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Chemical Composition, Phenolic Fractions, Protein Fractions and *In Vitro* True Dry Matter Digestibility of Fodder Top Foliages of District Poonch of Jammu and Kashmir

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ABSTRACT

Top foliages are a source of digestible energy, rumen degraded and undegraded protein, vitamins, and minerals in small ruminants' diets. However, the presence of phenolics and their interaction with protein degradability affects their utilization in the ration. Small ruminant production is a critical component of the agro-ecological scenario in hilly regions of Jammu and Kashmir. In these areas, top foliages are the primary feed resource for sheep and goats. In the present report, we scrutinized the ten commonly available top foliages of District Poonch of Jammu and Kashmir for nutrient composition, phenolic fractions and *in vitro* true dry matter digestibility to assess their utilizability as a feed resource. Crude protein content was found highest in *Melia azedarach* (21.20) and lowest in *Acacia nilotica* (10.20). The total phenolics content varied from 0.75 per cent in *Grewia optiva* to 7.32 per cent in *Zizyphus jujuba.* The protein fraction B_3 (per cent of total nitrogen), which indicates bypass protein fraction, varied from 6.96 in *Melia azedarach* to 36.32 in *Salix alba*. The unavailable protein fraction C (per cent of total N) was maximum in *Z. jujuba* (42.73) and minimum in *M. azedarach* (6.54). Based on these parameters, it is concluded that *Celtis australis, Ficus palmata, Ficus religiosa, Grewia optiva, Melia azedarach, Morus alba* and *Zizyphus jujuba* leaves of District Poonch have high nutritional potential.

HIGHLIGHTS

- Phenolic content and protein fractions of top foliages are key parameters that affect their utilization as a feed resource.
- *Celtis australis, Ficus palmata, Ficus religiosa, Grewia optiva, Melia azedarach, Morus alba* and *Zizyphus jujuba* leaves of District Poonch have high nutritional potential.

Keyword: Top foliages, Phenolic fractions, Protein fractions, Poonch, Proximate

Top foliages, *i.e.* tree and shrub leaves, are a common part of basal diet of ruminants, especially sheep and goats, in hilly areas (Khan *et al.*, 2014) and have been introduced into cropping and grazing systems to provide protein-rich fodder to supplement low protein feeds (Gaikwad *et al.*, 2017; Mataveia *et al.*, 2019). These resources fulfil nearly 60% of the total feeds required by sheep and goats (Delgado *et al.*, 2012; Habib *et al.*, 2013). Tree and shrub forages are a source of digestible energy, rumen degraded and undegraded protein, vitamins, and minerals, thereby reducing requirements for concentrates and reducing the cost of feeding (Yadav and Bisht, 2013). However, the presence of tannins limits their use as animal feed (Al-Masri and Mardini, 2013). The knowledge about the level of tannins and their ability to precipitate protein in various top foliages (Khan *et al.*, 2012; Khan *et al.*, 2014) for livestock feeding is essential as they have a negative effect on nutrients digestibility (Foster *et al.*, 2012). Stress is being laid on the research involving the utilization of

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tree leaves containing optimum amounts of condensed tannins for beneficial effects on ruminant health and production (Jan *et al.*, 2015; Khan *et al.*, 2017; Xiao *et al.*, 2018; Kalia *et al.*, 2021). The characterization of diets according to protein fractions is valuable in estimating the ruminal degradability of dietary protein and determining whether ruminal microbes are provided with proper types and amounts of nitrogenous nutrients (Higgs *et al.*, 2012), which concurrently adresses environmental concerns of excessive nitrogen extretion in faeces and urine of ruminants (Amburgh *et al.*, 2019).

In the present study, commonly available top foliages of District Poonch have been evaluated for their chemical composition, fiber fractions, phenolic fractions and *in vitro* true dry matter digestibility, as literature on these aspects is not available.

