



Nutritional evaluation of some Indian tree pods for livestock feeding

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ABSTRACT

The objective of this study was to evaluate promising tree pods for feeding to the livestock particularly for the small ruminants. Out of eight tested tree pods, seven tree pods, i.e. White siris (*Albizia procera*), Siris (*Albizia lebbek*), White kheri (*Acacia senegal*), Babul (*Acacia arabica*), Khejri (*Prosopis cineraria*), Vilayati babul (*Prosopis juliflora*) and Sajna (*Moringa oleifera*) were collected from semiarid region of Rajasthan while one tree pods e.g., Jungle jalebi (*Enterolobium timoba*) was collected from Dehradun, Uttarakhand, India. Most of the tree pods were rich in CP content. On an average, OM, CP, EE, NDF, ADF and cellulose content of these tree pods were found to be 91.1, 16.7, 2.5, 43.3, 34.7 and 25.4% on DM basis, respectively. Rumen protozoal number decreased due to inclusion of *Enterolobium timoba* tree pods in the incubation media. The TVFA and propionate production were higher for *Acacia Senegal*, *Acacia arabica* tree pods followed by *Moringa oleifera* tree pods while ammonia nitrogen concentration was lower due to inclusion of *Enterolobium timoba* tree pods in the incubation media. All the tested tree pods had no effect on xylanase, β -glucosidase and amylase enzyme activity. However, specific activity of carboxymethyl cellulase enzyme reduced due to addition of *Enterolobium timoba* tree pods in the incubation medium. Highest IVDMD was observed for *Acacia arabica* tree pods followed by *Acacia senegal* and *Moringa oleifera* tree pods. The results indicated that *Acacia arabica*, *Acacia senegal* and *Moringa oleifera* are good tree pods for feeding to the animals.

Keywords: Ciliate protozoa, Enzyme profile, *In vitro* digestibility, Rumen fermentation, Tree pods

Seasonal shortages particularly during dry season and low nutritive value of available feed resources are considered to be most widespread technical constraints to livestock production in arid and semi arid areas of the tropics (Gerbu *et al.* 2018). To overcome this challenge, the utilization of untapped feed resources can be an option. Leaves and other plant parts can constitute the cheaper and affordable supplements for ruminants particularly for small ruminants of resource poor livestock keeper in several regions of the world including in India (Singh *et al.* 2019). Leguminous shrubs are sometimes considered to be invasive plants but if properly integrated into feeding system of small ruminants, browse tree pods are cheaper and accessible supplementary feeds with potential to alleviate feed scarcity (Gebu *et al.* 2018). The utilization of plant foliage or tree pods in ruminant ration primarily depends on their chemical composition particularly the presence of plant secondary compounds like tannin, saponins, flavonoids, etc. and nutrient digestibility (Muhammad *et al.* 2018). The toxic factors may occur in all parts of the plant but the seed is normally the most concentrated source (D'Mello 1992). Abdalla *et al.* (2014) reported that pods of browse plants

have the potential to provide necessary nutrients to support maintenance requirement and even adequate to support production performances of ruminants. *Prosopis juliflora* pods can replace concentrate mixture up to 40% in sheep feeding without any adverse effect on nutrient intake and utilization as well as rumen fermentation characteristics (Chaturvedi and Sahoo 2013).

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 (Santra and Karim 2019). Tree leaves can provide green fodder almost throughout the year. During the lean period (April, May and June), animals of this region are maintained by feeding tree leaves along with tree pods lopped from multipurpose trees. However, information about the nutritive value of such feed resources and their effect on rumen fermentation, enzyme profile and protozoal population particularly for tree pods is very scanty. Some of the tree pods like *Prosopis juliflora*, *Albizia lebbek* were found to be rich in protein and showed potential as an alternate feed resource (Santra *et al.* 1998, Chaturvedi and Sahoo 2013). Therefore, this study was taken up to assess nutritive value of some promising Indian tree pods as livestock feeds.

MATERIALS AND METHODS

Collection and processing of tree pods: Mature pods of

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Jungle jalebi (*Enterolobium timoba*) tree was collected from Dehradun, Uttarakhand while, White siris (*Albizia procera*), Siris (*Albizia lebbeck*), White kheri (*Acacia senegal*), Babul (*Acacia arabica*), Khejri (*Prosopis cineraria*), Vilayati babul (*Prosopis juliflora*) and Sajna (*Moringa oleifera*) were collected from Avikanagar, Tonk, Rajasthan. Each tree pods were harvested manually from seven different trees, pooled and dried at 55°C for 72 h in a hot air oven. Ground in a hammer mill to pass through 1 mm sieve. These ground plant materials were tested for their effect on ruminal fermentation characteristics *in vitro*.

