

EVALUATION OF MICRO MINERALS COMPOSITION OF DIFFERENT GRASSES IN RELATION TO LIVESTOCK REQUIREMENTS

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Abstract

Four types of forages Bermuda grass (*Cynodon dactylon*), Bahia grass (*Paspalum notatum*), Star grass (*Hypoxis hirsute*), and Guinea grass (*Panicum maximum*) were collected from the Livestock Experimental Station Rakh Khaire Wala, located in the semi-arid region of central Punjab, Pakistan during two different seasons of 2003. Samples were collected and analyzed for iron (Fe), copper (Cu), zinc Zn), manganese (Mn), and selenium (Se) at eight sample dates after every two weeks during two consecutive seasons. No difference was found between winter and summer for forage Fe, Cu, Zn, Mn, and Se. Forage Cu concentrations increased in summer for Bahia grass from 20.3 to 23.1 µg/g. This species had the highest zinc concentrations 90.8 µg/g in winter and had the highest forage Fe and Cu concentrations 130.0 and 23.1 µg/g, respectively in summer. Star grass had the highest Mn concentrations 250.8 µg/g in winter and its Se concentrations increased in summer from 0.033 to 0.042 µg/g. Forage Se had the greatest increase in Guinea grass from 0.028 to 0.049 µg/g in summer. The summer season did not show difference for concentrations of the five micro-nutrients. It is concluded that there is not a significant variation in micro-nutrient status of the forage due to seasonal changes. Only the forage Se concentrations increased in summer. However, the Se level was still insufficient to meet the requirements for grazing ruminants; while other four micro-nutrients in these forages were sufficient to meet the requirements.

Introduction

Large variations in mineral content of different plant species growing on the same soil have been reported (Underwood 1981; Gomide *et al.*, 1969). Kaynogo-Male & Thomas (1975) demonstrated a wide range of nutrient concentrations among tropical grasses grown in Puerto Rico. As an example, large variations in concentrations were found for Mg (0.11-0.78%) and Cu (13-97 µg/g) in the various plant genera. *Brachiaria decumbens* doubled its P content when soil P was doubled, but P content of other pastures species such as *Sporobolus* spp., did not vary widely (Gomide, 1978).

Generally, legumes have higher amounts of Cu, Zn, Mo and Co than grasses whereas the reverse is true for Se (Fleming, 1973). Seasonal variations in micro-nutrient content of tropical grasses have been reported in some studies. For example, Gomide (1978) studied N, K, P, Ca, Mg, Mn, and Zn content of five tropical grasses of Brazil at different stages of maturity. It was concluded that as forages reached maturity, nutrient content declined for most minerals, and that K and P could be deficient and Zn could be borderline, for grazing cattle. In most circumstances, P, K, Mg, Na, Cl, Cu, Co, Fe, Zn, and Mo decline as the plant matures (Gomide *et al.*, 1969; Underwood, 1981).

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Climate limits the yield potential and responses to micro-nutrient fertilization. Where climate varies markedly between seasons, large seasonal variations in nutrient uptake have been observed. Under moist and warm temperatures of the wet tropics and semi-tropics, solubility of nutrients in soil is low (Allman & Hamilton 1949). Grass tetany primarily occurs in the cool season. Also the prevalence of Zn deficiency in cool and wet seasons has been associated with decreased solubility of soil Zn (Reuter, 1975).

Seasonal variation affects livestock seasonal production in different regions of the world by affecting forage dry matter accumulation (Arizmendi-Maldonado *et al.*, 2001a). Seasonal forage yield and nutrient contents stability may be achieved through successful breeding programmes (Mislevy *et al.*, 1999). It is important to know the micro-nutrient concentrations of new varieties because forage is the primary source of nutrients for livestock in rangelands (McDowell, 1992) and because micro-nutrients present in the soil are transported to livestock through the forages on which they feed. Generally, Fe and Se concentrations increase as the seasonal forage yield increases (Espinoza *et al.*, 1991; Santana & McDowell, 1994). In contrast Cu, Zn, and Mn contents were not affected by seasonal forage yield (Gomide *et al.*, 1969; Santana & McDowell, 1994; Arizmendi-Maldonado *et al.*, 2001b).

