Determination of copper status of grazing sheep: seasonal influence

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Summary

A study was conducted to determine the copper status of different classes of grazing sheep during two different seasons on a farm located in southern Punjab, Pakistan. A complete free-choice supplement was available to all animals throughout the year. Soil, forage and blood plasma from animals were taken eight times during the year (2005) (four times in both summer and winter seasons). Soil Cu^{2+} was affected by the seasonal changes ($P<0.001$), higher in summer than that in winter and was significantly higher than the need of plants during both seasons. While forage Cu^{2+} level showed non-significant seasonal ($P>0.05$) fluctuation in winter. It was adequate for ruminants' requirements during both seasons. plasma Cu^{2+} concentrations of all classes of sheep were significantly higher in winter than that in summer showing the seasonal changes ($P<0.001$). Higher plasma Cu^{2+} was found in male sheep than lactating and non-lactating sheep during both seasons. The low Cu^{2+} in plasma in lactating sheep may have been due to its secretion in milk. In winter forage Cu^{2+} contributed in enhancing the plasma Cu^{2+} levels, but in summer the forage Cu^{2+} level, although very high, was ineffective in elevating plasma Cu^{2+} levels in all classes of sheep. Based primarily on plasma analyses it was concluded that although, the adequate level of plasma Cu^{2+} was found, it was on borderline deficient levels. Thus supplementation is needed with mixture containing Cu^{2+}. The plus copper should be continuously supplemented to grazing animals in this semiarid region of Pakistan.

Key words: Seasonal variation, Soil, Plant, Animal, Pakistan

Introduction

Animal nutritionists and livestock producers have recognize that variation in nutrient profiles of feedstuffs is a common occurrence. However, few producers realize that the normal variation in energy, protein or macrominerals is relatively small compared to what has recently been reported for the trace minerals (McDowell, 2003) due the variation in trace mineral profiles among common feedstuffs leads to the consideration in supplementation programs. The identification of those factors contributing to this variation may be helpful to individual producers and nutritionist in preventing trace mineral deficiencies (Underwood and Suttle, 1999; Peterson *et al*., 2000).

Keeping in view the importance of Cu in animals, the present study was conducted to locate Cu deficiency or excess for grazing livestock by the use of pasture and animal samples. The final goal is to meet the Cu needs of grazing livestock in order to maximize the production of animal products by adopting the major way of adequate and balanced Cu supplementation.

Materials and Methods

Pasture description

Investigations were conducted, using a herd of sheep consisting of three classes according to physiological conditions and gender (lactating, non-lactating, and male) and grazing pasture of the farm in southern Punjab. The average of temperature during the experimental year was between 38 + 5°C during summer and 15 + 7°C during winter; relative humidity was 48 + 5% during summer and 80 + 8% during winter. These

animals were of variable degrees of cross breeding. Animals on this farm grazed predominately native grasses, along with new improved varieties of forages of higher qualities. In addition to these, some other plant species of inferior qualities such as creeping herbs, bark of trees, crop residues, plant hay, and some other crop wastes was also fed by grazing sheep in the pastures. The copper concentrations of these additional fodders have previously been reported (Khan, 2003).

The livestock farm was characterized by two pastures, denoted as feeding sites, one pasture was intensively managed with using fertilizer soils, irrigation with canal water and with grazing reserves characterized by the availability of sown forage species including: *Panicum*, *Andropogon*, *Pennisetum*, *Setaria*, *Medicago sativa*, *Trifolium alexandrium*, *Hordeum vulgare*, *Cichorium intybus*, *Cynodon genera*, vernal grass, imported velvet grass, tall fescue, orchard grass, molasses grass, elephant grass, pangola grass and jaragua grass.

The other pasture with unfertilized soils, barren and uncultivated area with natural weeds like vegetation and low intensity cropping were largely accessible to grazing animals. This pasture was overgrazed with extensive replacement of perennial grasses by annual grasses, and forbs and bush encroachment by *Accacia* spp., *Zyzyphus mucronata*, *Trachipogon* spp., *Cyperus rotandus*, *Tribulus terrestris*, *Chenopodium morale*, *Lathyrus odoratus*, *Alhagi* spp., *Salavadora* spp., *Calotropis* spp., and some wild species of plants; additional supplementations with free-choice mineral mixture having the following composition: Ca, 15.7%; P, 6.3%; Mg, 2.0%; K, 0.3%; Na, 11%; Mn, 2400 ppm; Fe, 6000 ppm; Zn, 2630 ppm; Cu, 448 ppm; Co, 16.4 ppm; Mo, 10.8 ppm and Se, 0.8 ppm. Forage Cu ranged between 7.81 and 25.7 ppm. All experimental animals aged from 3 to 4 years, were raised at the farm. Daily means of forage mineral consumption was approximately 50 g per animal.

