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A COMPARATIVE STUDY ON MINERAL STATUS OF BLOOD PLASMA OF SMALL RUMINANTS AND PASTURES IN PUNJAB, PAKISTAN

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Abstract

This paper reports the concentrations of some essential minerals (Mg, Cu and Zn) in soil, forage and blood plasma of grazing goats and sheep in two pastures (managed and unmanaged) in the south-western Punjab, Pakistan during two consecutive seasons of the year. The main aim was to evaluate and compare the mineral status of grazing ruminants on two different areas of the same farm in relation to seasonal variation. Mean mineral element concentrations in soil and forages in the managed pasture area tended to exceed levels over the unmanaged pasture area for almost all three investigated elements. Soil Mg and Cu concentrations were within the optimum range for active plant growth, but the levels of available Zn were below the critical limit. At both managed and unmanaged pastures, the effect of the season on both soil and forage mineral element concentrations were significant. The Zn concentrations in both summer and winter seasons in the managed pasture and Cu in the winter season were below the ruminants requirements, but Mg concentrations were above or within the recommended range. The plasma mineral profile of all animals fluctuated in relation to season and pasture. It was concluded that the mineral nutrition of the livestock at this region is almost adequate but at the marginal deficient level. This concentration may change abruptly to severe deficient levels at any time and may result in decreasing effective minerals particularly at the unmanaged location due to decrease in the levels of essential minerals in winter forages which are considered as the main reason for poor animal performance. Based on the information obtained from soil, pasture forage and plasma it is possible that low levels of Cu and Zn in soil and forage could potentially limit ruminant production. It is concluded that mineral supplement may continually be provided to the grazing animals to enhance the mineral status for maximizing the production potential of livestock at this site.

Introduction

Availability of minerals to animals in appropriate quantity is a pre-requisite for their health and productivity (Underwood, 1981; Khan *et al.,* 2006, 2007a, b). However, presence of minerals in higher concentration in a diet does not ensure full availability to the animals. This happens due to various dietary interactions in the metabolic processes. The levels of minerals in blood plasma and other body fluids also depend on various factors such as physiology of the guts, metabolic usage, homeostasis and excretion (Littledike & Goff, 1987). Herbivores under natural grazing conditions obtain minerals from forage plants. However, inadequate mineral intake or availability to ruminant results to reduced productivity (Minson *et al.,* 1976; Khan *et al*., 2005a, b; Khan *et al.,* 2007). Knowledge of mineral concentration in the body fluid or blood plasma is usually used as a diagnostic tool for assessing a variety of disorders. Sheep and goats have slightly different grazing or feeding habits. The differences in feeding behaviour have some nutritional implications. Sheep prefer to graze rather than to browse, while goats prefer slightly more browsing than grazing. The mineral contents of plants may vary widely among plant species and different places (Tolsma *et al.,* 1987; Ben-Shahar & Coe, 1992), and these may be pronounced seasonal changes (Tolsma *et al.,* 1987; Khan, 2003; Khan *et al.,* 2004). Thus feeding places and seasons may affect the mineral status. It is also important to examine the effect of management practices, which may differ between the reserves and availability of the nutrients.

In Pakistan, investigations regarding mineral responsive conditions among grazing ruminants are available but related survey from different agro-climatic zones are scanty. Keeping the present view in the mind, a base-line survey was conducted to assess the mineral status of grazing goats and sheep based on soil and forage mineral assay in addition to plasma. In this study, the differences in plasma mineral status between a herd of grazing sheep and goats with different feeding locations and seasons were evaluated on different classes of animals, in order to ascertain the needs of supplementation for maximizing the production potential of farm animals.

Materials and Methods

The study was conducted at the Livestock Research Unit located in south-western Punjab, Pakistan where the mean annual rainfall is approximately 250 mm; with most of rainfall in summer from June to August when the average daily temperatures are 49/25ºC (max/ min) while winters are cool (25/ 7ºC) and dry. Two different areas, managed and unmanaged, were selected in the northern and eastern parts of the complex having high populations of grazing animals. The vegetation of the managed area consisted of well developed native grasses and improved varieties of forages available to the animals for grazing in this area throughout the season. The unmanaged area of the complex was characterized by overgrazing with an extensive replacement of the perennial grasses by annual grasses, forbs, and bush encroachment of different species. Animals grazed on various grasses and improved forages in both pastures throughout the experimental period on the ranch.

