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FORAGE MINERAL STATUS EVALUATION: THE INFLUENCE OF PASTURES

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Abstract

Information is lacking on mineral nutritive potential of native pastures in arid pastures in different regions of Pakistan. An experiment, using wet nitric acid (HNO_3) digestion followed by an atomic absorption spectroscopy (AAS), was carried out to measure the levels of macro and micro minerals of herbage forages of a grazing pasture of Leiah district of south-western Punjab, Pakistan. The grazed forages had different concentrations of Ca and Mg, respectively, and were slightly higher than the minimum recommended levels in the diets of ruminants. Content of micro minerals varied among the grazing pasture forages. The forages had lower levels of Cu and slightly low to moderate contents of Zn as compared to recommended dietary requirements of ruminants. The feeds had moderate to high. The forages had levels of Fe considerably in excess of requirements. In order to optimize livestock productivity in ruminants fed on the pasture forages, specially, during winter or dry seasons, there is a need to supplement with micro mineral sources.

Introduction

All plants depend upon the soil for their supply of mineral nutrients, and grazing ruminant animals obtain the majority of their mineral nutrients from plants grown on these soils. Concentrations of mineral elements in forage are dependent upon the interaction of a number of factors, including soil, plant species, stage of maturity, yield, pasture management and climate (McDowell *et al.,* 1983). Most naturally occurring mineral deficiencies in herbivores are associated with specific regions and are directly related to soil characteristics (Underwood, 1981: McDowell *et al.,* 1983). When mineral nutrients in herbage are marginal in respect of animal requirements, changes in concentrations brought about by climatic, managerial or seasonal influences as well as plant maturity can be significant factors in incidence or severity of deficiency states by livestock wholly or largely dependent on these plants (Underwood, 1981).

In Pakistan, as in the rest of the Asia, forages still serve largely as a source of essential elements for grazing animals. Little coordinated research has been done in these areas to identify places of endemic mineral deficiencies and toxicities. Little information is available on the mineral status of grazing animals and of the forages upon which they subsist. Since the mineral status of these forages is a function of multiple factors which interact with one another to produce varied effects, it is vital to investigate how such factors influence the mineral status of different forages in a tropical environment. Many other studies have been conducted in different regions of the world on dry matter yield, nutritive value and persistence of different forages for animals, but very little information is available in the literature on the effect of some management practices on their mineral concentrations, especially on trace elements (Faria-Mamol *et al.,* 1997).

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Mineral concentrations in forage vary widely among species, and are influenced by many soil and other factors including growth stage, plant part, climate and fertilizer application (Minson, 1990, Judson & McFarlane, 1998). Mineral deficiencies or imbalances in soils and forages have long been held responsible for low production and reproduction problems among grazing animals (McDowell, 1985). In some countries a number of diseases severely inhibit animal production, with mineral deficiencies and imbalances suggested as at least part of the etiology of these conditions (McDowell, 1987).

Ruminant production during winter seasons in the sub-tropics including semi-arid areas of central and south-western Pakistan is limited by low productivity of pasture forages, which often contain too low mineral concentrations to meet the minimum requirement for optimal productivity of livestock (McDowell *et al.*, 1993, Annison & Bryden, 1998). Utilization of low quality roughages such as hays, straws and stover could also be further limited by their low contents of macro and micro minerals due to their effects on rumen microbial growth and activity, leading to lowered feed digestibility (Leng 1990 Judson & McFarlane, 1998). To alleviate feed shortages during winter seasons, some improved varieties of forages have been introduced recently to maximize the animal productivity in Pakistan for winter season livestock feeding for enhanced supply of fodder for livestock. The native and these improved species of forages and crop residues constitute the sole source of feeds for ruminants in central and south-western part of Punjab, province during winter seasons, which are characterized by low animal productivity (Vargas & McDowell, 1997; Rubanza, 1999).