MATERIALS AND METHODS

Ten tree and shrub forages *viz. Acacia nilotica*, *Celtis australis, Ficus palmate, Ficus religiosa, Grewia optiva, Salix alba, Melia azedarach, Morus alba, Quercus incana and Zizyphus jujuba* were collected from the Poonch district of Jammu and Kashmir having latitude of 33° 76’ N and longitude of 74° 09’ E. The collected samples were dried at 55 ± 1°C to constant weight and ground to pass through a sieve of 1mm diameter and stored in plastic containers with a lid for further analysis. Standard methods were followed for the determination of proximate (AOAC, 2012) and fibre composition (Van Soest *et al.*, 1991). Calcium and Phosphorus contents were determined as per the methods described by Talpatra *et al.* (1940) and AOAC (2012), respectively.

The percent soluble nitrogen of the selected top feeds was determined in 0.1 M borate phosphate buffer (pH 8.1) using standard procedure (Crooker *et al.,* 1978). The NDF and ADF residues were analyzed for nitrogen (Robertson and Van Soest, 1981). Protein fractions were estimated as per the method of Chalupa and Sniffen (1996). The fraction (A+ B1) soluble in borate phosphate was ammonia, nitrate, amino acids, peptides, globulins and some albumins. The content of most albumins and glutelins (B2) was estimated as the difference between borate phosphate buffer insoluble protein and neutral detergent insoluble protein. Prolamins, extension proteins and denatured proteins (B3) were the difference between NDIN and ADIN. Maillard products and Nitrogen bound to lignin (C) were ADIN.

The *in vitro* true degradability of dry matter (IVTDDM) was determined as per the method described by Blummel and Becker (1997). A sample weight of 500 mg was taken in glass syringes. Rumen liquor was collected from the slaughterhouse and was brought in the laboratory in a pre-warmed thermos flask. Buffered rumen fluid (40 ml) was pipetted into each syringe, which was immediately placed into thermostatically controlled incubator (39°C) with arrangements for holding the syringes (Blummel and Orskov, 1993) and the incubation was terminated at 24 hours. After termination of the incubation, syringe contents were quantitatively transferred into 600 ml spoutless beakers by rinsing syringes with a total of 50 ml of neutral detergent solution (double strength, Blummel and Becker, 1997) and refluxed for 1 hour. Residual dry matter was determined and IVTDDM was calculated.

RESULTS AND DISCUSSION

The proximate, calcium and phosphorus constituents (percent on DM basis) of selected top feeds are presented in Table 1. Crude protein content was found highest in *Melia azedarach* (21.20) and lowest in *Acacia nilotica* (10.20). *Acacia nilotica* and *Quercus incana* contained the CP content between 10-12% and hence are sufficient to meet the crude protein requirements for maintenance and minimum milk production, whereas *Salix alba* containing more than 13% crude protein is sufficient to meet the crude protein requirements for maintenance and medium milk production in ruminants. The remaining seven top feeds containing more than 14% crude protein are sufficient to meet the crude protein requirements for maintenance and high milk production in ruminants. The ether extract content was maximum in *Acacia nilotica* (5.40) and lowest in Z*izyphus jujuba* (3.20). The NFE content, which gives an idea about the soluble carbohydrates content was highest in *Salix alba* (63.90%) and lowest in *Ficus palmata* (42.95%). The crude fibre which indicates the bulk in a feed was found in the highest amount in the *Ficus palmata* (17.30), whereas it was minimum in *Acacia nilotica* (9.20%). The total ash, which indicates the total mineral content was maximum in *Morus alba* (12.70) and minimum in *Acacia nilotica* (6.10%). The acid insoluble ash content, which indicates feed contamination, is highest