The purpose of this study was to evaluate the seasonal variations in the concentrations of the micro-nutrients Fe, Cu, Zn, Mn, and Se in four grass species under irrigation in a semi-arid region of Pakistan. This information will lead to a better understanding of the likely micro-nutrient needs of grazing ruminants during particular seasons. Because of imbalanced nutrient availability, different physiological disorders and diseases are being observed in the livestock of this region.

Materials and Methods

The study was conducted with four different forage grasses during two seasons (summer and winter 2003) at the Livestock Experimental Station, Rakh Khiare Wala at 30.85 N latitude and 71.65 E longitude, on a sandy loam soil in the Leiah district, Punjab Province, Pakistan (Fig. 1). During winter and summer, day and night temperatures, relative humidity and temperature humidity index were recorded (Table 1). Forage plots were supplied recommended irrigations (70 mm water per irrigation) with canal water having EC 0.75 dS m⁻¹ and pH 7.5. The average rainfall during the experimental duration was 180 mm. Four soil samples from each plot were taken each at 15, 30 and 60 cm depths and mixed for soil analyses (Anon., 1954; Jackson, 1962) and soil characteristic are summarized in Table 2.

The experimental design was a spilt plot with season as main plots and sampling date as sub-plots. Forage samples were collected eight times, once every two-weeks during the experimental period. The harvests were collected from four locations each containing four different types of grass forages in the pasture. At each location, 4 replicates were harvested (1 m²) and sorted according to forage species during two consecutive seasons (winter and summer) and the sites where plant samples had previously been collected were avoided at each harvest. The forage samples were clipped to a height of 3-6 cm with a stainless steel knife and were placed in clean cloth bags and given a rapid wash with tap water followed by glass-distilled water to remove soil particles. The forage samples were oven dried at 60°C for 48 h and subsequently ground with a Wiley mill to pass through a 1 mm stainless steel sieve. To prepare samples for micro-nutrient determinations, 1 g of ground sample was heated in 10 ml HNO₃ and HClO₄ (3:1 ratio) at 250°C until the tissue was completely digested. The digested material was diluted to 50 ml with distilled water and stored at 4°C



Fig. 1. Location of experimental site at Rakh Khair Wala near Leiah, Punjab, Pakistan.

Table 1. Meteorological conditions (Mean \pm SD) recorded during the experimental periods.

	Winter				Summer		
	Temperature (°C)	Relative Humidity (%)	THI		Temperature (°C)	Relative Humidity (%)	THI
Day	11.8 \pm 2.9	67.9 \pm 19.5	53.7 \pm 4.6	Day	47.9 \pm 4.8	41.8 \pm 12.3	79.4 \pm 2.7
Night	6.8 \pm 3.9	85.6 \pm 13.7	44.6 \pm 6.3	Night	21.8 \pm 4.6	52.2 \pm 12.6	55.5 \pm 5.8

THI is the Temperature Humidity Index calculated as follows:
 THI=0.4(dry-bulb thermometer temperature °F + Wet-bulb thermometer temperature °F)+15

Table 2. Mean soil micro-mineral concentrations, pH, and organic matter for winter and summer seasons.

Variable	Winter	Summer
Soil texture	Sandy-loam	Sandy-loam
ECe (dS m ⁻¹)	1.45	1.67
PH	6.9	5.8
Organic matter (%)	5.2	6.3
Cu (mg/kg)	5.4	8.7
Fe (mg/kg)	16.5	12.8
Mn (mg/kg)	12.6	18.2
Zn (mg/kg)	6.5	20.9
Se (mg/kg)	0.077	0.055

(Anon., 1990). Copper, Fe, Mn, and Zn concentrations were determined using an atomic absorption spectrophotometer (Pye Unicam Ltd. York Street, Cambridge UK), whereas Se was determined by the fluorometric technique as described by Whetter & Ullrey (1978). As the variation in second and third fortnights was non-significant, data for only the first and last fortnights of each season were included and discussed in this paper.