Sample collection

Each composite of soil sample, which was derived from five sub-samples were taken at a depth of 20 cm as described by Sanchez (1976). As with soil samples, each of the composite forage sample came from five sub-samples of the same predominating forage species that was most frequently grazed by sheep on the farm. Forages were collected 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. Individual forage samples were collected at the same spots from where soil samples were collected. Representative samples of the forages then were placed in polyethylene bags at the laboratory where they were given a rapid wash with tape water followed by a glass-distilled water to remove any soil which was present. Soil and forage samples were placed in clean cloth bags for air drying.

For sampling purpose animals were divided into 3 classes, lactating/non-lactating and male animals, respectively with 10 animals per class. Blood plasma from animals were taken at the farm concurrently with the soil and forage samplings.

Blood samples were anaerobically collected by jugular vein puncture with a syringe and needle, then drawn by vacuum into evacuated tubes containing lithium heparin as an anticoagulant, plasma was separated by centrifugation and harvested into polyethylene tubes and frozen at -20^{o}C for subsequent analysis for copper. The samples of forages were dried in an oven at 60^{o}C for 48 hrs.

Sample preparation

Air and oven dried soil samples, were pulverized in a ceramic mortar to pass through a 2-mm sieve and were analysed for Cu concentrations using a Mehlich-1 (Hesse, 1972; Rhue and Kidder, 1983) extraction procedure: 5 g of soil were added to 20 ml of 0.05 M HCl in 0.025 M H_2SO_4 and final volume was analysed.

Air and oven dried samples of forage, then were ground with a Wiley mill to fit through a 1-mm mesh. To prepare samples for estimation of copper, representative dried and ground samples of about 2 g of each forages, were digested by nitric acid and perchloric acid (3:1) at 250^{o}C until the solution changed to colorless and thick

white fumes appeared in the flask. The contents of the flask were washed with pure water and diluted to constant volume. The supernatant obtained from centrifugation was used for analysis (Koh and Judson, 1986; Neathery *et al*., 1990). Direct dry or wet ashing of plasma was not possible because of high fat, protein and moisture as spattering and swelling might result in loss of sample. Therefore, appropriate quantity of each plasma samples was taken into crucible after thawing. To predigest, the samples were pretreated with 50% HNO_3 over an electric heater until smoking ceases to char the majority of organic matter. These samples then were ashed for 6 hrs at 550^{o}C in a muffle furnace.

The residues were dissolved in 1% HCL and transferred into a volumetric flask to make up a constant volume of 50 ml. Samples were poured into labeled plastic tubes suitable to fit the auto sampler of atomic absorption spectrophotometer. The samples were diluted to determine individual elements (Fick *et al*., 1979; Nockels *et al*., 1993; Mpofu *et al*., 1999).

All the samples were filtered through Whatman filter paper No. 42 and brought to appropriate volume with double distilled water and stored in polyethylene tubes. Samples were analysed for concentration of Cu by atomic absorption spectrophotometry (Perkin-Elmer Model 5000).

Statistical analysis

Data thus obtained during the study were analysed by statistical analysis system (SAS, 1987). Soil, forage, and animal samples were analysed as split-plot design (Steel and Torrie, 1980), with season as the main plot and sampling periods as sub-plots. Differences between means were ranked using Duncan's new multiple range test (Duncan, 1955). Soil, forage, and plasma copper concentrations were compared to established critical values to determine the various categories of deficient levels. The critical level for soils indicates the copper concentration below which normal growth and/or mineral composition of forage may be adversely affected. For forage samples, it indicates the lowest requirement of the element or organic constituent to avoid deficiency symptoms in animals. Plasma critical levels indicate the concentration below which specific signs of deficiency may occur. Interpretation of these critical values was done with caution taking into consideration the management, nutritional, environmental and individual factors that affect the availability, supply and utilization of this nutrient.

Results

Pasture samples

Soil

Cu^{2+} concentration in soil varied significantly in different seasons and at different fortnights (P<0.001) (Table 1). The soil Cu^{2+} was significantly higher in summer than that in winter and it remained unchanged at different fortnights during winter. In contrast, during summer soil Cu^{2+} was higher at the first fortnight than that at the other three fortnights (Table 3).Variations (P<0.01) due to interaction of season and fortnight were also found to be significant.

