVFA production pattern

Table 4. Effect of different tree leaves on TVFA production and acetate propionate (A:P) ratio

Tree leaves (Local/Scientific name)	TVFA (mM/dl)	Acetate (%)	Propionate (%)	Butyrate (%)	Acetate : Propionate
Kadam (*Anthocephalus cadamba)*	3.5^{CD}	67.6^{B}	20.7^{B}	11.7^{A}	3.2^{B}
Kaew (*Costus speciosus)*	3.8^{D}	67.2^{B}	20.9^{B}	11.9^{AB}	3.2^{B}
Karoi (*Albizia procera)*	4.6^{E}	65.3^{A}	22.4^{C}	12.3^{B}	2.9^{A}
Bakful (*Sasbania grandiflora)*	2.6^{AB}	68.3^{C}	19.5^{A}	12.2^{B}	3.5^{C}
Gamar (*Gmelina arborea)*	2.8^{B}	68.8^{C}	19.6^{A}	11.6^{A}	3.5^{C}
Barhar (*Artocarpus lakoocha)*	2.3^{A}	68.9^{C}	19.2^{A}	11.9^{BC}	3.5^{C}
SEM	0.12	0.21	0.14	0.17	0.03
Level of significance	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

^{ABCD}Values with different superscript in a column differ significantly (P<0.01); SEM, Standard error means, TVFA, Total volatile fatty acids.

Table 5. Effect of different tree leaves on rumen enzyme activity

Tree leaves (Local/ Scientific name)	Enzyme activity (u/ml/h)			
	CM-Cellulase	Xylanase	β-glucosidase	Amylase
Kadam (*Anthocephalus cadamba)*	2.3^{A}	3.5^{A}	2.5^{A}	14.6
Kaew (*Costus speciosus)*	2.5^{AB}	3.8^{AB}	2.6^{A}	13.7
Karoi (*Albizia procera)*	2.6^{B}	4.1^{B}	2.8^{A}	14.5
Bakful (*Sasbania grandiflora)*	3.5^{CD}	4.7^{C}	3.6^{B}	15.3
Gamar (*Gmelina arborea)*	3.4^{C}	4.9^{C}	3.5^{B}	13.9
Barhar (*Artocarpus lookacha)*	3.7^{D}	4.8^{C}	3.7^{B}	14.9
SEM	0.05	0.08	0.07	0.22
Level of significance	P<0.01	P<0.01	P<0.01	P<0.01

^{ABCD}Values with different superscript in a column differ significantly (P<0.01). SEM, Standard error means.

with the six different tree leaves in present experiment might be due to the difference in the plant secondary metabolites present in those tree leaves. Even the same active principle may behave differently because of its type and concentration like the effect of phenolics on TVFAs and its molar proportion are variable (Patra *et al*. 2006b). Sliwinski *et al*. (2002) observed no effect of *Castanea sativa* wood extract supplementation containing hydrolysable tannins on total as well as molar proportion of individual VFAs. Makkar *et al*. (1995) reported that supplementation of quabracho tannins up to 0.4 mg/ml had no effect on concentration of total and individual VFAs.

Effect of different tree leaves on rumen enzyme profile: Carboxymethyl cellulase enzyme activity was highest (P<0.01) due to incubation of Barhar (*Artocarpus lakoocha)* tree leaves while it was lowest due to incubation of Kadam (*Anthocephalus cadamba)* tree leaves followed by Kaew (*Costus speciosus*) and Karoi (*Albizia procera)* tree leaves (Table 5). Similarly activities of xylanase, β-glucosidase enzymes were also lower (P<0.01) in the incubation media due to incubation of Kadam (*Anthocephalus cadamba)* tree leaves in comparison to other tested tree leaves. However, amylase enzyme activity was similar due to incubation of all the tested tree leaves. The enzymes in the rumen are synthesized/liberated by bacteria, protozoa and fungi. Rumen protozoa produce or secrete various types of enzymes which are responsible for breakdown of the plant cell wall structural polysaccharide (Williams and Withers 1991). Agarwal *et al*. (1991) reported that in buffalo rumen the protozoal contribution in the activities of cellulase was 43.2% of the total activities of this enzymes, indicating a significant role of rumen protozoa in ruminal fibre digestion. However, the rumen bacteria are the most important microbial group of the rumen microbes for ruminal digestion of plant fibre (Amos and Akin 1978). Decreased protozoal population in the rumen are usually associated with higher bacterial and fungal population as evident from the defaunated animals (Chaudhary *et al*. 1995). Activity of fibre degrading enzymes e.g., carboxymethyl cellulase xylanase and β-glucosidase was lower due to incubation of Kadam (*Anthocephalus cadamba)* tree leaves which might be due to low rumen protozoal population.