In vitro incubation: 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 inoculums. The inoculum/incubation medium was prepared by mixing rumen liquor with buffer (McDougall buffer) in the ratio of 1:2. 1,000 mg air-equilibrated each tree pod was incubated with 100 ml of buffered rumen inoculums in a 250 ml conical flask and placed in an orbital shaker incubator at 39°C (Tilley and Terry 1963). The incubations were conducted in triplicate for each tree pods for 24 h and these were repeated three times at a 15 days interval.

Staining and counting of rumen ciliate protozoa: At the end of incubation (24 h), the contents of the conical flask were mixed properly and 1 mL sample was pipetted with a wide orifice pipette into a screw capped test tube containing 1 mL formalinized physiological saline (0.85% w/v sodium chloride solution containing 20% w/v formaldehyde and 2% w/v brilliant green dye), mixed thoroughly and allowed to stand overnight at room temperature. If necessary, further dilutions were made with 30% (v/v) glycerol. Total and differential count of protozoa were made in 30 microscopic fields at a magnification of 100 ×. Ciliate protozoa were identified according to the method of Hungate (1966) and were counted microscopically (Veira *et al.* 1983).

Estimation of in vitro dry matter degradability (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 tree pod was calculated as follows:

In vitro dry matter degradability (IVDMD) =

$$\frac{(\text{DM of tree pod taken for incubation} - \text{NDF residue})}{\text{DM of tree pod taken for incubation}} \times 100$$

Chemical analysis of biological samples: Tree pod sample was analysed for, organic matter (OM) and crude protein (CP) (AOAC 1995). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were estimated (Van Soest *et al.* 1991). Total volatile fatty acids (TVFA) estimation of incubation medium was carried out as described by Barnett and Reid (1957) while fractionation of VFA was carried out by the method of Cottyn and Boucque (1968). Processing and estimation of the enzymatic activity of incubation medium was done as described by Santra and Karim (2002). Ammonia nitrogen concentration in the flask content was estimated as per method of Weatherburn (1967).

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 DISCUSSIONS

Chemical composition of tree pods: Organic matter (OM) content of tree pods ranged 86.8% (*Albizia procera*) to 94.4% (*Enterolobium timoba*) (Table 1). The CP content was highest in *Moringa oleifera* pods (18.7%) followed by *Prosopis juliflora* (18.1%) and *Prosopis cineraria* (17.4%) tree pods. All the tree pods contained more than 9% CP below which the rumen fermentation is adversely affected (Datt *et al.* 2007). In general, all the tested tree pods were rich in CP content and contained higher CP than grasses and cultivated fodder. CP content of each tested tree pods was above 11.0% which is adequate to meet the maintenance requirement of ruminants (Singh *et al.* 2019). EE content was highest in *Acacia arabica* (3.2%) followed by *Acacia Senegal* and *Prosopis juliflora* tree pods while it was lowest

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

Tree Pod		Chemical composition (on % DM)										
Local name	Scientific name	OM	Ash	CP	EE	T-CHO	NDF	ADF	Cellulose	Lignin	ADF-N	Acid insoluble ash
White siris	<i>Albizia procera</i>	86.8	13.2	16.5	2.5	67.8	60.2	47.9	35.7	11.9	0.41	0.25
Siris	<i>Albizia lebbeck</i>	87.2	12.8	15.7	2.1	69.4	59.8	48.3	35.4	12.3	0.48	0.31
White kheri	<i>Acacia senegal</i>	90.8	9.2	17.2	2.9	71.7	35.8	30.5	25.2	4.9	0.52	0.29
Babul	<i>Acacia arabica</i>	91.4	8.6	16.8	3.2	71.4	34.6	28.3	24.2	3.5	0.57	0.21
Khejri	<i>Prosopis cineraria</i>	94.2	5.8	17.4	2.7	74.1	37.1	24.8	19.2	5.3	0.63	0.25
Vilayati babul	<i>Prosopis juliflora</i>	91.9	8.1	18.1	2.9	70.9	38.7	28.1	21.2	6.3	0.68	0.23
Sajna	<i>Moringa oleifera</i>	92.2	7.8	18.7	2.3	71.2	44.3	34.5	22.6	11.9	0.67	0.37
Jungle jalebi	<i>Enterolobium timoba</i>	94.4	5.6	13.2	1.9	79.3	46.1	35.2	20.3	14.3	0.71	0.29

in *Enterolobium timoba* (1.9%) pods. The overall mean contents of OM, CP, EE, NDF, ADF, cellulose and lignin were found to be 91.1, 16.7, 2.6, 43.3, 34.7, 25.4 and 8.8%, respectively. The values obtained in the present study were in the range as reported in other investigations in different parts of the country (Santra *et al.* 1998, Hassan *et al.* 2007, Chatuvedi and Sahoo 2013, Ahmad *et al.* 2017).