Table 1: Analysis of variance of data for Cu^{2+} concentration in soil, and forage plants in different sampling fortnights during winter and summer seasons at sheep ranch

Source of variation (S. O. V)	Degree of freedom (df)	Mean squares	
		Soil	Forage plants
Season (S)	1	4.5^{***}	1.04^{NS}
Error	28	0.09	38.02
Fortnight (FN)	3	0.35^{***}	68.57^{**}
S × FN	3	0.13^{**}	9.25^{NS}
Error	84	0.03	12.35

**, *** = Significant at 0.01 and 0.001 levels, respectively. NS = non-significant

Forage plants

Considerable variation in forage Cu^{2+} was observed at sampling intervals (P<0.01) accompanied with no seasonal effect (P>0.05) (Table 1). Non-consistent fluctuations were observed in forage Cu^{2+} level during both seasons at different fortnights (Table 3). A higher concentration of Cu^{2+} in forages was found at the first fortnight during both seasons than those at last three fortnights. The interaction (P>0.05) of season and fortnight was non-significant in this study.

Animal samples

Lactating sheep

Plasma

From analysis of variance it is evident that seasons ($P<0.001$) and fortnights ($P<0.05$) had significant effect on plasma Cu^{2+} (Table 2). The Cu^{2+} level was markedly higher in winter than that in summer, but there were non-consistent fluctuations at different fortnights in winter (Table 3). In contrast, a consistent increase in plasma Cu^{2+} was observed during summer. No variations were found due to interaction effect ($P>0.05$) of season and fortnight.

Table 2: Analysis of variance of data for Cu^{2+} in blood plasma of three different classes of sheep as related to season and sampling fortnights

Source of variation	Degree of freedom	Mean squares		
		Lactating	Non-lactating	Male
Season (S)	1	2.04^{***}	2.89^{***}	1.22^{**}
Error	18	0.04	0.05	0.102
Fortnight (FN)	3	0.09^{*}	0.003^{NS}	0.003^{NS}
S × FN	3	0.03^{NS}	0.014^{***}	0.38^{***}
Error	54	0.03	0.002	0.02

*, **, *** = Significant at 0.05, 0.01, and 0.001 levels, respectively. NS = non-significant

Non-lactating sheep

Plasma

Significant seasonal ($P<0.001$) and non-significant effects of fortnights ($P>0.05$) were observed on plasma Cu^{2+} concentration (Table 2). Cu^{2+} level was markedly higher during winter than that during summer. During winter, there was no significant change with time up to fortnight 3 but the Cu^{2+} level decreased at fortnight 4, whereas in summer, the Cu^{2+} level was uniform at the first two fortnights and also equal amount was observed at the last two fortnights, but higher than that at the first two fortnights (Table 3). Interaction effect ($P<0.001$) due to season and fortnight was found to be significant

Male sheep

Plasma

Cu^{2+} availability in blood plasma was affected ($P<0.001$) by the seasons, but in contrast, sampling intervals had no significant effect ($P>0.05$) on it (Table 2). The tendency of a gradual decrease in plasma Cu^{2+} in winter with time and increase in summer was found in this study (Table 3). The bioavailability of Cu^{2+} in plasma was significantly higher in winter as compared to that in summer. The effect of interaction due to season and sampling period was significant ($P<0.001$) in this study.

Discussion

Soil Cu^{2+} levels during seasons, winter and summer were above the critical level for the normal growth of plants (Rhue and Kidder, 1983). Similar levels of soil Cu^{2+} in winter and summer seasons have earlier been reported in Colombia (Pastrana *et al*., 1991), Guatemala (Tejada *et al*., 1987), and Nicaragua (Velasquez-Pereira *et al*., 1997). It has been suggested that soils with less than 0.6 mg/kg of extractable Cu^{2+} are

Table 3: Copper concentrations of soil, forage, and blood plasma of different animal classes as related to seasons and sampling times

Season/sampling time	Soil	Forage	Animal class		
			Lactating	Non-lactating	Male
Winter					
1-	1.81 ± 0.0230	14.70 ± 1.73	1.17 ± 0.033	1.35 ± 0.042	1.52 ± 0.057
2-	1.79 ± 0.024	12.45 ± 1.26	1.17 ± 0.090	1.32 ± 0.042	1.37 ± 0.035
3-	1.74 ± 0.065	12.65 ± 0.66	1.18 ± 0.031	1.33 ± 0.040	1.27 ± 0.065
4-	1.69 ± 0.027	11.08 ± 0.77	1.21 ± 0.071	1.29 ± 0.038	1.95 ± 0.058
Summer					
1-	2.39 ± 0.079	14.90 ± 0.87	0.753 ± 0.024	0.913 ± 0.032	0.937 ± 0.024
2-	2.14 ± 0.058	12.40 ± 0.69	0.824 ±0.032	0.919 ± 0.020	1.04 ± 0.054
3-	2.03 ± 0.057	10.89 ± 1.08	0.818 ± 0.032	0.964 ± 0.037	1.14 ± 0.066
4-	2.04 ± 0.087	11.91 ± 1.40	0.990 ± 0.091	0.973 ± 0.034	1.25 ±0.087

Means are based on following number of samples: soil (60), forage (60), during each season and plasma (120) during each season

considered deficient for pasture and crops. Based on this all the mean values of soil Cu^{2+} in the present study are not deficient.

