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CHEMICAL COMPOSITION, MINERAL PROFILE, PALATABILITY AND *IN VITRO* DIGESTIBILITY OF SHRUBS

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Abstract

This study determined the nutritive value of *Indigoferra gerardiana*, *Marisine affricana, Impashion bicolor* and *Adhatoda vesica* shrubs for ruminants. Five shrubs of each species were sampled and composite sample of leaves for each species was oven dried at 55°C for 48 h. Chemical analysis revealed that dry matter (DM) content varied from 24.3% (*Adhatoda vesica*) to 38.1% (*Indigoferra gerardiana, Impashion bicolor*). Maximum crude protein (14.7%) was observed for *Marisine affricana* while, minimum (15.6%) was noted for *Impashion bicolor* and *Adhatoda vesica.* Higher ash content (14.7%) and lower neutral detergent fiber contents (49%) were observed for *Marisine affricana*. Higher hemicellulose (42%) and lignin (7.9%) contents, and lower acid detergent fiber (22%) were observed for *Impashion bicolor*. The Ca, 1.01-2.7 %; P, 0.016-0.064 %; K, 0.47-1.29 %; Mg 0.012-0.032 %; Cu, 14-25 ppm; Zn, 12.4-41.3 ppm; Mn, 9-12 ppm and Co, 0.012-0.061 ppm were observed among shrub species. Potential intake rate (g/sheep/4min) was highest (64.5) for *Indigoferra gerardiana*, while, lowest (8.8) for *Adhatoda vesica*. Similar trend was observed regarding relative preference. Maximum *in vitro* DM digestibility (69.7%) and metabolizable energy (9.54 MJ/kg DM) were noted for *Impashion bicolor*, while, minimum *in vitro* DM digestibility (53.5%) and metabolizable energy (7.16 MJ/kg DM) were examined for *Adhatoda vesica*. Based upon the findings of present study, shrub species for use as ruminant feed were ranked as *Marisine affricana* > *Indigoferra gerardiana* > *Impashion bicolor* > *Adhatoda vesica*.

Introduction

Shrubs, a component of ruminant diet, play an important role in the nutrition of grazing animals (Meuret *et al*., 1990; Papachristou & Nastis, 1996). In the present scenario, Pakistan is deficient by 40 and 80% in forage and concentrates feed, respectively (Pasha, 1998). Moreover, the existing feed resources are only providing 62 and 74% of required crude protein (CP) and total digestible nutrients, respectively resulting low productivity of livestock in the country (Sarwar *et al*., 2009).

Shrubs offer a potential alternative forage source for small ruminants in tropical countries especially in periods of scarcity when quality and quantity of fodder is scarce, and can help to minimize the wide gap between availability and supply of nutrients resulting to improve livestock productivity. In addition to grasses and legumes, shrubs are widely available and inexpensive source of feed for small ruminants. Therefore, shrubs

being valuable supplements provid variety to grasses and have considerable potential in supporting economical animal production in developing countries (Devendra, 1989).

As little information regarding the nutritive value of shrubs widely used for small ruminants in hilly areas of the country is available so this study was lunched to envisage the nutritional evaluation of shrubs by their chemical composition, mineral profile, palatability and screening of *in vitro* digestibility to assertain their fitness to small ruminant feeding.

Materials and Methods

Collection of samples: Leaves of *Indigoferra gerardiana, Marisine affricana, Impashion bicolor* and *Adhatoda vesica* shrubs were collected from valley of Chagharzai, district Bunair, North Trans-Himalayan zone, Malakand Division, North West Frontier Provence, Pakistan. Five shrubs of each species were sampled. Approximately 1 kg of leaves sample was harvested from each species as a single sample harvested from a shrub. Composite sample of leaves was prepared for each species. The leaf samples were dried in an oven at 55^{o}C for 48 h and saved in polythene bags.