Sample collection and preparation for analysis

Soil sampling: Soil samples were collected from five sites of both managed and unmanaged pastures during both summer and winter seasons at regular intervals of one month (two times during each season). In both physiographic positions, five sub-samples were obtained at a depth of 15 cm using a soil auger. After thorough mixing of sub-samples, one composite sample was taken from each site. These soil samples were first dried under shade and then in an oven for analysis of Mg, Cu and Zn were determined by atomic absorption spectrophotometry following the standard dilution procedure Lindsay & Norvell (1978).

Forage sampling: Forage samples were taken during summer and winter seasons from the same sites where soil sampling was done. Each sample consisted of five sub-samples per feeding site. These forage samples were collected after careful observation of the grazing behaviour of ruminants in the pastures. After removing the non-edible plant species, forage samples were dried at 60º C to a constant weight in a forced air-dried oven. Prior to analysis, the samples were digested with perchloric and nitric acid. Following dilution with 0.1% $LaCl_3$ the concentrations of Mg, Cu and Zn were determined by an atomic absorption spectrophotometer (Anon., 1980).

Animal sampling: Twenty animals each of sheep and goats in different physiological conditions were selected within each ranch and ear tagged. Blood samples were collected by jugular vein puncture in heparinized trace mineral free vacationer tubes during two different seasons of the year. These samples were centrifuged for 20 minutes at 700 *Xg*; plasma was collected and frozen at -20°C for further analysis. Stored plasma samples were thawed and deproteinated using 10% trichloroacetic acid. The matrix effects were corrected for using 1% $LaCl_3$ (Fick *et al.*, 1979). Plasma Mg, Cu, and Zn were determined by and atomic absorption spectrophotometer.

The data so obtained were analyzed by the statistical analysis system (Anon., 1987). Animal blood plasma was analyzed as spilt plot-design with animal class as the main plot and season as a sub-plot. Significance level was limited from 0.05-0.01 in these statistical analyses. Duncan's New Multiple Range test was used to determine differences among means.

Results and Discussion

Soil: Mean soil mineral concentrations of both pastures as related to sampling seasons are shown in Table 1. The effect of season on Cu concentration of the managed pasture soils was not significant (p>0.05). Since the lower limit of available Cu exceeds the critical value of 0.20 mg/kg, this element is unlikely to limit plant growth in the study ranch. Cu uptake by forage plants is low in soil high in Zn (Katyal & Randhawa, 1983), but the concentration of this element was low in the study site in the pasture. Soil Zn concentration at all the sites of both pastures were below the requirement for optimum forage growth. Seasonal difference of Cu for the managed pasture soils was not significant (p>0.05). It is argued that forage Zn levels reflect soil Zn levels. Metallic cations such as Ca, Fe and Mn, which present in high concentration, interfere with forage plant Zn uptake (Tisdale *et al.,* 1985). Looking at the relatively high concentrations of these cations and corresponding low soil Zn level, the potential impairment of plant Zn uptake cannot be ruled out.

Soil Mg concentration between seasons for the managed pasture land did not differ (p>0.05), though the summer season mean value was slightly higher in both managed and unmanaged soil pastures above the critical level of 30 mg/kg (Rhue and Kidder, 1983). It has been suggested that Mg concentration of soil and the ratio of Mg to other cations affect forage Mg concentration (Minson, 1990) and the same holds true for K to Mg ratio that exceeds unity. The Ca to Mg ratio in excess can induce plant Mg deficiency (Tisdale *et al*., 1985).

