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LEVELS OF TOTAL AMINO ACIDS, SOLUBLE PROTEINS AND PHENOLIC COMPOUNDS IN FORAGES IN RELATION TO REQUIREMENTS OF RUMINANTS GRAZING IN THE SALT RANGE (PUNJAB), PAKISTAN

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Abstract

Soone Valley (Salt Range) Punjab, Pakistan is a rich habitat of a large number of plant and animal species. Various leguminous and non-leguminous species are indigenous to this Valley which are grazed by a large number of ruminants. Levels of total amino acids, soluble proteins and phenolic compounds were appraised in pods and leaves in the leguminous plant species therein, because metabolites are important constituents of nutrition for ruminants, The data obtained after analysis showed that amino acids and soluble proteins varied from 37.18 to 50.87 and 22.27 to 35.47 mg g^{-1} fresh weight in leaves respectively whereas in pods they ranged from 50.22 to 53.98and 30.67 to 35.48 mg g^{-1} fresh weight respectively in all species studied. Phenolic compounds ranged from 0.15 to 0.48 mg g^{-1} dry weight in leaves while they varied from 0.11 to 0.32 mg g^{-1} dry weight in pods, respectively, in all species under investigation in all pastures. Based on observations recorded for all the attributes, it was concluded that the forage plant species were palatable because all species, contained sufficient amount of amino acids and proteins. Furthermore, the effects of high levels of phenolic found in the leguminous species of the range need to be investigated on the grazing livestock therein particularly in relation to the toxicosis of these compounds on animals.

Introduction

An enormous environmental variability exists in Pakistan, which ranges from high snowy Himalayan peaks in the north to the hot humid climate of shores of the Arabian Sea in the south. The four provinces of Pakistan vary greatly in topography (Anon., 2003). The Punjab has diverse vegetation types and the major part of the natural vegetation comprises tropical thorn forests. This type of vegetation occurs in isolated patches like graveyards, forest plantation and some prohibited areas under armed forces and salinity/ sodicity affected soils (Khan, 1978; Hussain, 2002; Ahmad *et al.,* 2007). The southern edge of the Potohar plateau is demarcated by the famous Salt Range of Pakistan (Ahmad, 2002).

In the heart of this Range lies the Soone Valley (the study area). The climate of the valley is characterized by a relatively low annual precipitation (508 mm) and average minimum temperature is 1º C (January) while average maximum temperature is 36º C (June). Hot dry winds and prolonged periods of drought are frequent; winters are accompanied by frost. Summer and winter in the Range both are cooler than those of adjoining plains and the winter season is also longer than that in plains (Ahmad, 2002;

Hussain, 2002; Ahmad *et al*., 2007; 2008a; 2008b). Leguminous species are lavishly consumed by grazing animals because they are composed of large quantity of amino acids (Ramamohana *et al.,* 1983; Bowman and Asplund, 1988; Orr and Treacher, 1989; Andrighetto *et al*., 1993; Davendra, 1993; Muinga *et al.,* 1995). Some species of leguminous family are sources of cheap protein for livestock. For example bitter vetch (*Vicia ervilia*) is very important due to its high nutritional value and ability to grow in almost all types of poor soils (Lopeze Bellido, 1994). Most of the leguminous fodder tree species and shrubs have high protein levels and are potential supplements to overcome nutrient deficiencies caused by anti-nutritional factors such as tannins and other secondary compounds (El-Waziry, 2007).

Tannins and other poly-phenolics substances occur widely in plants. The effect of tannins either adverse or beneficial, for animals depends on the concentration and chemical structure (Makkar, 2003; Min *et al*., 2003). *Acacia* (*Acacia saligna*) a leguminous shrub, available in many countries, is high in phenolic compounds such as tannins (condensed tannins as well as hydrolysable tannins), reduce the nutritive value of foliage (Ben-Salem *et al*., 2002). Keeping in view the importance of amino acids, soluble proteins and phenolic compounds for animals grazing in the Soone Valley, Pakistan, the present study was planned to determine the critical values below which deficiency or toxicity of these compounds takes place. The information gathered will be useful for successful management of grazing livestock with respect to mineral nutrition for other regions of Pakistan as well as Asian countries with similar ecological conditions where forages are the only potential source of mineral nutrition.