Efforts made to enhance livestock productivity in semi-arid and arid areas of Pakistan include planting of promising exotic forages of different varieties and in native species. For example, supplementation of grazing animals with these improved forages could only overcome weight losses (Vargas & McDowell, 1997, Rubanza *et al.*, 2004). Information is also limited on mineral nutritive potential of these winter season feed resources. Therefore, there is a need to establish levels of mineral of these conventional feed resources in order to predict ruminant production responses in these arid and semi-arid areas of Pakistan.

Minerals are vital for normal growth, reproduction, health and proper functioning of the animal's body (McDowell, 1992). Minerals protect and maintain the structural components of the body, organs and tissues, and are constituents of body fluids and tissues as electrolytes. Minerals catalyze several enzymatic processes and hormone systems (Underwood & Suttle 1999), maintain acid-base balance, water balance and osmotic pressure in the blood and cerebral spinal fluids

Under pasture systems, animals depend on forages to satisfy all of their nutritional requirements. Unfortunately, forages often do not provide all of the needed minerals, which animal require throughout the year. Many incidences of mineral inadequacies in forages and soils have been reported which are principal causes of reproduction failure and low production rate (McDowell, 1985; McDowell *et al.*, 1993; Vargas & McDowell, 1997). Mineral deficiencies likely to affect production of grazing livestock at pasture in most of the regions of the world include those of the major elements Ca, P, Mg, Na, S, and the trace elements Co, Cu, I, Mn, Se, and Zn (Little, 1982; Judson *et al.*, 1987; Judson & McFarlane, 1998).

There is scanty information on the mineral nutritive potential of forages in this region of Pakistan despite of its importance in livestock production, and very little has been done to establish levels of minerals of these feed resources. Knowledge on mineral composition of improved pasture forages would form base-line data on mineral status of available feed resources for enhanced nutrition of grazing ruminants in arid and semi-arid areas of south-western Punjab, Pakistan. Analysis of forages for mineral composition is important for obtaining mineral status of an area with a view to provide mineral supplements to grazing animals. Adequate information on soil characteristics and mineral composition of forages in the province of Punjab is lacking despite the importance of this region to livestock production.

Content of minerals of native forage species that grow naturally in the pasture has already been established (Khan, 2003) This study was therefore conducted to assess the mineral nutritive potential of improved varieties of forages grazed by ruminants in the pastures in different districts of south-western Punjab, Pakistan.

Materials and Methods

Pasture description

This investigation was conducted in Leiah district of Punjab Province of Pakistan (located between 23° and 36° N latitude and 60° and 75° E longitude). Leiah district is located within an arid zone that receives low uni-modal rainfall of 300-850 mm per annum between mid July through mid August. Minimum and maximum temperatures during summer and winter as well as relative humidity during summer have been presented in Table 1. Leiah district is characterized by small sandy mountains with sandy and loamy. Vegetation is purely savanna, characterised by short grasses with scattered shrubs and trees, which are dominated by *Acacia* spp., *Prosopis* spp., *Zyzyphus* spp., *Salvadora* spp., and sown varieties of forages such as, *Panicum* spp., *Andropogon* sp., *Pennisetum typhoideum, Setaria* Sp., *Medicago sativa, Trifolium alexandrium, Hordeum vulgare, Cichorium intybus, Cynodon* spp., like Vernal grass, imported Velvet grass, Tall fescue, Orchard grass, Molasses grass, Elephant grass, Pangola grass, and Jaragua grass *etc.*

Table 1. Meteorological conditions (Mean ±SD) of climate parameters recorded during the experimental periods.

