Pak. J. Bot., 40(5): 1877-1883, 2008.

EVALUATION OF AVAILABLE SUGARS IN PLANT SPECIES INDIGENOUS TO SOONE VALLEY (PUNJAB) PAKISTAN

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Abstract

Soone Valley (Salt Range) in Pakistan is a habitat of a large number of flora and fauna. Various grasses and leguminous species are indigenous to this valley and are grazed by ruminants. Leguminous plant species were characterized for carbohydrates, starch and total sugar contents in pods and leaves as well. The data obtained after analysis showed that carbohydrates ranged from 54.37 to 64.43; starch contents varied from 30.85 to 40.55 mg g^{-1} dry weight in leaves while it ranged from 61.65 to 69.09 and 32.55 to 36.11 mg g^{-1} in pods respectively. Overall values of soluble sugars range from 23.88 to 31.42 in leaves and 35.65 to 40.70 in pods in species under investigation in different pastures. Based on observation recorded for total soluble sugars estimation, it was concluded that the forage plant species studied were found to be palatable and had significant amount of those elements required for the needs of livestock reared in that specific rangeland. The results of the present investigation provide key information regarding sufficient amount of sugars in forages, which seems to be excellent. Furthermore, investigations for toxicosis, if any of sugars on grazing livestock of the range are urgently needed.

Introduction

Plant diversity being a vital part of the world's natural heritage is a unique and most essential living source on this planet. Plants are considered as key component for the global sustainability (Goel, 2002). The Punjab (Pakistan) has diverse vegetation types and major portion of the natural vegetation of Punjab comprises of tropical thorn forest. This type of vegetations is still occurring 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). Seasonally flooded areas, especially near Indus, Chenab and Jehlum rivers were transformed into riverians or bela forests, swamps and wetlands. Desert and semi-deserts vegetation occurs in Cholistan and Thar areas, the mountain regions of Punjab; edges of Suleman Range near Rajan Pur have hot mountainous type of vegetation (Chaghtai *et al*., 1983; Khan, 1991). The southern edge of the Potohar plateau is demarcated by the famous salt range of Pakistan (Ahmad, 2002; Ahmad *et al*., 2007).

According to Ahmed (1964) the vegetation of plains of salt range, Soone Valley (investigation area) consists of an open low forest in which thorny usually hard woody, forage and fodder species pre-dominate. The trees usually have short trunks and low branching crowns. The dominant species vary from 12-20 feet in height and tend to be longer at elevation. However, higher altitude favourably affects the size and percentage cover of the plants. *Olea* and *Acacia modesta* association has been reported as the climax vegetation of the salt range (Ahmad *et al*., 2008a).

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Table 1 Ecological parameters of six pastures in Soone Valley, Pakistan.

Elevation (m)	632-890
Slope	15-60 %
Aspect	East-western, South-western and Northern
Soil Texture	Sand stone with clayed sand, lime stone and Sandy clay
Moisture contents	6.83-11.00 %
EC (dS m^{-1})	1.10-3.59
pH	7.14-8.78
N (mg g^{-1} dry soil)	99.12-131.09
P (mg g^{-1} dry soil)	82.02-94.64
K (mg g^{-1} dry soil)	58.00-69.99
Habitat description	Hills with steep slopes, Moderate slopes, More or less flattened peripheral Area
Vegetation Type	Dominant herbs with grasses, few shrubs, Sedges and trees
Plant Community	*Olea ferruginea, Dodonea viscosa, Acacia farnesiana* and *Acacia modesta*

The plants of this valley are very important from grazing point of view. The *Fabaceae* family contains a number of woody species and herbaceous weeds, which are of major significance regarding grazing livestock therein (Pearson *et al*., 1993; Hussain, 2002; Ahmad *et al*., 2007). Leguminous species are second only to grasses in importance as forage and pasture plants. The high nutritive value leads legumes to be used as a high protein supplement to livestock rations. Leguminous species are famous for having high quantity of sugars (carbohydrates and starch), fibre, mineral matter, dry matter, moisture contents and fats, so lavishly consumed by grazing animals. Moreover, the combination of leguminous and non-leguminous fodder reduces the concentrate requirement (Ramamohana *et al*., 1983; Bowman & Asplund, 1988; Orr & Treacher, 1989; Andrighetto *et al*., 1993; Davendra, 1993; Muinga *et al*., 1995; Hussain, 2002). From ruminant's point of view, the nutritive values of leguminous species in this salt range is high; however, digestibility of some species is higher in summer and autumn than that in spring. Mineral contents vary significantly among species ranging from inadequate to toxic for livestock production. Generally the concentrations in broadleaf plants including leguminous plants are more than those in grasses and other forages (Belesky *et al*., 2001; Khan *et al*., 2006). Mineral ion concentration decreases with increase in age both in the case of legumes and grasses (Gonzalez-V *et al*., 2006).

