



## Chemical composition and *in vitro* ruminal fermentation of common tree forages in the semi-arid range lands of India

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### ABSTRACT

The objective of study was to look for the promising tree leaves for feeding livestock particularly the small ruminants. Ten tree leaves were collected from semiarid region of Rajasthan and evaluated for their nutritional quality in terms of chemical composition as well as *in vitro* dry matter and organic matter digestibility (IVDMD and IVOMD). Most of the tree leaves were rich in CP content. OM, CP and ADF content of these collected tree leaves varied from 87.1 to 92.5%, 9.4 to 19.8% and 22.7 to 47.9% on DM basis, respectively. Rumen protozoal number decreased due to inclusion of *Sapindus mukorossi*, *Azadirachta indica* and *Prosopis cineraria* tree leaves in the incubation media. IVDMD, IVOMD, TVFA and propionate production significantly higher for *Ailanthus excelsa* tree leaves followed by *Acacia arabica* and *Acacia senegal* tree leaves. All the tested tree leaves had no effect on  $\beta$ -glucosidase and amylase enzyme activity. However, specific activity of carboxymethyl cellulase and xylanase reduced significantly due to addition of *Sapindus mukorossi*, *Azadirachta indica* and *Prosopis cineraria* tree leaves in the incubation medium. The results indicated that among the tree leaves tested in the present study *Ailanthus excelsa*, *Acacia arabica* and *Acacia senegal* are good tree fodder for feeding to the ruminants.

**Key words:** Ciliate protozoa, Enzyme profile, Fodder quality, *In vitro* digestibility, Rumen fermentation, Tree leaves

A major constraint to increase livestock productivity in developing countries like India is the scarcity and fluctuating quantity and quality of the year-round supply of conventional feeds (Bakshi and Wadhwa 2007). Due to the ever increasing human population and the consequent increase in demand for food, livestock feed tends to be derived from crop residues and by-products of the food industry. Tropical trees and shrubs can be used as alternative supplements to balance the diet of livestock in terms of protein, energy, vitamins and minerals (Devasena and Adilaxmamma 2016). The role of fodder trees and shrubs in the diets of ruminants is considered to be important in India and other South Asian countries where small land holdings and large ruminant densities result in severe problem of fodder availability from conventional sources (Patel *et al.* 2018). In India, the prominent role of multipurpose trees is related to fodder and fuel (Datt *et al.* 2008). Fodder trees have the potential for alleviating some of the feed shortages and nutritional deficiencies experienced in the dry season on small holder farmers. Tree leaves not only provide a cheaper source of nitrogen, energy and micro-nutrients to the livestock but also have

advantages like they can withstand severe adverse climatic conditions, they do not need heavy inputs (fertilizers, irrigation, labour, pesticides etc.), help in soil and moisture conservation and also protect environment (Datt *et al.* 2007). The supplementation of low quality roughage with tree leaves either fresh or as leaf meal to animals had been found effective in improving the animal performances during the dry season, while at the same time lowering the cost of production (Devasena and Adilaxmamma 2016).

The livestock of semi arid zone of Rajasthan are mostly dependent on the tree leaves and shrubs available in degraded pasture land to meet out their nutrient requirement. Tree leaves can provide green fodder almost throughout the year. During the lean period (April, May and June), animals of this region are mainly maintained by feeding tree leaves lopped from multipurpose trees. However, information about the nutritive value of such feed resources particularly for tree leaves is very scanty. Some of the tree leaves of semi arid region like *Azadirachta indica*, *Albizia lebbak*, *Acacia Arabica* and *Ziziphus mauritiana* were found to be rich in protein, soluble carbohydrates and minerals and showed greater potential as an alternate feed resources (Bakshi and Wadhwa 2007, Datt *et al.* 2007). Therefore, this study was taken up to asses nutritive value of promising tree leaves from semi-arid region of Rajasthan as livestock feeds.

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## MATERIALS AND METHODS

*Collection and processing of tree leaves:* Tree leaves were collected from Tonk, Rajasthan, and were dried at 50°C for 72 h in a forced hot air oven. Dried tree leaves were grinded in a hammer mill and passed through 1 mm sieve. Ground plant materials were stored in an air tight container for further chemical and biochemical analysis. These ground plant materials were tested for their nutritional evaluation as an animal feed.