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 (Bakshi and Wadhwa 2007). The NDF and ADF content varied between 34.6 and 60.2%, and 24.8 and 48.3%, respectively. The pods of *Albizia procera* were highly fibrous (highest NDF contents) which is an indicator of their potential for low voluntary dry matter intake. *Prosopis cineraria* tree pods possessed less amount of ADF e.g., 24.8%, among the tested tree pods, indicating good potential as livestock feed stuffs. Cellulose content of tested tree pods varied from 19.2 to 35.7%. The pods of *Enterolobium timoba* had the highest amount of lignin content (14.3%) followed by *Albizia lebbeck*, *Albizia procera* and *Moringa oleifera*. However, *Acacia arabica* and *Acacia senegal* pods had the lowest lignin content. The values for cell wall constituents of the tree leaves were comparable with those of other workers (Santra *et al.* 1998, Chatuvedi and Sahoo 2013, Gerbu *et al.* 2018).

Rumen protozoal population: Ciliate protozoa present in 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 165 × 87 µm (range 106–182 µm × 47–101 µm) and 54 × 31 µm (range 33–91 µm × 27–43 µm) while large and small spirotrich protozoa had an average cell size of 131 × 77 µm (range 83–169 µm × 54–107 µm) and 43 × 21 µm (range 25–69 µm × 13–34 µm). Numerically spirotrich protozoa comprised more than 80% of total protozoal population in the present experiment is also similar to the earlier findings from the same laboratory (Santra *et al.* 2014 and 2016).

Number of holotrich, spirotrich and total rumen protozoa was lowest (P<0.01) after inclusion of *Enterolobium timoba* pod in incubation media followed by *Moringa oleifera* and

Prosopis cineraria tree pods (Table 2). Rumen total as well as differential protozoal numbers was highest due to incubation of *Acacia senegal* pods. It has been reported that due to feeding *Enterolobium cyclocarpum* containing saponins, reduced rumen ciliate protozoal population (Diaz 1993). Agarwal *et al.* (2006) reported that methanol, ethanol and water extracts of berries of *Sapindus mukorossi* which contained saponin, inhibited rumen protozoal numbers *in vitro*. 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 might be due to presence of sterols in protozoa, but not in bacterial membrane. Thus, sterol binding capacity of saponin could cause destruction of protozoal cell membrane, causing leaking of cell content (Patra and Saxena 2009). Adverse effect of *Enterolobium timoba*, *Moringa oleifera* and *Prosopis cineraria* pods on total as well as differential rumen protozoal count might be due to the presence of plant secondary metabolites like saponin, tannin, flavonoids in those pods. *Prosopis cineraria* leaves contain high amount of tannin (Kumar 1990). Similarly, *Moringa oleifera* leaves also contain tannin and other phenolic compounds (Kholif *et al.* 2015) as well as flavonoids which has rumen antiprotozoal action (Alexander *et al.* 2008). 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*. The toxic factors or anti-nutritional factors like tannin, saponins, flavonoids, etc. may occur in all parts of the plant but the seed is normally the most concentrated source (D'Mello 1992).

Rumen fermentation, enzyme profile and feed digestibility: pH of the incubation media after termination of incubation, was lowest (P<0.05) due to inclusion of *Acacia arabica* pods followed by *Acacia senegal* and *Moringa oleifera* pods while TVFA production was highest due to incubation of *Acacia arabica* tree pods followed by *Acacia senegal* and *Moringa oleifera* pods (Table 3). It is well established that rumen pH and TVFA production are

Table 2. Effect of different tree pods on rumen protozoal number (× 10³) in sheep *in vitro*