Effect of different tree leaves on rumen microbial biomass production and feed digestibility: Microbial biomass production varied from 15.8 to 66.8 mg for different tree leaves. In terms of per gram of digested DM, microbial biomass production was highest due to incubation of Kaew (*Costus speciosus)* leaves and it was lowest in Gamar (*Gmelina arborea)*.

In vitro true dry matter (IVTDMD) digestibility was highest (P<0.01) for Karoi (*Albizia procera)* (52.3%) tree leaves. Lowest IVTDMD was observed in Barhar (*Artocarpus lookacha)* (29.9%) tree leaves, this might be due to higher content of lignin (17.8%). It is a well-established fact that digestibility of any feed is inversely related to its lignin content (Van Soest 1994, Van Soest *et al*. 1991). The data on IVTDMD of different tree leaves is comparable to that reported earlier (Sharma *et al*. 2000, Datt *et al*. 2006).

TDN content of these tree leaves varied from 32.2% to 60.3% on DM basis while metabolizable energy (ME) content varied from 1.1 to 2.1 Mcal/kg DM. ME and TDN values were highest in Karoi (*Albizia procera)* tree leaves. ME value was positively correlated with dry matter

Table 6. Effect of different tree leaves on microbial biomass production and dry matter digestibility

Tree leaves (Local /Scientific name)	Microbial biomass (mg)	Microbial biomass (mg/g DM)	Microbial biomass (mg/g DDM)	IVTDMD (%)	TDN (%)	ME (Mcal/kg DM)
Kadam (*Anthocephalus cadamba)*	58.9^{B}	315.1^{B}	718.3^{C}	43.8^{C}	32.2^{A}	1.1^{A}
Kaew (*Costus speciosus)*	66.8^{C}	351.6^{B}	734.1^{C}	47.9^{C}	47.5^{D}	1.7^{D}
Karoi (*Albizia procera)*	64.6^{C}	354.9^{B}	680.2^{B}	52.3^{D}	60.3^{E}	2.1^{E}
Bakful (*Sasbania grandiflora)*	18.1^{A}	94.8^{A}	317.5^{A}	30.3^{A}	41.4^{C}	1.5^{C}
Gamar (*Gmelina arborea)*	17.8^{A}	94.2^{A}	287.1^{A}	33.1^{AB}	45.6^{D}	1.6^{CD}
Barhar (*Artocarpus lookacha)*	15.8^{A}	85.9^{A}	287.3^{A}	29.9^{A}	36.9^{B}	1.3^{B}
SEM	3.43	18.29	27.35	1.54	1.33	0.05
Level of significance	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

^{ABCD}Values with different superscript in a column differ significantly (P<0.01). SEM, Standard error means; IVTDMD, *In vitro* true dry matter digestibility; ME, metabolizable energy; TDN, total digestible nutrients.

digestibility. Moreover, it was observed in present study that ME value of the tree leaves inversely related with the lignin content. As lignin causes depression in digestibility and thus lowering the rumen fermentation rate and hence reduce the ME value of the feeds.

On the basis of chemical composition, *in vitro* fermentation pattern and digestibility (IVTDMD), it was concluded that Karoi (*Albizia procera)* tree leave is an excellent tree fodder for feeding to the ruminants. Moreover, Kadam (*Anthocephalus cadamba)* tree leaves may be used as a rumen manipulator to reduce ruminal methanogenesis and protozoal population for better utilization of dietary protein and energy for improving productivity of ruminant animals.

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