*In vitro rumen fermentation studies:* Rumen liquor was collected just before morning feeding from two cannulated Malpura ram, fed on a diet (total mixed ration) containing *Cenchrus ciliaris* dried grass and concentrate mixture in 1:1 ratio. The rumen liquor, strained through muslin cloth, was pooled and used as the source of inoculum. The inoculum/incubation medium was prepared by mixing rumen liquor with buffer (McDough buffer) in the ratio of 1:2. 1000±5 mg air-equilibrated milled (<1.0 mm) each tree leave was incubated with 100 ml of buffered rumen inoculums in a 250 ml conical flask under anaerobic condition and placed in an orbital shaker incubator at 39°C (Tilley and Terry 1969). The incubations were conducted in triplicate for each tree leaves for 24 h and these were repeated three times at a 15 days interval.

*Enumeration of rumen ciliate protozoa:* At the end of incubation (24 h), the content of the conical flask was mixed properly and 1 ml sample was mixed with 1 ml brilliant green formal saline solution. The stained sample was kept overnight at room temperature and protozoa were counted microscopically (Veira *et al.* 1983). Rumen ciliates were identified according to Hungate (1966). Spirotrichs that were not identified to generic level were classified into small spirotrichs (mainly Entodinia with an average size 42 mm × 23 mm) and large spirotrichs (mainly Diplodinia with an average size of 132 mm × 66 mm).

*Estimation of in vitro dry matter digestibility (IVDMD):* For the estimation of IVDMD, the content of conical flask 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 IVDMD of feed was calculated as follows:

$$\text{In vitro dry matter digestibility (IVDMD)} = \frac{(\text{DM of feed taken for incubation} - \text{NDF residue})}{\text{DM of feed taken for incubation}} \times 100$$

*Chemical analysis:* Each tree leaf sample was analyzed for organic matter (OM) by ashing at 550°C for 4 h and crude protein (CP) by Kjeldahl technique (AOAC 1995). The neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were estimated following the method of Van Soest *et al.* (1991). Total volatile fatty acids (TVFA) estimation of incubation medium after termination of incubation was carried out as described by Barnett and Reid (1957) and fractionation of VFA as well as estimation of the enzymes activity was done as described by Patra *et al.* (2006). Ammonia nitrogen concentration in the incubation media after termination of incubation, was estimated as per method of Weatherburn (1967).

*Statistical analysis:* Data were analyzed by the method 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 DISCUSSIONS

*Chemical composition of tree leaves:* Results of nutrient composition indicated that organic matter content of the various tree leaves ranged from 87.1% (*Prosopis cineraria*) to 92.5% (*Sapindus mukorossi*) with an average value of 89.6% (Table 1). *Albizia lebbeck* and *Bauhinia racemosa* had organic matter comparable to *Sapindus mukorossi*. The crude protein content of the tree leaves varied between 9.4 to 19.8%. Ether extract content of tree leaves varied from 2.4 to 3.4% with an average value of 2.9%. The feed resources e.g., tree leaves evaluated in the present study, 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 metabolic activity for rumen fibre degradation (Leng 1990). All tree leaves contained more

Table 1. Chemical composition of different tree leaves

Tree		Chemical composition (% DM basis)									
Local name	Scientific name	OM	CP	EE	T-CHO	NDF	ADF	Cellulose	Lignin	ADF-N	Acid insoluble ash
Siris	<i>Albizia lebbeck</i>	91.3	19.8	3.4	68.1	47.8	32.7	24.3	7.9	0.9	2.6
White kheri	<i>Acacia senegal</i>	88.9	13.6	2.9	72.4	31.4	27.9	20.8	6.8	0.8	3.5
Jinja	<i>Bauhinia racemosa</i>	91.2	14.7	2.4	74.1	38.3	28.9	19.2	8.9	0.6	4.6
Khejri	<i>Prosopis cineraria</i>	87.1	15.2	2.7	69.2	42.7	33.6	21.4	11.7	0.8	5.4
Babul	<i>Acacia arabica</i>	89.3	16.7	3.2	69.4	31.2	23.1	15.9	6.5	1.7	1.9
Neem	<i>Azadirachta indica</i>	88.7	14.6	2.9	71.2	48.7	28.2	21.2	6.3	0.7	3.1
Kankera	<i>Gymnosporia spinosa</i>	87.5	9.4	2.7	75.4	30.9	24.4	13.7	9.9	0.5	2.9
Pala	<i>Ziziphus nummularia</i>	89.8	11.2	2.5	76.1	61.4	47.9	36.9	10.2	0.6	4.9
Ritha	<i>Sapindus mukorossi</i>	92.5	17.9	3.1	71.5	58.6	39.3	30.7	8.1	0.4	2.7
Ardu	<i>Ailanthus excelsa</i>	89.3	16.9	3.2	68.2	30.2	22.7	20.3	5.7	0.6	1.4