Chemical analysis: The samples of shrubs species were ground through 2 mm screen in Willey mill and analyzed for dry matter (DM), N, ash (AOAC, 1995), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL; Van Soest *et al*., 1991). The CP was calculated as CP= N x 6.25. Hemicellulose was determined by difference of NDF and ADF. Following the wet digestion (nitric acid and perchloric acid), Na and K were determined by the flame photometer (Jenway PFP7). The Ca, P, Mg, Cu, Co, Mn and Zn were determined by atom absorption spectrophotometer. All chemical analyses were done in triplicate.

Palatability: Four mature sheep (average body weight 40 kg) were used to evaluate palatability. The sheep were adapted for 5 weeks to the mixture of dry leaves of selected shrubs and trained in the experimental procedure by offering the test samples to them alone or in pairs daily. During preliminary periods, animals were fed a mixed diet of 80% dry leaves and 20% concentrate (10% CP and 8.37 MJ/kg metabolizable energy; ME) along with mineral mixture to meet sheep requirements (NRC, 1985). The potential intake rate and relative preference for different shrub leaves was determined by the procedure of Sultan *et al*., (2007). Leaves of each shrub species were offered to sheep for a set of comparisons consisting of 4 consecutive periods of 1 minute duration at 10 minute intervals. Sufficient feed was offered in plastic containers to ensure that residues remained after one minute of intake. After a delay of 1 h, a new set of comparisons was started with a maximum of 4 sets in a day. Positioning of the containers of forages in a pair were reversed for each successive offering to avoid left- or right-hand bias. The preference for a particular forage was determined by the standard procedure developed by Bell (1959) for 2-choice tests, as the intake of shrub leaves expressed as a percentage of the combined intake of both test and standard forage.

$$\text{Relative preference} = \frac{\text{Amount of test forage eaten}}{\text{Amount of test + standard forage eaten}} \times 100$$

***In vitro* dry matter digestibility and metabolizable energy:** Oven-dried ground samples (0.5g) were incubated at 37±1°C for 48 h at pH 6.7-7.0 in an all-glass system using 45 mL of inoculum. The inoculum consisted of 36 mL of McDougal's artificial saliva and 9 mL of strained fresh rumen liquor collected from ruminally fistulated sheep (fed diverse shrubs and grasses and supplemented with concentrate). After incubation and centrifugation, the residue was treated for 48 h with pepsin in weak acid. All incubations were carried out in triplicate with a blank (without sample). The final residue was composed of undigested plant cell wall and bacterial debris and yield values were supposed to be comparable to *in vivo* apparent digestibility (Tilley & Terry, 1963). *In vitro* DM digestibility (IVDMD) was determined by the method described by Sultan *et al.*, (2007). The ME was determined by the following equation (Sultan *et al.*, 2008):

$$\text{ME (MJ/kg DM)} = 0.15 \text{ IVOMD\%}$$

whereas, *in vitro* organic dry matter digestibility (IVOMD%) = 0.98 IVDMD% - 4.8

Statistical analysis: The data collected on chemical composition, mineral profile, *in vitro* DM digestibility, ME, potential intake rate and relative preference were analyzed by SPSS program (SPSS, 1999).

Results and Discussion

Chemical composition: Chemical composition of shrubs is presented in Table 1. The DM contents varied from 24.3% (*A. vesica*) to 38.1% (*I. gerardiana, I. bicolor*). Highest CP concentration (23.7%) was observed for *M. affricana*, while, lowest CP concentration (15.6%) was noticed for *I. bicolor* and *A. vesica*. The NDF contents varied from 49% (*M. affricana*) to 64% (*I. bicolor*). Lowest ADF (22%) was observed for *I. bicolor*, while, highest (37%) for *I. gerardiana*. Hemicellulose ranged from 21% (*A. vesica*) to 42% (*I. bicolor*). Lignin contents varied between 4.6% (*I. gerardiana*) to 7.9% (*I. bicolor*). Highest (14.7%) ash contents were noticed for *M. affricana* and lowest (10.2%) for *A. vesica*. The chemical composition of shrubs species analyzed here provides a good source to be used as the nutrient source of ruminant feed.

Table 1. Chemical composition of shrubs.