Attribute	Tree pod								SEM
	<i>Albizia procera</i>	<i>Albizia lebbeck</i>	<i>Acacia senegal</i>	<i>Acacia arabica</i>	<i>Prosopis cineraria</i>	<i>Prosopis juliflora</i>	<i>Moringa oleifera</i>	<i>Enterolobium timoba</i>	
Small holotrich protozoa	0.9 ^f	0.8 ^{be}	0.6 ^{cd}	0.7 ^{de}	0.5 ^c	0.5 ^c	0.4 ^{bc}	0.3 ^{bab}	0.03
Large holotrich protozoa	1.9 ^g	1.4 ^d	1.7 ^f	1.5 ^e	1.2 ^c	1.3 ^c	0.9 ^b	0.6 ^a	0.05
Total holotrich protozoa	2.8 ^e	2.2 ^d	2.3 ^b	2.2 ^d	1.7 ^c	1.8 ^c	1.3 ^b	0.9 ^a	0.08
Small spirotrich protozoa	6.5 ^D	5.5 ^C	5.7 ^{CD}	5.2 ^{BC}	4.9 ^{BC}	5.1 ^{BC}	4.3 ^B	3.1 ^A	0.42
Large spirotrich protozoa	31.9 ^{BC}	32.6 ^C	33.9 ^C	33.3 ^C	30.7 ^{AB}	31.1 ^{AB}	29.5 ^{AB}	26.4 ^A	1.19
Total spirotrich protozoa	38.4 ^{BC}	38.1 ^{BC}	39.6 ^C	38.5 ^C	35.6 ^B	36.6 ^B	33.8 ^{AB}	29.5 ^A	1.35
Total rumen protozoa	41.2 ^C	40.3 ^{BC}	41.9 ^C	40.7 ^C	37.3 ^B	38.4 ^B	35.1 ^B	30.4 ^A	1.51

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

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

Attribute	Tree pod								SEM
	<i>Albizia procera</i>	<i>Albizia lebbbeck</i>	<i>Acacia senegal</i>	<i>Acacia arabica</i>	<i>Prosopis cineraria</i>	<i>Prosopis juliflora</i>	<i>Moringa oleifera</i>	<i>Enterolobium timoba</i>	
<i>Rumen metabolites</i>									
pH	6.51 ^b	6.49 ^b	6.41 ^a	6.39 ^a	6.75 ^d	6.78 ^d	6.42 ^a	6.68 ^c	0.015
TVFAs (MEq/dl)	5.1 ^C	4.9 ^C	5.9 ^D	6.2 ^D	3.8 ^A	4.1 ^A	5.8 ^D	4.3 ^B	0.07
Acetate (% of TVFA)	69.2 ^B	69.1 ^B	67.5 ^A	67.6 ^A	69.4 ^{BC}	69.8 ^C	67.9 ^A	69.3 ^B	0.21
Propionate (% of TVFA)	21.1 ^B	21.4 ^B	22.9 ^D	22.7 ^{C^D}	20.5 ^A	20.7 ^A	22.3 ^C	20.8 ^{AB}	0.16
Butyrate (% of TVFA)	9.7 ^{AB}	9.5 ^A	9.6 ^A	9.7 ^A	10.1 ^B	9.5 ^A	9.8 ^{AB}	9.9 ^B	0.13
Acetate: Propionate ratio	3.28 ^{bc}	3.22 ^b	2.95 ^a	2.98 ^a	3.39 ^c	3.37 ^c	3.05 ^a	3.33 ^c	0.07
Total nitrogen (mg/dl)	24.9 ^b	24.4 ^b	25.9 ^c	25.2 ^{bc}	26.1 ^c	26.5 ^c	27.4 ^d	23.2 ^a	0.41
TCA-ppt.-N (mg/dl)	5.8 ^A	6.2 ^A	5.6 ^A	6.1 ^A	7.4 ^B	7.7 ^B	7.9 ^B	8.8 ^C	0.26
NH3-N (mg/dl)	13.5 ^{CD}	12.9 ^C	13.8 ^D	13.2 ^{CD}	9.6 ^A	10.9 ^B	10.5 ^B	9.8 ^A	0.29
<i>Enzyme activity (IU/dl/h)</i>									
Carboxy methyl cellulase	24.9 ^B	24.6 ^B	25.1 ^B	25.4 ^B	24.5 ^B	26.1 ^B	24.8 ^B	20.8 ^A	0.86
Xylanase	31.2	28.9	30.8	29.7	28.3	29.1	28.5	27.3	1.95
β-glucosidase	10.1	9.8	10.5	9.4	7.8	8.1	8.4	7.2	1.62
Amylase	158.3	156.2	159.7	162.3	145.5	147.3	152.9	139.4	14.39
<i>Feed digestibility</i>									
IVDMD (%)	50.2 ^C	49.7 ^C	52.8 ^D	53.2 ^D	45.1 ^A	45.7 ^A	52.5 ^D	47.2 ^B	0.37
IVOMD (%)	52.5 ^C	51.5 ^C	54.7 ^D	55.1 ^D	47.8 ^A	48.3 ^A	54.6 ^D	49.5 ^B	0.45