than 9% CP below which the rumen fermentation is adversely affected (Datt *et al.* 2007). The values obtained in the present study were in the range as reported (Bakshi *et al.* 2007, Datt *et al.* 2007, Ramachandran *et al.* 2015, Patel *et al.* 2018).

The cell wall analysis based on detergent extraction can predict the nutritional value of fibrous feed resources, because voluntary dry matter intake and its digestibility are related to cell wall constituent NDF. The NDF and ADF content of the different tree leaves varied from 30.2 to 61.4%, and 22.7 to 47.9% while lignin content was varied from 5.7 to 11.7%. The leaves of *Ziziphus nummularia* were highly fibrous (highest NDF and ADF contents) an indicator of their low voluntary dry matter intake. The leaves of *Ailanthus excelsa* had significantly lower NDF, ADF and lignin content, indicating good potential as livestock feed stuffs. Cellulose content of the tree leaves varied from 13.7 to 36.9% with an average value of 22.4%. The values for cell wall constituents of the tree leaves were comparable with those of other workers (Sharma *et al.* 2000, Bakshi and Wadhwa 2007, Datt *et al.* 2007, Ramachandran *et al.* 2015). Highest amount of acid insoluble ash content was observed in *Prosopis cineraria* (5.4%) followed by *Ziziphus nummularia* (4.9%) and *Bauhinia racemosa* (4.6%) tree leaves while lowest acid insoluble ash content was observed in *Ailanthus excelsa* tree leaves (1.4%).

*Effect of different tree leaves on rumen protozoal population:* 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 multivesiculatum* (Coleman 1980). The large and small holotrich protozoa had an average cell size of  $157 \times 76 \mu\text{m}$  (range  $104 - 178 \mu\text{m} \times 43 - 104 \mu\text{m}$ ) and  $59 \times 29 \mu\text{m}$  (range  $37 - 96 \mu\text{m} \times 24 - 39 \mu\text{m}$ ) while large and small spirotrich protozoa had an average cell size of  $135 \times 73 \mu\text{m}$  (range  $87 - 171 \mu\text{m} \times 48 - 103 \mu\text{m}$ ) and  $47 \times 25 \mu\text{m}$

(range  $28 - 72 \mu\text{m} \times 15 - 36 \mu\text{m}$ ). Numerically spirotrich protozoa comprised more than 80% of total protozoal population is also similar to the earlier findings from (Santra *et al.* 2014, 2016). Number of holotrich, spirotrich and total rumen protozoa was lowest ( $P < 0.01$ ) for *Sapindus mukorossi* followed by *Azadirachta indica* and *Prosopis cineraria* tree leaves (Table 2). Rumen total as well as differential protozoal numbers were similar among the other tested tree leaves. Agarwal *et al.* (2006) reported that methanol, ethanol and water extracts of berries of *Sapindus mukorossi* inhibited rumen protozoal numbers in an *in vitro* gas production system. Further, it was also reported that supplementation of *Sapindus mukorossi* leaves @ 3% in the diet as herbal feed additives in growing calves reduced the rumen protozoal number (Meel *et al.* 2015). *Sapindus mukorossi* leaves contain saponin which inhibit the rumen protozoal population. It has been postulated that saponins have the property of binding with lipids. The sensitivity of protozoa towards saponins may be due to presence of sterols in protozoa, but not in bacterial membrane. Thus, sterol binding capacity of saponin most probably causes destruction of protozoal cell membrane, causing leaking of cell content (Patra and Saxena 2009). Adverse effect of *Azadirachta indica* and *Prosopis cineraria* leaves on total as well as differential rumen protozoal count might be due to the presence of bitter principles as well as tannin in those leaves. *Prosopis cineraria* leaves contain high amount of tannin (Kumar 1992). Patra *et al.* (2006), Bhatta *et al.* (2012) and Santra *et al.* (2012) reported that rumen protozoal number decreased due to addition of tannin containing plants in the incubation medium *in vitro*.