Lighting condition	Winter			Summer		
	Temperature (^{o}C)	Relative humidity (%)	THI	Temperature (^{o}C)	Relative humidity (%)	THI
Day	11.8±2.9	67.9±19.5	53.7±4.6	47.9±4.8	41.8±12.3	79.4±2.7
Night	6.8±3.9	85.6±13.7	44.6±6.3	21.8±4.6	52.2±12.6	55.5±5.8

Pasture selection and forage sampling

Livestock Experimental Station of Leiah district was selected for forage vegetation sampling and study of forage nutritive value based on its high population of cattle, sheep and goats, and due to its characteristic winter or dry season forages shortages (Khan, 2003) A total of six grazing pastures (A, B, C, D, E & F) of Leiah district, were selected and demarcated for the forage nutritive study to represent the rest of the districts of south-western, Punjab, Pakistan. The grazing pastures were selected to represent the animal feed in the pastures. Forage samples, which were used for the mineral study, were harvested from three-places in each of randomly selected six grazing pastures of region in early December *i.e*., early winter season in 2005 up to March, 2006. A total of 120 samples *i.e.* 20 samples per grazing pasture; 15 samples per sampling during each season, were harvested from the six grazing pastures using 0.25 m^2 quadrates selected at random at five places in each of three places in each pasture. The forage samples consisted of grasses legumes, and crop residues and hay.

Each of the composite forage samples came from five sub-samples of the same predominating forage species that was most frequently grazed by sheep on the farm. Forages were collected fortnightly, after careful observation of sheep grazing pattern. The forage samples were clipped to a height of 3-6 cm, from the ground to simulate the grazing behaviour of animal. Representative samples of the forages then were placed in polyethylene bags at the laboratory where they were given a rapid wash with tap water followed by a distilled water to remove any soil which was present. Forage samples were placed in clean cloth bags for air drying. The samples of forages were dried in an oven at 60^oC for 48 h. Soil samples were randomly taken from the different pastures during different seasons to assess soil mineral levels and prepared for mineral analysis as described by Black (1965). The mineral concentrations in the extracts were determined using the same procedures described above.

Analysis of minerals

Mineral contents of forages were determined by wet ashing using a digestion method as described by Mullis *et al.*, (2003) and Fick *et al.*, (1979). Forage samples (0.5 g) were weighed into Teflon-lined digestion vessels to which 5 ml of HNO_3 was added, and were allowed to digest for 25 min., in a CEM MDS-2000. The microwave oven was set at four times and pressure phases of 40 PSI for 5 min (step 1); 80 PSI for 5 min; 120 PSI for 5 min (step 3) and 160 PSI for 10 min (step 4). After cooling, the samples were filtered through an ash-free filter paper, and diluted in ion exchanged distilled water to a final volume of 50 ml. Mineral contents were detected by atomic absorption spectrophotometry (AAS) (Anon., 1980). The samples were analyzed for macro minerals: calcium (Ca), magnesium (Mg) and micro minerals: iron (Fe), zinc (Zn), and copper (Cu).

Statistical analysis

The data thus obtained during the study were analyzed by Statistical Analysis System (Anon., 1987). Forage samples were analyzed as split-plot design (Steel & Torrie, 1980), with pasture as the main plot and sampling periods as the sub-plots. Significance levels ranged from 0.05-0.001 for statistical analyses. Differences between means were ranked using Duncan's New Multiple Range Test (Duncan, 1955).

Results

The results on soil chemical composition indicated the soil pH as acidic (Table 2). Soil exchangeable magnesium concentrations were higher ($p<0.01$) during the dry season than during the wet season. Available Ca, and Zn were lower ($p<0.01$) during the dry season than during the wet season.

Table 2. Chemical composition of soils from experimental site at different periods.

Variables S	Sampling periods				
	Early wet season	Late wet season	Late dry season	Mean	SE
Soil pH	5.5	5.4	5.9	5.6	0.18
Ca (g/g)	1611.7[a]	1452	1372.3[b]	1478.7	87.08
Mg (g/g)	241.3[b]	251.3[b]	287.7[a]	260.1	17.4
Fe (g/g)	275[a]	300	225[b]	266.7	27.28
Zn (g/g)	70[a]	32.5[b]	12c	38.2	17.30
Cu (g/g)	7.1	6.4	6.1	6.5	0.37

a, b, c in the same row bear different superscripts having different (p<0.01).

Forages harvested from grazing pasture -A had the lowest (p<0.001) Ca (7.1 g kg^{-1} DM) compared to forages harvested from D (8.8 g kg^{-1} DM) and effect of sampling time was also significant (p<0.05; Table 3).