The data were analyzed using Analysis of Variance (Table 3) for a split-plot design (Steel & Torrie 1986). For those variables which were significant ($p \leq 0.05$), the means were separated using Fisher's Least Significant Difference test (LSD).

Results

Day and night temperatures were 47.9/21.8°C with relative humidity 41.8/52.2% during the summer, and 11.8/6.8°C with relative humidity 67.9/85.6 % during the winter at the experimental site (Table 1). The rainfall during the experimental period was 180 mm.

The soil analysis data showed that pH of the soil decreased while ECe and organic matter increased with time (Table 2). Similarly variations in micro-nutrient (Cu, Fe, Mn, Zn and Se) concentrations with time were also recoded in soil extracts. The copper (Cu), Mn, and Zn increased and Fe and Se of the soil decreased with time (Table 2).

The grass species had similar seasonal Fe concentrations (Table 3). Bahia grass and Guinea grass showed the highest Fe ($p \geq 0.05$) contents (118.7 and 107.7 µg/g, respectively) (Table 4). There were no differences in Fe concentrations among the four grass species during winter, but differences emerged in summer (Table 5). Generally, Fe content remained declined as the season advanced (Table 6). Bahia grass showed a decrease in Fe concentration with time during both seasons from 133.9 to 81.0 µg/g and from 156.0 to 103.9 µg/g (Table 6). The Fe content of Guinea grass decreased as winter season advanced (127.9 to 85.2 µg/g), whereas, Fe content of Bermuda grass decreased as summer season advanced from 113.0 to 72.9 µg/g (Table 6).

There was a significant seasonal difference in Cu content of the four grass species (Table 3). Bahia grass had the highest Cu concentration (21.7 µg/g), whereas, Bermuda grass, Star grass and Guinea grass had similar concentrations (Table 4). The summer Cu content (23.1 µg/g) of Bahia grass was higher in summer than that in winter (20.3 µg/g). Other species had similar seasonal trend (Table 5). Mainly, Cu contents during the late sampling dates were lower than those of early sampling dates (Table 6).

Zinc contents significantly varied during summer and winter (Table 3) in grass species. Bahia grass (88.5 µg/g) and Bermuda grass (83.5 µg/g) had the highest Zn concentrations than others. Whereas, Star grass had the lowest (61.5 µg/g) Zn concentration (Table 4). Bahia grass had the highest Zn concentration during winter and summer (90.7 and 86.3 µg/g, respectively). Star grass had the lowest Zn concentration during winter and summer 63.8 to 59.2 µg/g, respectively (Table 5). Zinc concentration decreased in late sampling dates during both seasons in all grass species (Table 6).

There was a significant seasonal difference in Mn content of the four grass species (Table 3). Star grass had the highest forage Mn concentration (243.9 µg/g), whereas, Guinea grass had the lowest Mn concentration of 115.0 µg/g (Table 4). During winter season Star grass had the highest Mn concentration 250.8 µg/g, same was the case during summer 237.0 µg/g, while the minimum Mn concentration was in Guinea grass during both the seasons 114.1 and 115.9 µg/g (Table 5). There was a decrease in the Mn concentration between the first and last samplings during both winter and summer seasons for Bahia grass, and Bermuda grass Guinea grass, respectively (Table 6), except Star grass where Mn increased in last sampling dates during summer.