Materials and Methods

The investigated area (Soone Valley) is situated between longitude 71º30′ and 73º30′ E and between the parallels of 32º23′ and 33º N latitude and the altitude is about 670 m. Ecological investigations were done to explore the nutritional status of forage plants in the valley for need-based assessment of grazing livestock by the forage species. The pastures or feeding sites were designated as Pasture A, B, C, D, E and F respectively. These native pastures are composed of a variety of plant species including *Acacia farnesiana, A. hydaspica, A. modesta, A. nilotica, Albizzia lebbeck, Argyrolobium stenophyllum, Dalbergia sissoo, Medicago laciniata, M. polymorpha, Melilotus alba, M. indica, Prosopis glandulosa, P. juliflora, P. spicigera, Rhyncohsia minima, Sophora mollis, Trigonella monantha, Vicia monantha,*and *V. Sativa* as the dominant but grasses such as *Cynodon dactylon, Saccharum munja, Saccharum spontaneum, Cyperus rotundus* make up the bulk of herbaceous cover. These native pastures are the major sources of food for different ruminants in the valley (as described in Ahmad *et al*., 2008b).

Sample collection: The grazing animals were followed and forages consumed by the ruminants were collected. Five predominant samples of different forage species were taken from each pasture four times after an interval of three months. Each sample was comprised of five sub-samples of each plant species. The collected plant samples consisted of green leaves and pods of all available species. The analytical procedures followed are described below:

Total amino acids: Total amino acids were estimated according to the method of Moor and Stein (1957). One gram fresh plant material (leaves or pods) were chopped in 10 mL

of citrate buffer (pH 5.0) and incubated for 1 h at room temperature and centrifuged at 15000 rpm at 15^{o} C for 10 minutes. The supernatant was separated and used to measure the total free amino acids. One mL of the extract was taken in 20 mL test tube and then 1 mL of ninhydrin solution added to it. The tubes heated for 20 minutes in boiling water bath. Then they were cooled and 5 mL of the diluents were mixed and again incubated at room temperature for 15 minutes. The optimal density was read at 570 nm using a spectrophotometer (Hitachi, 220 Japan)

Total soluble proteins: Total soluble proteins were determined following Lowry *et al.*, (1951). Fresh plant material, leaves or pods (0.2 g) each were chopped in 5 mL phosphate buffer (pH 7.0). One mL of sample extract of each treatment was taken in a test tube. The blank contained only 1 mL distilled water. One mL of the solution was added to each test tube. The reagents in the test tube were thoroughly mixed and allowed to stand for 10 minutes at room temperature. Then 0.5 mL of Folin-Phenol reagent (1:1 diluted) was added, mixed well and kept for 30 minutes at room temperature. The optical density (OD) was read at 620 nm on a spectrophotometer (Hitachi, 220).

Total phenols: Total phenols were estimated according to the method of Julkunen-Titto, (1985). Dried ground material of leaves or pods (1g) was taken and then 10 mL of 80 % methanol were added to it. All samples were heated at 40-45º C for 5 minutes and filtered. The residue was taken and washed it with 20 % sodium carbonate. The methanol was evaporated through rotary evaporator and 10 mL of distilled water was added, and then washed it with *n*-hexane. The aqueous layer (extract) was evaporated. One mL of extract was taken and then 1 mL of 20 % sodium carbonate was added to it, incubated for 5 minutes at room temperature and then 0.5 mL Folin Denis Reagent (1:1 diluted with distilled water) was added to it. It was incubated for another 10 minutes at room temperature. Dilution was made with distilled water, keeping in view the colour intensity read at 735 nm. Total phenols were calculated by using a standard curve developed through various concentrations of tannic acid in micrograms following the above method for the colour development.

Statistical analysis: The data collected were analyzed by analysis of variance technique. Duncan's New Multiple Range test at 5 % level of probability was used to test the differences between the mean values (Steel & Torrie, 1980).

Results and Discussion

The data regarding total amino acids, soluble proteins & phenolic contents of all forage species collected from six pastures pooled and presented in Table 1. Mean forage total amino acids ranged from 37.18 to 50.87 mg g^{-1} in leaves and from 50.22 to 53.98 mg g^{-1} in pods, whereas soluble proteins values varied from 22.27 to 35.47 and 30.67 to 35.48 mg g^{-1} in leaves and pods respectively. Amino acids concentrations were lower in pods of samples collected from pasture-C and the highest level was observed in the pods of the forages from pasture-B. The maximum value of leaf amino acids was recorded in pasture-A while minimum in pasture-D. Soluble proteins were lower in leaves in the samples collected from pasture-D and the highest level observed in the leaves of forages from pasture-A but in case of pods, the maximum value was recorded in species from pasture-B while minimum in pasture-A.