*Rumen fermentation, enzyme profile and feed digestibility:* *In vitro* dry matter digestibility (IVDMD) as well as *in vitro* organic matter digestibility (IVOMD) was highest in *Ailanthus excelsa* followed by *Acacia arabica* and *Azadirachta indica* tree leaves (Table 3). However,

Table 2. Effect of different tree leaves on rumen protozoal population ( $\times 10^3/\text{ml}$ ) *in vitro*

Attributes	Tree leaves										SEM
	Siris ( <i>Albizzia lebeck</i> )	White kheri ( <i>Acacia senegal</i> )	Jinja ( <i>Bauhinia racemosa</i> )	Khejri ( <i>Prosopis cineraria</i> )	Babul ( <i>Acacia arabica</i> )	Neem ( <i>Azadirachta indica</i> )	Kankera ( <i>Gymnosporia spinosa</i> )	Pala ( <i>Ziziphus nummularia</i> )	Ritha ( <i>Sapindus mukorossi</i> )	Ardu ( <i>Ailanthus excelsa</i> )	
Large Holotrich protozoa	0.4 <sup>c</sup>	0.4 <sup>c</sup>	0.5 <sup>c</sup>	0.2 <sup>b</sup>	0.3b <sup>c</sup>	0.2 <sup>b</sup>	0.4 <sup>c</sup>	0.3 <sup>c</sup>	0.1 <sup>a</sup>	0.5b <sup>c</sup>	0.02
Small Holotrich protozoa	0.9 <sup>c</sup>	0.8 <sup>c</sup>	0.8 <sup>c</sup>	0.6 <sup>b</sup>	0.8 <sup>c</sup>	0.5 <sup>b</sup>	0.9 <sup>c</sup>	0.9 <sup>c</sup>	0.2 <sup>a</sup>	0.9 <sup>c</sup>	0.05
Total holotrich protozoa	1.3 <sup>c</sup>	1.2 <sup>c</sup>	1.3 <sup>c</sup>	0.8 <sup>b</sup>	1.1 <sup>c</sup>	0.7 <sup>b</sup>	1.3 <sup>c</sup>	1.2 <sup>c</sup>	0.3 <sup>a</sup>	1.4 <sup>c</sup>	0.08
Large Spiotrich protozoa	7.1 <sup>C</sup>	7.3 <sup>C</sup>	6.8 <sup>C</sup>	5.9 <sup>C</sup>	7.4 <sup>C</sup>	4.1 <sup>B</sup>	7.2 <sup>C</sup>	6.8 <sup>C</sup>	2.9 <sup>A</sup>	7.2 <sup>C</sup>	0.26
Small Spirotrich protozoa	37.2 <sup>D</sup>	36.1 <sup>D</sup>	37.3 <sup>D</sup>	34.6 <sup>C</sup>	37.8 <sup>D</sup>	31.5 <sup>B</sup>	36.4 <sup>D</sup>	37.1 <sup>D</sup>	27.7 <sup>A</sup>	37.3 <sup>D</sup>	1.03
Total Spirotrich protozoa	44.3 <sup>D</sup>	43.4 <sup>D</sup>	44.1 <sup>D</sup>	40.5 <sup>C</sup>	45.2 <sup>D</sup>	35.6 <sup>B</sup>	43.6 <sup>D</sup>	43.9 <sup>D</sup>	30.6 <sup>A</sup>	44.5 <sup>D</sup>	1.15
Total rumen protozoa	45.6 <sup>D</sup>	44.6 <sup>D</sup>	45.4 <sup>D</sup>	41.3 <sup>C</sup>	46.3 <sup>D</sup>	36.3 <sup>B</sup>	44.9 <sup>D</sup>	45.1 <sup>D</sup>	30.9 <sup>A</sup>	45.9 <sup>D</sup>	1.24

Mean with different superscripts in a row differ significantly among treatment; <sup>ABCD</sup>( $P < 0.01$ ), <sup>abc</sup>( $P < 0.05$ ).