Table 3. Analysis of variance of data for micro-nutrient concentrations in different forages at different sampling periods (fortnights) during winter and summer seasons at semi-arid goat ranch

Source of variation	Degree of freedom	Mean squares				
		Copper	Iron	Zinc	Manganese	Selenium
Season (S)	1	17.535**	24.503 ^{NS}	236.006**	1636.20**	0.001**
Error	3	0.999	7.555	0.960	2.562	0.000
Fortnight (FN)	1	191.476**	17969.40**	786.101**	33470.702**	0.000 ^{NS}
S x FN	1	4.358 ^{NS}	14.822	4.254 ^{NS}	95.063*	0.000 ^{NS}
Error	6	5.581	0.347	2.141	11.48	0.000
Grass (G)	3	249.916**	1440.891**	2514.53**	49317.47**	0.000 ^{NS}
G x S	3	8.371 ^{NS}	934.511**	7.418 ^{NS}	280.809**	0.000**
G x FN	3	4.726 ^{NS}	821.577**	80.003**	4476.91**	0.000**
G x S x FN	3	3.51 ^{NS}	710.451**	39.429**	337.469**	0.000 ^{NS}
Error	36	10.651	10.789	6.196	13.087	0.0000028
Total	63					

*, **, *** = Significant at 0.05, 0.01, and 0.001 levels respectively.

NS = Non-significant.

Table 4. Means of forage Mineral concentrations (µg/g) averaged over two seasons.

Forage	Mineral				
	Fe	Cu	Zn	Mn	Se
Bermuda grass	98.40 c	12.9 c	83.5 a	151.5 c	0.033 b
Bahia grass	118.70 a	21.7 a	88.5 a	192.8 b	0.041 a
Star grass	99.05 c	14.4 bc	61.5 c	243.9 a	0.037 ab
Guinea grass	107.70 b	14.8 b	68.8 b	115.0 d	0.039 ab

Means with different letters differ significantly according to Fisher's least significant difference at $p \geq 0.05$ **Table 5. Mineral concentrations (µg/g) during summer and winter seasons.**

Season/Forage	Mineral				
	Fe	Cu	Zn	Mn	Se
Winter					
Bermuda grass	103.8b	12.5 d	85.5 ab	156.2 c	0.029 c
Bahia grass	107.45ab	20.3 b	90.7 a	201.8 b	0.038 bc
Star grass	103.5b	14.5 cd	63.8 cd	250.8 a	0.038 b
Guinea grass	106.5b	14.1 cd	69.7 c	114.1 d	0.028 c
Summer					
Bermuda grass	93.0b	13.4 cd	81.0 b	146.8 c	0.037 bc
Bahia grass	130.0a	23.1 a	86.3 ab	183.7 b	0.045 ab
Star grass	94.6b	13.7 cd	59.2 d	237.0 a	0.036 bc
Guinea grass	108.8ab	15.6 c	67.9 c	115.9 d	0.049 a

Means with different letters differ significantly according to Fisher's least significant difference at $p \geq 0.05$

Significant difference in forage Se concentrations during winter and summer seasons were recorded (Table 3). Similarly, Bahia grass, Star grass and Guinea grass had similar values for Se (0.041, 0.037 and 0.039 µg/g, respectively) and Bermuda grass was the lowest in forage Se concentration 0.033 µg/g (Table 4). Guinea grass had the highest forage Se concentration during the summer season 0.049 µg/g (Table 5). Although all forages had a decrease in Se concentration with sampling dates during both the seasons but only Guinea grass had a significant increase from 0.036 to 0.049 µg/g (Table 6) during summer. Generally, Bahia grass contained the highest Fe, Cu, Zn, and Se concentrations.

Table 6. Means of forage micro-nutrient concentrations ($\mu\text{g/g}$) during winter and summer seasons at 1st and last samplings dates.