Forage: Mean forage mineral concentrations of both pastures as related to sampling seasons are shown in Table 2. There was no significant (p>0.05) differences between summer and winter season forage Mg concentrations in the managed pasture. Summer season managed pasture Mg levels in both managed and unmanaged locations would be adequate for all physiological forms of livestock except lactating animals, for which this element was at marginal deficient level (Anon., 1996). Summer season Mg concentration in the managed pasture, however, is only adequate for growing ruminants. Since managed pasture constitutes the largest grazing area, winter season mineral supplementation may have a beneficial effect on livestock production. The winter season Mg level in the unmanaged pasture was at the high end of the range that meets the ruminant need but the present contribution of unmanaged grazing lands is small and is expected to further decline with expression of forage cropping annually. Earlier works on the seasonal change in pasture Mg concentration were also consistent with the findings of Khan *et al*., (2003, 2007). Both decrease and increase in pasture Mg levels were reported with the progress of season (Pastrana *et al*., 1991a; Espinoza *et al*., 1991a, b).

Table 1. Mean mineral concentrations of soils as recorded during winter and summer seasons at both managed and unmanaged pastures.

Pasture	Element DM basis (mg/kg)	Season		Critical level*
		Winter	Summer	
Managed	Mg	38.50 ± 4.28	46.30 ± 7.25	30
	Cu	0.72 ± 0.24	0.64 ± 0.17	0.20
	Zn	0.06 ± 0.02	0.08 ± 0.05	<0.60
Unmanaged	Mg	34.50 ± 3.20	40.30 ± 9.25	30
	Cu	0.82 ± 0.11	0.94 ± 0.06	0.20
	Zn	0.07 ± 0.04	0.11 ± 0.04	<0.60

*Rhue and Kidder (1983), McDowell *et al*., (1984), Lindsay (1972)

Table 2. Mean mineral concentrations of forages as recorded during winter and summer seasons at both managed and unmanaged pastures.

Pasture	Element DM bases	Season		Critical level*
		Winter	Summer	
Managed	Mg %	0.18 ±0.01	0.15 ± 0.01	0.10-0.20
	Cu (mg/kg)	7.15 ±2.11	18.76 ± 1.90	10
	Zn (mg/kg)	25.30 ±2.80	27.65 ± 1.74	30
Unmanaged	Mg %	0.16 ± 14.60	0.11 ± 0.02	0.10-0.20
	Cu (mg/kg)	10.24 ± 0.02	25.30 ± 0.24	10
	Zn (mg/kg)	19.80 ± 4.60	28.55 ± 4.22	30

*Rhue and Kidder (1983), McDowell *et al*., (1984)

Mean summer season Cu concentration in the managed pasture was higher than that in the winter season, but the difference was not significant (p>0.05). Espinoza *et al*., (1991a,b) reported a low Cu value in winter season forage. Summer season Cu concentration in both managed and unmanaged pastures were well above the minimum (10 mg/kg) recommended for ruminants (Anon., 1996). Winter season pastures do not appear adequate in Cu for grazing livestock. The availability of Cu for grazing livestock may be limited further due to intricate interactions with other nutrients such as sulphur, molybdenum and iron (McDowell, 2003). High levels of Cu may be needed in the presence of high concentration of antagonists such as Fe, Mo and S (Phillippo *et al*., 1987a, 1987; Underwood & Suttle, 1999). So it can be concluded that high levels of these antagonists could affect the Cu utilization in both seasons.

Summer and winter season pastures Zn values in the managed site were not appreciably different (p>0.05). In contrast, a seasonal change in pasture Zn concentration was reported by Espinoza *et al*., (1991a,b). Forage Zn levels in managed and unmanaged pastures were comparable, but these were below ruminant requirements at both pastures. High concentration of Ca in pasture may further aggravate Zn deficiency (Alfaro *et al*., 1988). In order to meet the ruminant's requirement, forage should contain 20 mg/kg Zn (Anon., 1996).

Lactating animals: Mean plasma mineral concentrations of lactating sheep and goats as related to areas and sampling seasons are presented in Table 3. With the exception of Cu, the rest of the minerals (Mg and Zn) in sheep exhibited seasonal variation. Higher plasma concentrations of Mg and Zn were observed in winter than that in summer. Plasma mineral concentrations were different in both seasons, and managed and unmanaged locations. Furthermore, plasma mineral concentrations of lactating sheep and goats also differed with areas and seasons. Goats at the managed area had higher concentrations of Cu and Zn than that at the unmanaged area. However, Zn was higher in summer than that in winter while the reverse was true for Cu at the managed areas. Plasma Mg of goats at the managed area was higher than that at the unmanaged areas during both seasons.