Table 3. Content of macro-and micro-minerals of pasture grazing land forages of a specific animal ranch.

Pasture	Ca	Mg	Cu	Fe	Zn
	g kg^{-1} DM		mg kg^{-1} DM		
A	7.1	2.8	6.8	812	34.8
B	6.4	2.9	5.6	452	33.99
C	7.2	2.3	5.9	635	28.50
D	8.8	1.9	6.3	225	28.00
E	7.6	2.6	5.8	428	30.6
F	7.5	1.9	6.6	672	35.7
Mean	7.43	2.4	6.1	534	31.9
SEM	0.82	0.38	0.75	218	4.50
Significance	P***, S*, PXS**	P***, S**, PXS^{ns}	P^{ns}, S^{ns}, PXS**	P***, S^{ns}, PXS^{ns}	P***, S**, PXS**

P = pasture, **S =** sapling period, **P x S =** pasture and sampling period interaction.

The grazing pasture forages had variable (P<0.001) levels of Mg that ranged from 2.6 (E) to 2.9 g kg^{-1} DM in forages harvested from B, which had (P<0.01) highest levels of Mg (Table 3). There was no difference in the content of Mg between forages harvested from pasture-A and pasture-B as compared to the rest of the grazing pasture forages. Both the effects of pasture and sampling periods were found to be significant.

There was no significant pasture difference (P>0.001) in the content of Cu among the grazing pasture forages (Table 3). Copper content ranged from 5.9 to 6.6 mg kg^{-1} DM (pasture-C and F, respectively). Forages harvested from pasture-D had the lowest Fe (225.0 mg kg^{-1} DM) as compared to pasture-A, which had the highest (P<0.001) level of Fe (812 mg kg^{-1} DM). There was no difference (P>0.05) in the content of Fe among grazing pasture forages. except pasture-A among sampling period. Content of Zn varied from 28.50 (Pasture-C) to 35.7 mg kg^{-1} DM (pasture-F) showing significance (p<0.001) of pasture and non-significance (p>0.05) of sampling time (Table 3).

Discussion

The investigated grazing pastures forages had slightly higher levels of Ca than the rain fed forage pastures (Minson, 1990; Evitayani *et al.*, 2004). Variations in the levels of Ca between findings in this study with values reported in the literature could be partly explained by different forage species, species composition, stage of maturity and season, and variations in soil characteristics due to location of the different grazing pastures at the ruminant ranch in this arid region of Punjab-Pakistan. Higher contents of Ca could probably be explained by proportion of forage species. Forages that were used in this study were harvested to include a mixture of grasses crop residues and legumes species that form the major portion of the diet consumed by grazing ruminants. In this region it could be assumed that legumes contain more minerals concentrations than would be in grass species crop residues and legumes during winter seasons, which are richer in minerals, including Ca (Minson 1990; Aregheore, 2002.) than stem fractions of grasses (Underwood & Suttle 1999). Mean forage Ca^{2+} concentrations were adequate and sufficiently higher than the requirements of ruminants. Forage Ca^{2+} requirements of grazing ruminants is a subject of considerable debate as the requirement is influenced by animal type and level of production, age and weight. Reuter & Robinson (1997) suggested Ca^{2+} requirement for maintenance, growing and lactating sheep to be 1200-2600 mg/kg. Thus the forage Ca^{2+} values found in this study was considered adequate for the optimum performance of ruminants. Similar forage Ca^{2+} values as found in summer were reported by Pastrana *et al*., (1991a,b) in Colombia, Tiffany *et al*., (2000, 2001) in North Florida, Espinoza *et al*., (1991) in Central Florida and Cuesta *et al.* (1993) in North Florida. It is generally recommended that diets of livestock should have Ca: P ratio of about 1:1 to 2:1 (Underwood, 1981). Livestock will tolerate dietary Ca:P ratios of more than 10:1 without any serious effect provided the P intakes are adequate (Ternouth, 1990). Temperate forages generally contain more Ca^{2+} than those grown in the tropics. However, hay from Ireland had a mean Ca^{2+} concentration almost similar to that found in this study during winter from the forage which was also similar to that reported by the Pennsylvania State Forage Test Service (Adams, 1975). The forage means level of Ca^{2+} was found higher than that the requirements of grazing ruminants. However, efficiency of Ca utilisation from these forages, and its bioavailability in ruminants, would depend on presence of adequate level of P, active form of vitamin D, and calcitonin and parathyroid hormone (PTH). Calcitonin and PTH mobilise conversion of vitamin D to its active form.