Forage Cu^{2+} concentrations were found to be sufficiently high to meet the demand of animals during both seasons. The forage Cu^{2+} had no relationship with soil Cu^{2+} levels during both seasons. Forage Cu^{2+} values found in this study were not sufficiently high, 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*., 1991) and lower than that 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).

Dietary copper requirements vary greatly among species. The recommended levels for one species may cause toxicity in another. For example, 10 ppm is the NRC recommended level for dairy cattle but under certain conditions, 10 ppm can cause toxicity in sheep (Church and Pond, 1988). By comparison, growing pigs are often fed 100 to 250 ppm of copper in the diet to improve growth. According to the National Research Council, poultry require approximately 8 ppm copper (NRC, 1984).

Sheep are unique in that they accumulate copper in the liver more readily than other farm animals. Over a period of time, 1,000 - 3,000 ppm copper on a dry basis may be achieved. Usually there are no clinical signs until there is a sudden release of copper into the blood. Plasma copper levels then increase 10 to 20 fold. These elevated blood copper concentrations (500-2000 mg/dl) usually precede clinical signs by 24 to 48 hrs (Undrwood and Suttle, 1999). Because of the variation in recommended copper concentrations, it is difficult to have one copper level in a trace mineralized salt for all species. One alternative is to have a low-copper product for sheep and a high-copper product for the other species. This would insure that all species would receive an appropriate amount of copper without the risk of copper toxicity in sheep. Those swine producers feeding copper as a growth promotant will continue to supplement copper in addition to that in the trace mineralized salt (NRC, 1984).

The Cu^{2+} sources were not significantly different during both seasons, but during summer slightly higher concentration was found than that in winter. The plasma Cu^{2+} concentrations of all classes of sheep were significantly higher in winter than that in summer showing the seasonal as well as physiological effects. Higher plasma Cu^{2+} was found in male sheep than lactating and non-lactating sheep during both seasons. The low Cu^{2+} in plasma in lactating sheep may have been due to its secretion in milk and faeces. In winter the forage Cu^{2+} contributed in enhancing the plasma Cu^{2+} levels, but in summer the forage Cu^{2+} level, although very high, was ineffective in elevating plasma Cu^{2+} levels in all classes of sheep.

The low plasma Cu^{2+} levels were not due to the Cu^{2+} status in the diet, since the forage and feed collectively had higher Cu^{2+} content in summer than that in winter and thus the source Cu^{2+} level remained ineffective in raising the plasma Cu^{2+}. Nevertheless, plasma Cu^{2+} concentrations were above the critical level in all classes of sheep during both seasons (McDowell, 1985).

High level of sheep plasma Cu^{2+} in winter had already been reported in Colombia (Pastrana *et al*., 1991) and in cattle plasma in Nicaragua (Velasquez-Pereira *et al*., 1997). According to Suttle (1986) and Mills (1987) the plasma Cu^{2+} is of limited value in diagnosing Cu^{2+} status because of certain diseases responsible for altering these levels. In this study there were small seasonal differences in concentration

of dietary Cu^{2+}, which seemed to have no effect on plasma Cu^{2+} of sheep. This was in agreement with the observation of Underwood (1981) that plasma/serum Cu^{2+} does not reflect dietary Cu^{2+} and the observations found in this study could not be attributed with the finding of Rowlands (1980) who suggested that plasma Cu^{2+} is affected by dietary Cu^{2+} intake.

Plasma Cu^{2+} levels in male animals were slightly higher during both seasons than in other groups of animals, Goodrich *et al*., (1972) reported that extent of Cu^{2+} absorption may be influenced by age, some hormones, pregnancy, and some diseases. In addition, various nutrient interrelationships have to be found to affect the absorption of Cu^{2+}. Cu^{2+} deficiency is widespread for grazing ruminants throughout the world (McDowell, 1985). Most deficiencies are conditioned by the presence of dietary factors, which interfere with the utilization of Cu^{2+} by the animals. The low level of plasma Cu^{2+} during summer found in this study may have been due to its rapid tissue distribution and inhibitory effect of Fe^{2+} and the active process of its excretion through faeces and urine in summer.

This study indicated that seasonal fluctuations were found only in soil and plasma Cu^{2+} concentrations in all classes of sheep. Higher plasma levels were found in winter than that in summer. Although, the adequate level of plasma Cu^{2+} was found, it was on borderline deficient levels. Thus, supplementation is needed with mixture containing Cu^{2+}.

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