On the average, plasma Mg concentrations of goats at the unmanaged area were low during both seasons and most of the samples from both sheep and goats were below the critical level (2.0 mg/dL) as suggested by McDowell *et al*., (1984) at the unmanaged ranch and is considered at marginal deficient levels for sheep and severe deficient levels for goats particularly during summer. Similar concentrations were reported by Espinoza *et al*., (1991a,b) with a range of 1.7-2.3 mg/dL in Florida. It has been found that plasma Mg concentration depends mainly on its dietary intake (Pastrana *et al*., 1991b). Levels below 2.0 mg/dL in plasma are taken as hypomagnesaemic, but Mg concentration in blood plasma does not fall until there is a severe deficiency (Anon., 1973).

A large number of goat samples and a few of sheep samples at the unmanaged area were Cu deficient below the critical value of 0.65 mg/L (McDowell and Conrad, 1977). These samples were marginally deficient for sheep during summer and for goats during both seasons at the unmanaged pasture. Similar concentrations were reported by Epinoza *et al*., (1991a,b) with a range of 0.76-1.12 mg/L, Tejada *et al*., (1985) with a range of 0.58-0.80 mg/L Prabowo *et al*., (1991), with a range of 0.6-0.8 mg/L, Rojas *et al*., (1993) with a range of 0.61-1.06 mg/L in various groups of domestic animals. Only a small number of samples were below the critical level (0.6-0.8 mg/L) of plasma Zn (McDowell, 1987) to meet the requirement of ruminants during both seasons. Similar concentrations were reported by Epinoza *et al*., (1991a,b) with a range of 0.44-0.97 mg/L, Tejada *et al*., (1987) with a range of 0.71-1.02 mg/L, Prabowo *et al*., (1991) with a range of 0.90-0.115 mg/L, Pastrana *et al*., (1991a,b) with a range of 0.68-0.85 mg/L, Tiffany *et al*., (2001) with a range of 0.77-1.06 mg/L, Velasquez-Pereia *et al*., (1997) with a range of 0.74-1.08 mg/L, and Merkel *et al*., (1990) with 0.77 mg/L.

Non-lactating animals: On the average, non-lactating sheep and goats were low in plasma Mg only in summer and also at the managed area the sheep were low in winter (Table 4). Higher proportions of the samples from non-lactating sheep and lower proportions of the samples from non-lactating goats were below the critical levels of 2.0 mg/dL (McDowell, 1997). Mean plasma Mg concentrations found in this study were similar to those reported for Colombia (Pastrana *et al*., 1991a,b), and Florida (Tiffany *et al*., 2001), while lower than those found by Rojas *et al*., (1993) for Florida.

During summer, non-lactating goats were deficient in plasma Cu, but small proportions of the samples from non-lactating sheep and larger proportion from non-lactating goats were below the critical level of 0.65 mg/L (McDowell & Conrad, 1977; McDowell, 1997). Mean plasma Cu ranged from 0.75-1.19 mg/L in non-lactating sheep and 0.34-1.05 mg/L in non-lactating goats. These concentrations are similar to those (0.59 mg/L) reported by Merkel *et al*., (1990). Plasma Cu differed with areas, seasons and animal class. Copper deficiency was found in grazing ruminants and hypocuprosis could be expected by excess intake of Mo, S, Fe, Zn and Ca which affects all stages of growth and reproduction (Graham, 1991). Balbuena *et al*., (1989) found a high percentage of plasma Cu deficiency as compared to the present findings.

Table 3. Plasma mineral concentrations of lactating sheep and goats as related to ranch and sampling season.