Soluble sugars are very important bio-molecules involved in tolerance of water shortage during seed maturation and in storage as well (Obendorf, 1997). Raffinose, stachyose and verbascose are galactosyl sucrose oligosaccharides are next to sucrose in the plant kingdom (in distribution) and are accumulated during seed development in different forage crops and involved in various important physiological roles (Amuti and Pollard, 1977; Horbowicz *et al*., 1998). The sugars, carbohydrates and starch in maturing seeds have been thought to be non-toxic (in the form of storage products for the seeds) and also plays significant role in structural stability of different macromolecules. Methyl-ether derivatives of cyclitols may also form liquid crystals (Obendorf, 1997; Peterbauer and Richter, 2001). The contents of stored sugars in seeds varied dramatically, decreasing initially and being restored by the end of the analysis period (Nkang, 2002). Mineral composition of all species is satisfactory for animals.

Table 2. Total soluble sugars, starch, carbohydrates in leaves and pods of selected plant species of Soone Valley.

Organic Compound	Pasture Type	Leaves	Pods
		mg g^{-1} (dry weight)	
Soluble Sugars	A	27.92 ± 1.68	36.54 ± 1.81
	B	28.10 ± 1.19	38.39 ± 1.85
	C	23.88 ± 1.27	39.53 ± 2.45
	D	25.90 ± 1.70	40.70 ± 1.48
	E	26.80 ± 2.20	35.65 ± 2.46
	F	31.42 ± 1.66	37.26 ± 1.92
Starch	A	40.55 ± 5.47	32.82 ± 1.48
	B	38.35 ± 1.86	34.11 ± 2.42
	C	40.54 ± 2.84	36.11 ± 2.36
	D	38.34 ± 1.90	33.18 ± 0.96
	E	36.97 ± 1.55	32.55 ± 1.36
	F	30.85 ± 2.00	36.09 ± 1.44
Carbohydrates	A	54.37 ± 1.52	68.96 ± 3.18
	B	58.05 ± 2.65	69.09 ± 1.65
	C	64.43 ± 2.79	65.31 ± 2.19
	D	60.70 ± 2.02	66.30 ± 2.54
	E	62.10 ± 2.20	61.65 ± 2.27
	F	56.68 ± 1.94	62.97 ± 3.08

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

Keeping in view the importance of soluble sugars in leguminous forage and pasture plants in terms of nutrition for ruminants grazing in this particular range, the present study was undertaken to determine whether the contents of soluble sugars are below or above the critical levels for normal growth of the indigenous livestock in the Soone Valley the salt range of Punjab, Pakistan.

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 2200 ft. 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 found with leguminous plant species (*Acacia farnesiana, Acacia hydaspica, Acacia modesta, Acacia nilotica, Albizzia lebbeck, Argyrolobium stenophyllum, Dalbergia sissoo, Medicago laciniata, Medicago polymorpha, Mellilotus alba, Mellilotus indica, Prosopis glandulosa, Prosopis juliflora, Prosopis spicigera, Rhyncohsia minima, Sophora mollis, Trigonella monantha, Vicia monantha,*and *Vicia sativa* as the dominant but grasses (*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.

Soil Characteristics: The soil texture was determined by the hygrometer method (Dewis & Freitas, 1970). The physico-chemical characteristics are presented in Table 1. Electrical conductivity, pH and ions of saturation extracts were determined according to Jackson (1962).

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 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:

Digestion: Dried ground material 0.5 g was taken in digestion tubes and 5 mL of concentrated H_2SO_4 added and incubated overnight at room temperature. Then ½ mL of H_2O_2 (35 %) poured down the sides of the digestion tube, ported the tubes in a digestion block and heated at 350º C until fumes were produced and continued to heat for another 30 minutes. The digestion tubes were removed from the block and cooled. Slowly added ½ mL of H_2O_2 and placed the tubes back into the digestion block. The above step was repeated until the cooled digested material become colourless. The volume of the extract was maintained up to 50 mL in volumetric flasks. The extract was filtered and used for analysis.
Following two types of analysis were conducted

Determination of total soluble sugars: Total soluble sugars were determined according to the method of Yem & Willis (1954).

Extraction: Plant material (0.1 g) was extracted in 80 % ethanol solution. Dried plant material was ground so as to pass through 1 mm sieve of mill micro mill (Model Culatti, DFH-48) and it was taken for 6 hrs at 60^o C. This extract was used for the estimation of total soluble sugars.

Reagents: Anthrone reagent was prepared by dissolving 150 mg of anthrone in 72 % H_2SO_4. This reagent was freshly prepared whenever needed.

Procedure: Plant extract (100 mL) was taken in 25 mL test tubes and 6 mL anthrone reagent was added, and then heated in boiling water bath for 10 min. The test tubes were ice cooled for 10 min. The test tubes were incubated for 20 min., at room temperature (25º C). Optical density was read at 625 nm on a spectrophotometer (Hitatchi, 220). Blank was also read in the same way. The soluble sugars were calculated from the standard curve developed by using following the above mentioned method.