Items	*I. gerardiana*	*M. affricana*	*I. bicolor*	*A. vesica*
Dry matter, %	38.1	36.2	38.1	24.3
Crude protein, %	16.6	23.7	15.6	15.6
Neutral detergent fiber,%	61	49	64	56
Acid detergent fiber, %	37	23	22	35
Hemicellulose, %	24	26	42	21
Lignin, %	4.6	5	7.9	5.2
Ash, %	10.6	14.7	12.4	10.2

Table 2. Minerals composition of shrubs.

Shrubs	Ca, %	P, %	Ca:P	K, %	Mg, %	Cu, ppm	Zn, ppm	Mn, ppm	Co, ppm
I. gerardiana	1.28	0.042	30.48	0.47	0.014	20	17.6	9	0.061
M. affricana	1.02	0.064	15.94	0.71	0.012	18	12.4	11	0.029
I. bicolor	2.7	0.016	168.75	1.29	0.032	25	41.3	10	0.012
A. vesica	1.01	0.061	16.56	0.56	0.023	14	18.4	12	0.053

Mineral profile: Mineral profile of shrubs is presented in Table 2. The Ca contents varied from 1.01% for *A. vesica* to 2.7% for *I. bicolor* and were higher than the dietary requirements of dairy cattle (0.43-0.60% of DM of diet) recommended by NRC (2001). However, ruminants can tolerate Ca up to 2% of diet DM (NRC, 1985). The P contents ranged from 0.016% in *I. bicolor* to 0.064% in *M. affricana*. The values of P observed in this study were lower than the maximum value (0.38%) of P examined for most of tree leaves reported earlier (Mandal, 1997) and were also lower than the dietary requirements of dairy cattle (0.31-0.40% of DM of diet) recommended by NRC (2001). The P in shrubs was low (0.031–0.054% of DM) in comparison with the required minimum of 0.20% (McDowell *et al.*, 1984). The Ca:P ratio in *I. gerardiana, M. affricana, I. bicolor* and *A. vesica* was much wider (30.48, 15.94, 168.75 and 16.56, respectively) compared to those recommended for ruminants (McDowell, 1997), however, this factor can be overcome by feeding cereal byproducts supplemented diets containing low Ca and high P (Prakash *et al.*, 2009). Ruminant can tolerate Ca:P ratio as wide as 7:1 (NRC, 1985). Rogosic *et al.*, (2006) observed wide Ca:P ratio among shrub species. Saha and Gupta (1987) reported that tree are rich in Ca and poor in P. High Ca:P ratio reduces absorption of P (NRC, 2001). Therefore, P supplementation appears to be essential (Breves & Schroder, 1991). Durand and Komisarczuk (1988) reported that available P should be at least 5 g/kg organic matter digested to optimize degradation of cell walls by microbes. Concentration of Ca >1% have been associated with lower DM intake, and excess Ca can interfere with trace mineral absorption (especially Zn) and lower performance of dairy cattle (NRC, 2001). The K value was highest in *I. bicolor* (1.29%) and lowest (0.47%) in *I. gerardiana*. Dietary requirement of K for dairy cattle is 0.80% of diet on DM basis (NRC, 2001). Maximum tolerable level of K is 3% diet DM (NRC, 1980). Increasing the level of K from 0.7 to 3% linearly decreased the energy and weight gain in lambs (NRC, 1985). The Mg contents varied from 0.012% (*M. affricana*) to 0.032% (*I. bicolor*) and were lower than the required level (0.12-0.18% of diet DM) of Mg for sheep (NRC, 1985). The Cu contents ranged from 14 ppm (*A. vesica*) to 25 ppm (*I. bicolor*) and were below the toxic level as Underwood (1981) reported that Cu concentration as low as 40 ppm can cause toxicity in sheep if level of Mo and S are low in the diet, however, cattle are more tolerant. Dietary requirements of Cu are 10mg/kg (NRC, 1985) and 0.2-7mg/kg (Church, 1988). The Cu values determined for shrubs in this study were higher than required level. Sheep requirement for Zn varied between 20-33 mg/kg of diet DM (NRC, 1985). The Zn contents of *I. gerardiana, M. affricana* and *A. vesica* (17.6, 12.4 and 18.4 ppm, respectively) were lower than the required level, whereas, the Zn content (41.3 ppm) of *I. bicolor* was higher than the recommended level, however, this may be controlled by its high level of Ca as it affect the Zn utilization (Mills & Dalgarno, 1967). Less variation was observed in Mn contents among *I. gerardiana, M. affricana, I. bicolor* and *A. vesica* shrubs. However, Mn values observed in this study were lower than the recommended level (20mg/kg of diet DM) for sheep (NRC, 1985). The Co contents