Legumes are an important source of protein and minerals in animal nutrition but in various leguminous species the protein content varies from 26 to 41 percent (Ferna´ndez *et al*., 1996). Some legumes contain more protein at vegetative stages as compared to the later stages (Khan *et al*., 2002). In the present studies all the legume forage species from all six pastures had amino acids and proteins within the beneficial range for grazing livestock as already reported by Hove *et al.,* (1978). Comparison of our analytical data with values recommended by the Anon., (1978) shows that all species provide sufficient amounts of essential amino acids and proteins to ensure normal development in grazing livestock.

Table 1. Total amino acids, soluble proteins & phenolic compounds in leaves and pods of selected plant species of Soone Valley.

Compound	Pasture Type	Leaves	Pods
	A	**50.87** ±3.15	**50.70** ±2.35
	B	**50.17** ±1.86	**53.98** ±1.86
	C	**38.42** ±1.32	**50.22** ±1.25
Total amino acids	**D**	**37.18** ±2.48	**51.82** ±1.45
(mg g^{-1} fresh.wt)	**E**	**41.49** ±2.12	**51.09** ±1.73
	F	**44.33** ±2.10	**51.27** ±2.57
	A	**35.47** ±3.02	**30.67** ±1.16
	B	**31.40** ±2.07	**35.48** ±1.50
	C	**24.97** ±1.71	**30.88** ±2.21
Total Soluble Proteins	**D**	**22.27** ±1.50	**31.09** ±1.45
(mg g^{-1} fresh.wt)	**E**	**29.60** ±1.49	**33.01** ±1.80
	F	**32.36** ±1.71	**31.77** ±1.46
	A	**0.15** ±0.040	**0.32** ±0.038
	B	**0.21** ±0.046	**0.21** ±0.033
	C	**0.48** ±0.105	**0.16** ±0.033
Total Phenols	**D**	**0.25** ±0.034	**0.18** ±0.027
(mg g^{-1} dry.wt)	**E**	**0.19** ±0.032	**0.13** ±0.032
	F	**0.36** ±0.038	**0.11** ±0.016

Means are average ± standard error; Species = NS (Non-significant at $p>0.05$)

Mean forage phenolics compounds values ranged from 0.15 to 0.48 mg g^{-1} in leaves and from 0.11 to 0.32 mg g^{-1} in pods respectively, in various species reported from the investigation area. Phenolics concentrations were lower in pods of samples collected from pasture-F and the highest level observed in the pods of forages from pasture-A. In case of leaves the maximum value of phenolic compounds was recorded in pasture-C, while minimum in pasture-A.

Polyphenolic compounds are widely distributed in plants and play a vital role in protection mechanisms against various bacterial, fungal, viral or chemical attacks (Thompson, 1993). The phenolic acids are either derivatives of benzoic acid or of cinnamic acid, which are commonly found as esters of caffeic and quinic acids, among polyphenolic compounds, flavonoids (*e.g.* catechin, epicatechin *etc.*) are the most common and widely distributed group of plant phenolics. They are the monomeric constituents of condensed tannins but are also very common as free monomers. Isoflavones are present in high concentrations in soybean products but in much lower amounts in most common legumes (Champ, 2002).

Although some species like *Dilbergia sissoo* and *Melilotus alba* had phenolic contents a little higher than other species; this amount is below the toxic value or they are within the safe limits. So they are not dangerous for animal health. Some species have very low amount of phenolic compounds that may be due to soil composition of different selected sites. The plants of *Dilbergia sissoo* and *Melilotus alba* are growing on soils, which may contain some residues of animals and plants with some phenolic compounds. As indicated above that these compounds are not up to the dangerous limit so they are not harmful for both plants and animals. Phenolic compounds of all plants of present study were not significant compared to the toxic values reported by Caramori *et al.,* (2004). It can be concluded that the plant species studied in the present paper are palatable and contain reasonable amount of organic compounds *e.g.* amino acids and proteins for livestock.

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