Table 3. Effect of different tree leaves on rumen fermentation, enzyme activity and feed digestibility *in vitro*

Attributes	Tree leaves										SEM
	Siris ( <i>Albizia lebbek</i> )	White kheri ( <i>Acacia senegal</i> )	Jinja ( <i>Bauhinia racemosa</i> )	Khejri ( <i>Prosopis cineraria</i> )	Babul ( <i>Acacia arabica</i> )	Neem ( <i>Azadirachta indica</i> )	Kankera ( <i>Gymnosporia spinosa</i> )	Pala ( <i>Ziziphus nummularia</i> )	Ritha ( <i>Sapindus mukorossi</i> )	Ardu ( <i>Ailanthus excelsa</i> )	
IVDMD (%)	47.1 <sup>d</sup>	56.4 <sup>f</sup>	42.7 <sup>c</sup>	34.9 <sup>a</sup>	57.5 <sup>f</sup>	52.9 <sup>e</sup>	39.7 <sup>b</sup>	38.5 <sup>b</sup>	46.5 <sup>d</sup>	61.2 <sup>g</sup>	0.59
IVOMD (%)	49.8 <sup>d</sup>	58.1 <sup>f</sup>	44.2 <sup>c</sup>	36.5 <sup>a</sup>	59.2	55.4 <sup>e</sup>	41.3 <sup>b</sup>	39.8 <sup>b</sup>	48.3 <sup>d</sup>	63.6 <sup>g</sup>	0.63
TVFAs (MEq/dl)	5.1 <sup>d</sup>	5.9 <sup>f</sup>	4.9 <sup>c</sup>	4.1 <sup>a</sup>	6.1 <sup>f</sup>	5.4 <sup>e</sup>	4.6 <sup>b</sup>	4.5 <sup>b</sup>	5.1 <sup>d</sup>	6.7 <sup>g</sup>	0.09
Acetate (% of TVFA)	67.3 <sup>d</sup>	66.9 <sup>b</sup>	67.8 <sup>f</sup>	68.3 <sup>g</sup>	66.8 <sup>b</sup>	67.1 <sup>c</sup>	67.8 <sup>f</sup>	68.2 <sup>g</sup>	67.5 <sup>e</sup>	66.5 <sup>a</sup>	0.09
Propionate (% of TVFA)	23.4 <sup>d</sup>	23.9 <sup>ef</sup>	23.1 <sup>c</sup>	22.4 <sup>a</sup>	24.1 <sup>f</sup>	23.7 <sup>e</sup>	22.9 <sup>b</sup>	22.7 <sup>b</sup>	23.3 <sup>cd</sup>	24.4 <sup>g</sup>	0.11
Butyrate (% of TVFA)	9.3	9.2	9.1	9.3	9.1	9.2	9.3	9.1	9.2	9.1	0.12
Acetate: Propionate ratio	2.88 <sup>d</sup>	2.83 <sup>c</sup>	2.93 <sup>e</sup>	3.05 <sup>f</sup>	2.77 <sup>b</sup>	2.79 <sup>b</sup>	2.96 <sup>e</sup>	3.01 <sup>f</sup>	2.89 <sup>d</sup>	2.72 <sup>a</sup>	0.02
Total nitrogen (mg/dl)	39.8 <sup>d</sup>	32.4 <sup>ab</sup>	34.1 <sup>b</sup>	34.7 <sup>bc</sup>	36.5 <sup>c</sup>	34.3 <sup>b</sup>	29.4 <sup>a</sup>	31.9 <sup>a</sup>	37.2 <sup>cd</sup>	36.8 <sup>c</sup>	1.38
NH <sub>3</sub> -N (mg/dl)	10.4 <sup>D</sup>	10.2 <sup>D</sup>	10.5 <sup>D</sup>	9.1 <sup>C</sup>	10.9 <sup>D</sup>	8.3 <sup>B</sup>	10.3 <sup>C</sup>	10.1 <sup>C</sup>	7.5 <sup>A</sup>	10.7 <sup>D</sup>	0.32
Enzyme activity (IU/dl/h)											
Carboxy methyl cellulase	22.1 <sup>C</sup>	20.5 <sup>C</sup>	21.7 <sup>C</sup>	15.3 <sup>B</sup>	21.8 <sup>C</sup>	16.8 <sup>B</sup>	21.2 <sup>C</sup>	20.7 <sup>C</sup>	13.5 <sup>A</sup>	21.3 <sup>C</sup>	0.85
Xylanase	31.5 <sup>C</sup>	32.7 <sup>C</sup>	33.2 <sup>C</sup>	28.1 <sup>B</sup>	31.8 <sup>C</sup>	27.5 <sup>B</sup>	31.6 <sup>C</sup>	31.9 <sup>C</sup>	23.2 <sup>A</sup>	32.1 <sup>C</sup>	1.17
β-glucosidase	6.8	7.1	7.4	6.5	7.1	6.9	7.2	6.6	6.9	7.2	0.47
Amylase	136.9	138.6	134.9	131.5	142.5	140.9	132.7	129.8	137.1	146.8	9.58

Mean with different superscripts in a row differ significantly among treatment; <sup>ABC</sup>(P<0.01), <sup>abcdefg</sup>(P<0.05).