ranged from 0.012 ppm for *I. bicolor* to 0.061 ppm for *I. gerardiana* and were lower than requirement (0.1mg Co/kg diet DM) for sheep (NRC, 1985). Inam-ur-Rahim *et al*., (2008) observed 0.001 to 0.02 ppm Co concentrations in mature marginal land grasses and reported that the concentrations were below the recommended requirements for livestock. Vercoe (1987) analyzed mineral contents of 23 tree species used for livestock feeding and reported range of Ca, 0.29-3.52%; P, 0.05-0.18%; K, 0.41-1.78%; Mg, 0.21-062%; Cu, 4-152 ppm; Zn, 22-123 ppm and Mn, 30-917 ppm. In present study, Ca, K and Cu values were within the range as reported by Vercoe (1987), however, Mg and Mn values were below this range.

Palatability: Potential intake rate (g/sheep/4min) was highest (64.5) for *I. gerardiana*, while, lowest (8.8) for *A. vesica* (Table 3). Similar results were observed regarding relative preference. Potential intake rate and relative preference are considered the main indicators for palatability (Rehman, 1995; Sultan *et al.*, 2007). Potential intake rate is affected by degree of tenderness, while, relative preference is influenced by chemical factors (Sultan *et al.*, 2007; Inam-ur-Rahim *et al.*, 2008). Lower potential intake rate in for *I. bicolor* and *A. vesica* might be due to the presence of any antinutritional factor having particular taste and smell (Rehman *et al.*, 1995). Sheep preferred diet having faster intake rates when the effects of taste and odor were removed (Kenney & Black, 1984).

***In vitro* dry matter digestibility and metabolizable energy:** Highest *In vitro* DM digestibility (69.7%) and ME (9.54 MJ/kg DM) were observed for *I. bicolor* (Table 3), whereas, lowest *in vitro* DM digestibility (53.5%) and ME (7.16 MJ/kg DM) were examined for *A. vesica*. The higher ME contents of *I. bicolor* than other shrub species might be due to its higher (69.7%) *In vitro* DM digestibility. Lower *in vitro* DM digestibility of *A. vesica* might be due to the presence of any antinutritional factor which inhibited the rumen microbial enzymes (Moore & Jung, 2001; Sultan *et al.*, 2007).

Conclusion

On the basis of results regarding chemical composition, high availability of ME, *in vitro* DM digestibility and palatability, shrub species for use as ruminant feed were ranked as *M. affricana* > *I. gerardiana* > *I. bicolor* > *A. vesica*. Wide Ca to P ratio suggests supplementing cereal byproducts having high level of P along with these shrubs when fed to ruminants. The concentration of P, Mg, Mn and Co among these shrub species were less than required level. Further research is needed to evaluate these shrubs for any antinutritional factor.

Table 3. *In vitro* digestibility and metabolizable energy, potential intake rate and relative preference.

Shrub species	IVDMD	ME	PIR	PR
I. gerardiana	57.8 [b]	7.78 [b]	64.5 [a]	73.18 [a]
M. affricana	57.1 [b]	7.66 [c]	53.0 [b]	66.35 [b]
I. bicolor	69.7 [a]	9.54 [a]	34.0 [c]	43.8 [c]
A. vesica	53.5 [c]	7.16 [d]	8.8 [d]	2.63 [d]
Standard error	2.65	0.33	2.29	2.6

IVDMD: *In vitro* dry matter digestibility (%), ME: metabolizable energy (MJ/kg DM), PIR: Potential intake rate (g/sheep/4mintues), RP: Relative preference (%)

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