Table 1: Chemical composition (% in DM) of selected top feeds

Attributes	OM	CP	EE	CF	NFE	TA	AIA	Ca	P
Acacia nilotica	93.9	10.2	5.4	9.2	69.1	6.1	3.3	1.7	0.28
Celtis australis	90.4	15.7	4.3	16.1	59.4	9.6	1.6	3.2	0.19
Ficus palmata	87.8	14.3	4.7	17.3	49.5	12.2	1.9	2.8	0.22
Ficus religiosa	90.9	13.4	3.5	10.8	63.2	9.1	2.4	2.3	0.14
Grewia optiva	89.8	20.3	3.8	13.7	55.0	10.2	1.2	2.1	0.31
Melia azedarach	89.6	21.2	3.7	11.2	53.5	10.4	0.81	1.9	0.24
Morus alba	87.3	18.8	4.5	11.3	52.7	12.7	2.4	3.1	0.25
Quercus incana	92.1	11.2	5.3	14.3	61.3	7.9	2.1	1.6	0.21
Salix alba	93.1	12.8	3.7	12.7	63.9	6.9	1.0	2.3	0.16
Zizyphus jujuba	92.8	17.6	3.2	11.4	60.6	7.2	0.51	3.6	0.31

Table 2: Phenolics, fiber fractions and fiber bound nitrogen (% of total nitrogen) of selected top feeds

Attributes	TP	HT	CTP	NDF	ADF	HC	NDIN	ADIN
Acacia nilotica	1.78	0.69	1.09	32.4	16.1	16.3	45.33	19.75
Celtis australis	1.02	0.65	0.37	47.1	39.2	7.9	57.04	33.82
Ficus palmata	1.62	0.64	0.98	41.8	34.3	7.5	58.26	33.04
Ficus religiosa	2.22	1.12	1.10	38.3	29.6	8.7	49.44	23.92
Grewia optiva	0.75	0.58	0.17	33.9	22.4	11.5	52.93	17.67
Melia azedarach	1.98	1.34	0.64	29	21.1	7.9	13.50	6.54
Morus alba	1.00	0.79	0.21	31.1	23.4	7.7	58.99	49.77
Quercus incana	4.25	2.04	2.21	52.1	43.8	8.3	51.22	10.97
Salix alba	1.17	0.74	0.43	41.4	24.7	16.7	59.68	23.36
Zizyphus jujuba	7.32	3.02	4.3	36.3	25.7	10.6	56.05	42.73

in *Acacia nilotica* (3.30%) and minimum in *Zizyphus jujuba* (0.51%). The calcium content of the selected feeds varied from 1.7 percent (*Acacia nilotica*) to 3.6 per cent (*Zizyphus jujuba),* whereas phosphorus content varied from 0.14 percent (*Ficus religiosa*) to 0.31 percent (*Grewia optiva and Zizyphus jujuba*). The NDF content (Table 2) was found highest in *Quercus incana* (52.10%) and lowest in *Melia azedarach* (29.00%). Al-Kirshi *et al.* (2013) and Dias *et al.* (2022) analysed chemical composition of *M. alba* and reported values similar to our findings except that of CP(%), which is relatively lower in our samples. However, our observations are similar to that reported by Wang *et al.* (2017). These variations could be attributed to various environmental factors (Dias *et al.*, 2022). High calcium and low phosphorus content of tree leaves is a common observation (Habib *et al.*, 2013), therefore grain supplementation to balnce the calcium:phosphorus ratio is recommended along with incorporation of tree leaves in livestock ration.

The phenolic, fiber fractions and fiber bound nitrogen (% of total nitrogen) of selected top feeds is shown in Table 2. The values for fibre fractions observed in the present study are relatively less from that reported by Gaikwad *et al.* (2017) for the tree leaves of Mahrashtra.