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

inversely related. The VFA concentration may generally follow the trend of feed digestion in the rumen. However, a part of the digested substrate may be partitioned to microbial mass, decreasing VFA concentration (Makkar *et al.* 1998). The TVFA production was positively correlated with the digestibility of the tree pods. 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* pods in incubation media might be higher content of tannin in that pods. Tannic acid has been reported to reduce TVFA production (Hristov *et al.* 2003). Higher (P<0.05) propionate production due to inclusion of *Acacia senegal* and *Acacia arabica* pods followed by *Moringa oleifera* tree pods might be at the expense of acetate production.

The highest total nitrogen concentration in the incubation media due to inclusion of *Moringa oleifera* pods followed by *Prosopis juliflora* and *Prosopis cineraria* tree pods might be due to higher CP content of those tree pods. TCAT-ppt.-N concentration was also highest due to inclusion of *Moringa oleifera* pods in the incubation media. Lower (P<0.01) NH₃-N concentration due to inclusion of *Enterolobium timoba* and *Moringa oleifera* tree pods in the incubation media might be 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. Therefore, decreased protozoal population in the rumen is 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). Tannins are recognized as protein binder. In the absence of tannin, degradability of protein was higher resulting in greater NH₃-N concentration

possibly because of inhibition of microbial deaminase by tannins (Bhatta *et al.* 2012). Comparatively lower ammonia nitrogen concentration due to inclusion of *Prosopis cineraria* pods in the incubation media might be because of higher content of tannins.

The ligno-cellulosic feed stuffs are degraded in the rumen by the synergistic activities of the bacteria, protozoa and fungi, with bacteria and fungi contributing approximately 80% of the degradative activity, and the protozoa only 20% (Dijkstra and Tamminga 1995). Ruminal fungi produce a broad array of enzymes and generally degrade a wide range of substrates than do rumen bacteria (Trinci *et al.* 1994). Furthermore, ruminal fungi are able to degrade the most resistant plant cell wall polymers and the cellulase and xylanase produce by them are among the most active fibrolytic enzymes (Forsberg and Cheng 1992). The ruminal protozoa also contribute to the degradation of plant cell wall polymers but their contribution in fibre degradation is considered not as important as that of the bacteria and fungi (Lee *et al.* 2000). Lower (P<0.01) activities of carboxymethyl cellulase enzyme due to inclusion of *Enterolobium timoba* pods in the incubation media might be due to their antiprotozoal activity as about 38% of cellulase enzyme activity is associated with protozoa fraction of rumen liquor (Agarwal *et al.* 1991). Activities of xylanase, β-glucosidase and amylase enzyme in incubation medium were not influenced by the inclusion of tree pods.

In vitro dry matter digestibility (IVDMD) as well as *in vitro* organic matter digestibility (IVOMD) was highest in *Acacia arabica*, *Acacia senegal* and *Moringa oleifera* followed by *Albizia procera* and *Albizia lebbbeck* tree pods (Table 3). However, IVDMD as well as IVOMD were

lowest for *Prosopis cineraria* followed by *Prosopis juliflora* and *Enterolobium timoba* tree pods. Lower IVDMD and IVOMD in *Prosopis cineraria* and *Prosopis juliflora* tree pods might be due to higher content of tannin. *Prosopis cineraria* as well as *Prosopis juliflora* tree leaves contain tannin which adversely affect on its digestibility (Kumar and Vaithyanathan 1990). The toxic factors may occur in all parts of the plant but the seed is normally the most concentrated source (D'Mello 1992). Tannins have been implicated for their inhibitory effect on feed digestion, microbial population and enzyme activity in many experiments (Hristov *et al.* 2003, Patra *et al.* 2006, Bhatta *et al.* 2017). Lower IVDMD and IVOMD of *Enterolobium timoba* pods might be due to higher content of saponins which also reduced the rumen protozoal population.

It was concluded that tree pods like *Acacia arabica*, *Acacia senegal* and *Moringa olifera* are excellent unconventional feed stuffs for livestock particularly for small ruminants. *Enterolobium timoba* tree pods may be used as a rumen manipulator to reduce rumen protozoal population.

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