Element	Critical level*	Ranch	Animal class							
			Sheep				Goats			
			Summer		Winter		Summer		Winter	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Mg	2.0	Managed	2.6	0.09	3.63	0.05	2.1	0.08	2.8	0.04
(mg/dL)		Unmanaged	2.0	0.09	2.2	0.05	1.8	0.09	2.1	0.04
		Mean	2.3**		2.9*		1.9**		2.5*	
Cu	0.65	Managed	0.69	0.06	0.71	0.04	0.82	0.07	0.85	0.04
(mg/L)		Unmanaged	0.66	0.06	0.68	0.04	0.60	0.07	0.64	0.04
		Mean	0.68		0.70		0.71*		0.75**	
Zn	0.6	Managed	0.70	0.04	0.98	0.04	1.18	0.05	1.06	0.04
(mg/L)		Unmanaged	0.73	0.04	0.92	0.04	0.92	0.06	0.96	0.04
		Mean	0.71**		0.94*		1.05		1.01	

* = McDowell, 1985, 1987; *, ** = Significant at 0.05 and 0.01 levels, respectively.

Table 4. Plasma mineral concentrations of non-lactating sheep and goats as related to ranch and sampling season.

Element	Critical level*	Ranch	Animal class							
			Sheep				Goats			
			Summer		Winter		Summer		Winter	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Mg	2.0	Managed	1.9	0.04	2.1	0.03	2.0*	0.04	2.1*	0.03
mg/dL		Unmanaged	1.8*	0.04	2.0*	0.03	1.8**	0.03	2.0**	0.03
		Mean	1.85		2.0		1.9		2.0	
Cu	0.65	Managed	0.75	0.03	1.02**	0.02	0.34**	0.03	0.90**	0.03
mg/L		Unmanaged	0.80	0.03	1.19*	0.02	0.72*	0.03	1.05*	0.02
		Mean	0.76		1.15		0.53		0.98	
Zn	0.6	Managed	0.92*	0.03	0.58*	0.02	1.36*	0.05	0.75	0.02
mg/L		Unmanaged	0.83**	0.03	0.48**	0.02	1.28**	0.04	0.72	0.02
		Mean	0.88		0.53		1.32		0.74	

* = McDowell, 1985; McDowell, 1987; *, ** = Significant at 0.05 and o.o1 levels, respectively

On the average, only non-lactating sheep were low in plasma Zn in winter, however, a large number of samples from non-lactating sheep and a few from non-lactating goats were below the critical level of 0.6-0.8 mg/L (McDowell, 1987). Mean plasma Zn ranged from 0.48-0.92 mg/L in non-lactating sheep and 0.72-1.36 mg/L in non-lactating goats. These concentrations are similar to the value, 0.77 mg/L, as reported by Merkel *et al*., (1990), Espinoza *et al*., (1991), with a range of 0.44-0.97 mg/L, Velasquez-Pereira *et al*., (1997) with a range of 0.74-1.08 mg/L, Prabowo *et al*., (1991) with a range of 0.61-.80 mg/L, and Tiffany *et al*., (2001) with a range of 0.93-1.08 mg/L , for non-lactating sheep during both the seasons and non-lactating goats only during winter. However, plasma Mg in non-lactating goats, during summer was found higher than those reported in the earlier reports. Mean plasma mineral concentrations lower than the critical levels, were found more often in sheep as compared to those in goats. The lower mineral concentrations in sheep plasma are related to lower forage mineral contents. The mineral contents were affected by forage maturity, and particularly, in this study might have been the result of decreased mineral levels in grass forage, which are mostly liked by these animals. The present study indicates the higher minerals concentrations in the plasma of goats than that in sheep. This is probably due to their slightly different adaptations and feeding habits, though these two animal species have a same type of digestive system. Goats prefer more browsing, while sheep do grazing. It may be attributed to difference in dietary intake and feeding habits of these ruminants. It has been observed that browsed forages have higher mineral contents than grasses. Also as forages mature and dry up, their mineral concentration declines. The rate of this decline is higher in grasses than that of browsed forage plants as earlier reported by Kapu, (1976) and Khan *et al*., (2007c).

The levels of soil Fe and Cu did not seem to limit pasture production in the study area. Soil, however, is seriously deficient in Zn and its amendment by fertilization needs consideration. The information collected from the soil pasture and plasma analysis, shows that the livestock grazing may be benefited from supplementation of Zn and Cu during the winter season. These elements were deficient in soil, pasture, and also in the blood plasma of grazing goats and sheep therein.

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