Carbohydrates (sugars and starch): Soluble sugars and starch were estimated following Malik & Srivastava (1979). For estimation of soluble sugars and starch, 0.1 g of well ground dry material was homogenized and centrifuged at g × 290 C. The residue was retained, which was repeatedly washed with 80 % ethanol to remove all the traces of soluble sugars. The filtrate thus obtained was used for the determination of soluble sugars. The residue was used for the determination of starch. Five mL of distilled water and 6.5 mL of 52 % perchloric acid

were added to the residue. Extraction of starch with perchloric acid was carried out at 0º C for 20 minutes, then centrifuged at g × 2900 and the extract was retained. With the residue the above step was repeated using fresh perchloric acid and the extract of this step was combined with the extract of the first step and then the volume of each of the sugar and starch extracts were made up to 100 mL by the addition of distilled water.

Anthrone solution: (i) 0.4 g of anthrone and 200 mL analar H_2SO_4 were taken in 250 mL volumetric flask. (ii) 60 mL distilled water and 15 mL ethyl alcohol (95 %) was taken in 500 mL flask. The large flask was placed in ice and poured the solution (i) into (ii) slowly with constant stirring by means of an electromagnetic stirrer.

Glucose solution: 0.2 g glucose was taken in a volumetric flask of 100 mL. The volume was made up to 100 mL. 5 mL of this solution was diluted to 100 mL by adding 95 mL distilled water.

Blank solution: 1 mL distilled water was taken in a test tube and then added 10 mL of anthrone solution. The tube was covered with cap and shaken at room temperature.

Glucose solution: 1 mL glucose solution and 10 mL of anthrone solution were taken in a test tube and was shaken as usual.

Sample solution: 1 mL sample solution and 10 mL of anthrone solution were taken in a test tube and was shaken. All the tubes were heated in boiling water for 12 minutes. Cooled and absorbance was read at 625 nm.
Total soluble sugars and starch was calculated by the following formula:

$$\text{Total soluble sugars and starch (Glucose Unit) (mg g}^{-1}\text{ of dry wt.)} = \frac{\text{Conc. of glucose solution}}{\text{Absorbance of glucose}} \times \text{Absorbance of sample} \times \text{Dilution factor}$$

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 significance of means (Steel and Torrie, 1980).

Results and Discussion

The data regarding total sugar contents of all forages collected from six pastures pooled and presented in Table 2. Mean forage carbohydrates values ranged from 54.37 to 64.43 mg g^{-1} in leaves and from 61.65 to 69.09 mg g^{-1} in pods whereas amount of starch varied from 30.85 to 40.55 mg g^{-1} and 32.55 to 36.11 mg g^{-1} in leaves and pods respectively in various species reported from investigation area. Over all sugar values lies between 23.88 to 31.42 mg g^{-1} in case of leaves and in case of pods there is an increasing trend (35.65-40.70 mg g^{-1}) among different species in different pastures. Forage carbohydrates concentrations were higher in pods of samples collected from pasture-B and the lowest level was found in the leaves of forages from pasture-A, in case of starch the maximum value for leaves are recorded in pasture-A and for pods in pasture-E, whereas overall sugar values are in the range of (23.88 to 40.70 mg g^{-1}) for pasture-C and

pasture-D respectively. Soluble sugars are important bio-molecules reported from different forages in present investigation involved in water shortage tolerance during seed maturation and as storage product (Obendorf, 1997).

The concentration of different plant nutrients plays a key role in various metabolic activities. With the availability of essential nutrients, the plant are able to produce the starches and soluble sugars as a product of photosynthesis (Khan *et al.,* 2005c). Other reported plant species also had reasonable amount of total sugars in pods and leaves which is a clear indication of good feed for livestock.

In the present research finding, the sugars, carbohydrates and starch in leaves and as well as in pods (mature seeds) are in sufficient amount and thought to be non-toxic for grazing live stock and had significant role in structural stability of different macromolecules (Peterbauer and Richter, 2001). The contents of stored sugars in seeds varied dramatically, decreasing initially and being restored by the end of the analysis period (Nkang, 2002).

According to Areghore & Hunter (1999) and Ahmad *et al.,* (2008b), the plant species maintaining higher total sugar contents have higher growth and are useful for grazing ruminants because they supply the easily available source of energy to the ruminants. As concluded in present research study, the concentration of different plant nutrients plays a key role in different metabolic activities. With the availability of essential nutrients the plants are able to produce the starches and soluble sugars as a product of photosynthesis (Khan, 2007). Most of reported plant species had reasonable amount of total sugars in pods and leaves, which is a clear indication of good feed for livestock. It is also noted from the present study and many other studies that the species are of very importance from nutritional point of view (Khan *et al*., 2005c; Ahmad *et al*., 2008b). The leguminous pastures are a very good source of food and energy and animals feeding on them can maintain higher growth and disease resistance (Bowman & Asplund, 1988; Orr & Treacher, 1989; Ahmad *et al*., 2008b).

It may be concluded that the studied plant species are palatable and contain appreciable amount of energy for livestock grazing thereupon.

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(Received for Publication 9 January 2008)