Season/ Forage	Date	Mineral				
		Fe	Cu	Zn	Mn	Se
Winter						
Bermuda grass	1 st	103.7 bcde	14.9 def	89.9 ab	184.2 cd	0.031 c
	Last	103.9 bcde	10.0 h	81.3 bcd	128.2 efg	0.032 bc
Bahia grass	1 st	133.9 ab	21.4 ab	96.0 a	242.6 ab	0.035 b
	Last	81.0 de	19.3 bc	85.5 ab	161.0 d	0.029 c
Star grass	1 st	120.9 bc	15.8 cde	65.1	256.4 a	0.036 bc
	Last	86.1 cde	13.1 efgh	62.5 ef	245.2 ab	0.036 bc
Guinea grass	1 st	127.9 ab	17.4 cd	71.9 de	136.1 ef	0.029 c
	Last	85.2 de	10.9 gh	67.5 ef	92.2 h	0.028 c
Summer						
Bermuda grass	1 st	113.0 bcd	15.1 def	89.4 ab	185.7 cd	0.035 bc
	Last	72.9 e	11.7 fgh	72.7 cde	107.9 fgh	0.038 bc
Bahia grass	1 st	156.0 a	24.6 a	88.6 ab	217.9 fgh	0.036 bc
	Last	103.9 bcd	21.6 ab	84.1 bc	150.4 c	0.030 c
Star grass	1 st	102.7 bcde	14.9 def	59.6 f	231.0 ab	0.045 ab
	Last	86.5 de	12.5 efgh	58.8 f	242.9 ab	0.043 ab
Guinea grass	1 st	123.5 bc	17.0 cd	72.0 de	137.0 ef	0.036 bc
	Last	94.2 cde	14.2 defg	63.8 ef	94.8 gh	0.049 a

Means in column with different letters differ significantly according to Fisher's least significant difference at $p \geq 0.05$

Means in column with different letters differ significantly according to Fisher's least significant difference at $p \geq 0.05$

Discussion

It is a common observation that ECe and organic matter increased with time in cultivated soils (Ashraf *et al.*, 2006) and finding of present study are in agreement. The enhancement in ECe may be due to irrigation with water having EC of 0.75 dS m^{-1} and due to seasonal variation because in summer evapotranspiration rate of soil was very high and all soluble salts accumulated in the upper layers of the salts (Ashraf *et al.*, 2005). The soil chemical analysis confirmed this statement that out of five tested micro-nutrient, three (Cu, Mn, Zn) significantly increased. The reduction in soil pH and increase in organic matter in the present studies may be due the root activities of the grasses cultivated in winter on this soil. Ashraf *et al.*, (2006) also reported that with constant cultivation the soil organic matter increased and pH decreased.

It has been earlier reported that Fe deficiency is rare in grazing livestock due to a generally adequate content in soils and forages together with contamination of plants by soil (McDowell *et al.*, 1984). The soil contains 20-100 times the Fe content found in pastures grown on a particular soil (Khan *et al.*, 2006). In many parts of the world, under grazing conditions, annual ingestion of soil can reach 75 kg for sheep and 600 kg for dairy cows (Healy, 1974). Acid soil conditions favour availability and plant uptake of Fe. Even plants grown on neutral or slightly alkaline soils often contain quite high levels of Fe. In this experiment, Fe content during both seasons was sufficient for the maintenance and production-requirements of grazing ruminant livestock (Anon., 1984).

Forage Cu concentrations were found to be sufficiently high to meet the demand of animals during both seasons. Forage Cu values found in this study were not higher than

the critical limits, but these values were higher than those reported by Tiffany *et al.*, (2001) in north Florida, and Espinoza *et al.*, (1991) in Venezuela and central Florida. However, Cu values reported in the present study were similar to those reported from Indonesia (Prabowo *et al.*, 1990) and lower than those reported by Tejada *et al.*, (1985, 1987) in Guatemala. McDowell *et al.*, (1993) reported that Cu interacts strongly with trace minerals and macro-nutrients for absorption by the plants. Calcium in the form of carbonate precipitates Cu, making it unavailable for the plants. In addition, the Cu content is inversely related to increasing plant maturity (McDowell *et al.*, 1983).