IVDMD as well as IVOMD were lowest for *Prosopis cineraria* followed by *Ziziphus nummularia* and *Gymnosporia spinosa* tree leaves. Although the rumen protozoal number decreased due to inclusion of *Azadirachta indica* tree leaves in the incubation media, but it did not effect on its digestibility (IVDMD and IVOMD), which might be due to increase in rumen fungi and bacterial numbers. It was reported that rumen fungi and bacterial number increased due to complete removal of rumen protozoa (defaunated animal) or reduce rumen protozoal number (partial defaunation) as the rumen protozoa are the predator of rumen fungi and bacteria (Williams and Coleman 1997). Lower IVDMD and IVOMD in *Prosopis cineraria*, *Ziziphus nummularia* and *Gymnosporia spinosa* tree leaves might be due to higher content of lignin in those trees leaves as it is well known that digestibility of any feed inversely related with its lignin content. Moreover, *Prosopis cineraria* tree leaves contained tannin which also adversely affected digestibility. Tannins have been implicated for their inhibitory effect on feed digestion, microbial population and enzyme activity in many studies earlier (Hristov *et al.* 2003, Patra *et al.* 2006, Bhatta *et al.* 2017).

TVFA and propionate production was highest due to inclusion of *Ailanthus excelsa* followed by *Acacia arabica* tree leaves in incubation media while it was lowest for *Prosopis cineraria* followed by *Ziziphus nummularia* and *Gymnosporia spinosa* tree leaves. Acetate:propionate ratio was lowest in *Ailanthus excelsa* while it was highest for *Prosopis cineraria* followed by *Ziziphus nummularia* tree leaves. It was observed that TVFA production was positively

correlated with the digestibility of the tree leaves. In general, an increase in TVFA production is expected as the digestibility of feed increases (Bhatta *et al.* 2017). Moreover, lowest TVFA production due to inclusion of *Prosopis cineraria* leaves in incubation media might be due to higher content of tannin in leaves. Tannic acid has been reported to reduce TVFA production (Hristov *et al.* 2003). Higher (P<0.05) propionate production due to inclusion of *Ailanthus excelsa* tree leaves might be due to in expense of acetate production. Highest nitrogen concentration in the incubation media due to inclusion of *Albizia lebbek* followed by *Sapindus mukorossi*, *Ailanthus excelsa* and *Prosopis cineraria* tree leaves might be due to higher CP content of those tree leaves. Lower (P<0.01) NH<sub>3</sub>-N concentration due to inclusion of *Sapindus mukorossi* and *Azadirachta indica* tree leaves in the incubation media probably due to lower 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 concentration, primarily as a result of a decrease in proteolysis of bacterial protein by ruminal protozoa (Hristov *et al.* 2005). Tannins have been recognize as protein binder. In the absence of tannin, degradability of protein was higher, resulting in greater NH<sub>3</sub>-N concentration, possibly because of inhibition of microbial deaminase by tannins (Bhatta *et al.* 2012). Lower ammonia nitrogen concentration due to inclusion of *Prosopis cineraria* leaves in the incubation media might be due to higher content of tannin in that leaves. Lower (P<0.01) activities of carboxymethyl cellulase and xylanase

due to inclusion of *Sapindus mukorossi*, *Azadirachta indica* and *Prosopis cineraria* leaves in the incubation media might be due to their antiprotozoal activity, as it has been reported that about 38% of cellulose activity is associated with protozoa fraction of rumen liquor (Agarwal *et al.* 1991).

On the basis of chemical composition, *in vitro* fermentation pattern and digestibility (IVDMD and IVOMD), it was concluded that tree leaves like *Ailanthus excelsa*, *Acacia arabica* and *Acacia senegal* are excellent un-conventional feed stuffs for small ruminants. Moreover, *Sapindus mukorossi* tree leaves may also be used as a rumen manipulator to reduce rumen protozoal population for better utilization of dietary protein and energy.

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