The pasture forages had lower levels of Mg than most species of grasses However, differences in the content of Mg in this study with those in the literature could be partly explained by differences between forage species, level of Mg in the soil, influences of locality and climate, growth stage, proportion of leaf and stem fractions collected for mineral analysis, and season when forage sampling was done. The grazing pasture forages had slightly higher levels of Mg than the recommended requirement (ARC, 1980; Islam *et al.*, 2003). These forages would therefore meet the theoretical requirement of Mg for beef cattle (Anon., 1996) and for lactating cows (1.2-2.1 g kg^{-1} DM), (Anon., 2001). These forages had also higher levels of Mg than the recommended requirements for growing lambs and lactating ewes and goats (Meschy, 2000). The high forage Mg^{2+} level in this study was above the requirement of ruminants as reported earlier (Anon., 1985). Similar values in winter forage Mg^{2+} were reported by Salih *et al*., (1983) in Florida.

According to Dua & Care (1995) the dietary Mg^{2+} availability to stock is markedly affected by other dietary components, especially K^{+}. High dietary levels of K^{+} and N will inhibit Mg^{2+} absorption from the rumen. Ca^{2+} and soluble carbohydrates may respectively increase and decrease dietary Mg^{2+} requirements of livestock, whereas raised dietary P levels appears to lower the requirements for both Ca^{2+} and Mg^{2+} (Judson & McFarlane, 1998). All grazing land forages except pasture-F had lower levels of Cu than the mean content of 6.1 mg Cu kg^{-1} DM of most species of grasses (Minson, 1990; Kumagai *et al.*, 1990).

Differences in Cu observed in the present study with literature values could be partly explained by genotypic differences, vegetative parts, stage of maturity, levels of available Cu in the soil and soil pH. Forages' Cu content declines with forage maturity, and is higher in leaf vs. stem fractions (McDowell 1996). The pasture forages had lower levels of Cu than the minimum recommended requirements of ruminants for different production purposes (Spears, 2003; Tudsri & Kaewkunya, 2002). Ruminants that depend on pasture forage feed as their sole feeds, for example, cattle especially during dry seasons, could be deprived of Cu (McDowell, 1996; Manyayu *et al.,* 2003). Copper-deficiency of grazing cattle, sheep and goats in these pastures could be further intensified by its reported low bioavailability already reported (Khan, 2003). Forage Cu^{2+} concentrations were found to be sufficiently low to meet the demand of animals during the study season. The forage Cu^{2+} had no relationship with soil Cu^{2+} levels during the study season which has already been studied in these pastures in Pakistan (Khan, 2003). Forage Cu^{2+} values found in this study were not sufficiently higher, but were within the range and higher than those reported previously in north Florida (Tiffany *et al*., 2001); Venezuela (Rojas *et al.,* 1993), and central Florida (Espinoza *et al.,* 1991).