Forage Zn concentration was higher than the Zn requirements of ruminants during winter. However, the level in summer was considered slightly deficient for growing and lactating animals (Prabowo *et al.*, 1991; Reuter & Robinson 1997; Tiffany *et al.*, 2001). Cox (1973) reported the low level of Zn in soil and plants. Plant maturity has also been reported to affect Zn concentration of forage and it also depends upon the tissue type of plants (Underwood, 1981; Kabata-Pendias & Pendias, 1992). The Kenya standing committee on Agriculture (Corbett, 1990) indicates that Zn requirement is approximately 25 µg/g of dry matter. The summer and winter values for Zn exceed this considerably. But according to NRC (Anon., 1984) the required Zn during summer is 70- 75 µg/g dry matter and so the values for Zn in summer were within the referenced NRC.

Forage Mn levels were above the critical level during both seasons and were sufficient to meet the requirements of animals. Similar levels of forage Mn with seasonal fluctuations have already been reported in Nicaragua (Velasquez-Pereira *et al.*, 1997) with no seasonal effects in Indonesia (Prabowo *et al.*, 1990) and Guatemala (Tejada *et al.*, 1987).

Forage Se concentration was not sufficiently high during winter, whereas during summer these were adequate for the normal Se requirement of animals. The Se contents of the 4 forage species were not different significantly and were slightly higher in summer than those in winter. There was some positive association between soil and forage Se contents found during both seasons. Mean forage Se concentrations found in this study were similar to those reported for Guatemala (Tejada *et al.*, 1987), Colombia (Pastrana *et al.*, 1991), and for Indonesia (Prabowo *et al.*, 1991) in different forage species. Underwood (1977) stated that the differences in Se concentrations between accumulator and non-accumulator plants are marked, but differences among non-accumulators may be minor. It appeared that the site of the farm having goats was the region with high Se deficiency problem particularly in summer when forage contained Se on borderline deficient levels.

Most micronutrients tended to be similar regardless of season and the differences were not statistically significant. Larger differences in nutrient concentrations were found between 1st and last sampling periods within both seasons. A similar general trend for a reduction in micro-nutrients concentration with increasing plant maturity has been previously reported by some researchers in different parts of the world (Arizmendi *et al.*, 2001a,b; Faria-Marmol, 1983; Predomo *et al.*, 1977; Underwood, 1981).

The forages during this study were higher in micro-nutrients than those reported in forages grown on infertile soils in Venezuela (Feria-Marmol, 1993; McDowell *et al.*, 1984, 1987), and in Indonesia (Little, 1989). Similar significant variations for sampling periods among some genera and species of forages in the concentrations of Co, Cu, Fe, Mo, and Se as found in this study were reported in Puerto Rico (Ramos-Santana & McDowell, 1993), Uganda (Kayongo-Male & Thomas 1975; Reid *et al.*, 1979),

Guatemala (Tejada *et al.*, 1985), Costa Rica (Vargas *et al.*, 1992) and Trinidad (Youssef & Brathwaite, 1987). These variations in the micro-nutrient concentrations of forages reflect differences in nutrient uptake (Reid & Horvath, 1980). When the supply of nutrients is marginal, it is useful to select forages capable of extracting and supplying the required amount of the nutrients for the grazing ruminants (Youssef, 1988).

The Se level in the forages studied may explain the poor reproductive performance and retained placenta in grazing animals at the Livestock Experimental Station where the forages studied were grown. Possibly, because SAC (The Society of Core Analysts) suggests a level of 1 µg/g of dry matter.

There may be no practically significant differences in micro-nutrient concentrations between winter and summer seasons for all the forage species studied. All the forages would generally meet the micro-nutrient requirements for grazing ruminants in this ranch for Fe, Cu, Zn, and Mn. Selenium, however, was extremely deficient in all forages being grazed at this region. Therefore, there is a need for micro-nutrient supplementation of Se for grazing animals in this semiarid region.

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