The total phenolics content (Table 2) varied from 0.75 per cent in *Grewia optiva* to 7.32 per cent in *Zizyphus jujube.* The total phenolics content (per cent) of *Melia azedarach, Quercus incana and Zizyphus jujuba* was 1.98, 4.25 and 7.32. The total phenolics and tannins content of *Q. incana* observed in the present study did not agree with that observed by Al-Masri and Mardini (2013), with significant lower values reported by them. The contents of hydrolyzable tannins varied from 0.64 per cent in *Ficus palmata* to 3.02 per cent in Studies have also been conducted on tree leaves of Karnataka based on their content of polyphenols and rumen *in vitro* gas production (Giridhar *et al.,* 2018). The levels and types of tannins in

Table 3: Protein fractions (% of Total N) and in vitro true dry matter digestibility (24 hours) of selected top feeds

Attributes	NDIN	A	B_1	B_2	B_3	C	IVTDMD (%) (24 hours)
Acacia nilotica	45.33	25.18	17.27	12.22	25.58	19.75	74.34
Celtis australis	57.04	15.26	13.48	14.22	23.22	33.82	62.12
Ficus palmata	58.26	13.22	12.27	16.25	25.22	33.04	69.12
Ficus religiosa	49.44	30.72	10.62	12.22	25.52	23.92	60.18
Grewia optiva	52.93	35.18	6.28	5.61	35.26	17.67	76.92
Melia azedarach	13.5	41.18	3.10	42.22	6.96	6.54	78.88
Morus alba	58.99	33.21	5.19	2.61	9.22	49.77	79.56
Quercus incana	51.22	42.48	2.65	3.65	40.25	10.97	52.22
Salix alba	59.68	30.02	2.18	8.12	36.32	23.36	51.58
Zizyphus jujuba	56.05	28.48	4.25	11.22	13.32	42.73	75.12

plants vary greatly between species, within species, stage of development, from location and from year to year (Tong *et al.*, 2022). They are found in the wood, bark, leaves and fruits of many species but are more abundant in some than others (Mora *et al.*, 2022). It is found in higher concentration in tropical plants as light intensity and high temperature stress enhances synthesis. Changes in environmental factors like climate, water availability and soil fertility can drastically affect CT levels (Foster *et al.*, 2012; Al-Masri, 2013; Al-Masri and Mardini, 2013). These variations can be high and erratic. Mobilization of tannin pool of plants can sharply increase the levels in grazed plants. The tannin-protein complexes are specific both in terms of tannins and proteins. Therefore, behaviour of different sources of plant tannins with proteins varies significantly (Singh *et al.*, 2020).

The NDIN (per cent of total Nitrogen) was maximum in *Salix alba* (59.68) and minimum in *Melia azedarach* (13.50). The various protein fractions (% of total nitrogen) and *in vitro* true dry matter digestibility of selected top feeds is shown in Table 3. The protein fraction B_3 (per cent of total nitrogen), which indicates bypass protein varied from 6.96 in *Melia azedarach* to 36.32 in *Salix alba*. The unavailable protein fraction C (per cent of total N) was maximum in *Z. jujuba* (42.73) and minimum in *M. azedarach* (6.54). The *in vitro* true dry matter digestibility (%; 24 hours) ranged from 79.56% in *Morus alba* suggesting excellent utilizability to 51.58% in *Salix alba* indicating poorly degraded dry matter in rumen. Tree leaves are known to affect ruminal degradability of nutrients (Kumar *et al.*, 2022) by modifying the rumen microbiome (Chanu *et al.*, 2020) through various plant secondary metabolites including condensed tannins (Vuong *et al.*, 2015). These effects are usually dose-dependent and may even have associative effects (Singh *et al.*, 2020) when leaves are fed as meal mixture. Variable IVTDMD (%) of tree leaves observed in the present study is therefore a reflection of their total phenolics and condensed tannins concentration.

CONCLUSION

Based on the chemical composition, phenolics content and *in vitro* degradation values for different tre leaves, the *Celtis australis, Ficus palmata, Ficus religiosa, Grewia optiva, Melia azedarach, Morus alba* and *Zizyphus jujuba* leaves of District Poonch are recommended as the candidate feed supplement in ruminant ration.

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