These values were similar to those reported for Indonesia (Prabowo *et al.,* 1990) and lower than reported by Tejada *et al.* (1985, 1987) in Guatemala. Low forage Cu^{2+} in this study may have been due to its interaction with other elements in soil. McDowell *et al*., (1993) reported that Cu^{2+} interacts strongly with trace minerals and macro minerals for absorption by the plants. Fe^{2+} and Ca^{2+} are some of the elements that could have had an effect on the absorption of $Cu^{2+,}$ because the concentrations of these elements were very high, observed in this work. Ca in the form of carbonate precipitates Cu^{2+}, making it unavailable for the plants. In addition, the content of this element often is inversely related to increasing plant maturity, possibly one of the causes of low levels of Cu^{2+} in forage (McDowell *et al.,* 1983). Trace mineral concentrations are affected by four interdependent factors: 1) the genus, species or variety of crop, 2) type and mineral concentration of the soil, 3) stage of plant maturity, and 4) climatic or seasonal conditions. The following discussion is intended to give nutritionists and livestock producers a review of our current understanding on how these factors cause variation in trace mineral content of feeds.

High contents of Fe of the grazing land forages were comparable to high levels of Fe of most forages (100-700 mg kg^{-1} DM) reported by McDowell (1992). Variations in the contents of Fe among grazing land forages could be partly explained by forage species' differences and the influence of grazing lands on the level of Fe in the soil. All the grazing pasture forages had higher levels of Fe than the critical content of Fe in animal tissues (30-50 mg kg^{-1} DM). Differences in the contents of Fe between the grazing different pastures forages and literature values could be partly explained by variations in the content of Fe in the soil, and climatic conditions between localities. Forage Fe content is a function of forage species, soil Fe content, nature and type of soil on which forages are grown (McDowell, 1992). These forages had higher levels of Fe than the normal requirements of 30-60 mg Fe kg^{-1} DM of ruminants (Anon., 1980; McDowell, 1992). However, Fe bioavailability in ruminants would depend on feed mixture fed together and form of Fe in these feeds. Forage Fe^{2+} levels during the study season were sufficient for the requirements of ruminants for optimal performance. These levels of forage Fe^{2+} in the present study may support the reports of various researches who found similarly higher concentrations of Fe^{2+} with similar levels being higher in winter than that in summer in Guatemala (Tejada *et al*., 1987), North Florida (Cuesta *et al*., 1993), Nicaragua (Velasquez-Pereira *et al*., 1997) and Indonesia (Prabowo *et al*., 1991). The generally high forage Fe^{2+} found in this study is in agreement with the higher forage Fe^{2+} value (650 mg/kg) (Khan, 2003). This coincides with zero incidence of deficiency in soil samples. According to Kabata-Pendiaus & Pendias (1992) the changing conditions of soil and climate as well as physiological state of plants affect the Fe^{2+} absorption by the plants.

The pasture forages had slightly lower levels of Zn than mean content of required for ruminants (McDowell, 1985, Nasullah *et al.,* 2003, Serra *et al.,* 1997,). The Zn content in these forages could be sufficient for recommended requirement for sheep However, efficiency of Zn utilization of these forages would depend on zinc bioavailability, and its interaction with other mineral elements. Forage Zn^{2+} concentration was also found above the requirements of ruminants during this study in winter (Reuter & Robinson, 1997). Almost similar results were reported by Prabowo *et al*., (1991) in South Sulawesi Indonesia and Tiffany *et al*., (2001) in North Florida. A number of factors including soil, plant species, pasture management, and climate, may affect the likelihood of Zn^{2+} deficiency in ruminants. Cox (1973) reported the low level of Zn^{2+} in soil and plants. Plant maturity has also been reported to affect Zn^{2+} concentration of forage and it also depends upon the tissue type of plants (Underwood, 1981; Kabata-Pendias & Pendias, 1992; Church & Pond, 1988).

Conclusions

Forages harvested from selected grazing pastures of Leiah district of south-western Punjab-Pakistan had almost adequate levels of minerals for grazing ruminants. The pasture forages had adequate levels of macro minerals: Ca and Mg However, these pastures forages contained lower contents of Cu and Zn than the dietary requirements for most ruminants. Based on soil and forage analyses it is concluded that trace-minerals are needed for supplementation of grazing ruminants during the winter or dry season particularly when there is fall of these minerals profile of different forages at this specific region of Pakistan. These results would have application to the remaining Asian countries as well as other countries having similar ecological regions.

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