Pak. J. Bot., 39(4): 1103-1112, 2007.

SEASONAL DYNAMICS OF ZINC IN SOIL, DIETARY FACTORS AND GRAZING SHEEP FROM SOUTHWESTERN, PUNJAB, PAKISTAN

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Abstract

This study has been conducted in a sheep farm to determine the zinc status in a semiarid region of south western Punjab, Pakistan, during two different seasons. The purpose of this was to investigate as function of the season and the sampling period, the transfer of Zn from soils and forages to sheep grazing in this semiarid region in order to evaluate if the Zn requirement of the grazing sheep was met or if Zn deficiency occurred. The final goal was to maximize the production of animals by adopting if necessary, an adequate and balanced Zn supplementation. Soil, forage, water and feed samples as well as blood, urine, milk and faeces from lactating/ non-lactating and male grazing animals have been taken fortnightly, 4 times during summer and 4 times during winter. Zn concentrations of the samples were determined by atomic absorption spectrometry. Extractable soil Zn concentrations were found adequate for normal plant growth during both seasons. Non-significant effect of season but significant effect of sampling periods was observed on soil zinc level and was slightly higher in winter than that in summer. Forage zinc level was affected both by the season and fortnights and also found above the requirements of the ruminants only in winter. However, the level in summer was considered slightly deficient for growing and lactating animals. No effect of seasons or fortnights was observed on water Zn and that of feed zinc was affected only by the seasonal variation. Zinc content in dietary sources was higher in summer than that in winter. In summer, the plasma contained higher zinc only in non-lactating sheep than that in other two groups. Plasma zinc showed a response to the diet zinc content in summer in non-lactating sheep while the higher zinc concentration in the diet during summer remained ineffective increasing the plasma zinc levels in lactating and male sheep. The milk zinc concentration was higher in early lactation period than late lactation. The levels of faeces and urine zinc were not affected by the seasonal variation. The zinc content of faeces has reflected its pasture levels in all classes of sheep. Only plasma and milk zinc was affected by seasonal changes and urine zinc by the sampling period only in lactating sheep. Relationships between soil, forages and plasma Zn levels and the seasons and period of sampling have been discussed. Based on this study it is concluded that supplementation of grazing sheep should be done on this ranch with a specifically tailored mixture of high bioavailability to maximize the animal potential.

Introduction

Trace elements play very important role in animals and it has been suggested that any imbalance in trace elements level in the cell or body would affect structural and functional activities (Valee & Galdes, 1984). Zinc is a trace mineral and important for animal metabolism. It has been reported by various veterinarians that animals whose diet contained inadequate level of zinc developed red and cracked skin with loss of hair or wool, as well as other problems. In animals, it has been reported that zinc supplementation has proved a

very effective treatment for relieving the disease of acne (McDowell, 2003). Nutritional zinc imbalance in the developing countries does not occur in isolation in grazing animals. It plays a major role in the functioning of many enzymes that are concerned with nucleic acid, carbohydrate and protein metabolism (Parasd, 1995).

Zinc deficiency has now been recognized to be associated with many diseases for example malabsorption syndrome, chronic liver and renal diseases as well as reproduction abnormalities (Prasad, 1993). In these conditions, deficiencies of micronutrients such as vitamins and other trace elements may also be associated in manifestation of zinc imbalances. Maternal zinc deficiency is associated with abnormal foetal development and other reproductive problems in different animals (Keen,1996). In spite of its great importance in animal nutrition, very little work has been done to determine its level in the diet for animals in many developing countries including Pakistan. In Pakistan livestock production depends largely on the use of natural pastures throughout the year and the pastures consists of a variety of forages ranging from grasses, legumes, tree leaves and crop residue available for grazing animals. Minerals and other nutrients status of forage are still completely unknown in Pakistan. The purpose of this investigation was to identify zinc concentrations of soil, pasture and animals during two seasons of the year to know the effect of seasonal variation in dietary sources and its availability in blood plasma in order to assess the need of fomulation of supplements for grazing animals. We intended to document the success of a general feeding strategy for free grazing sheep rather than measuring the exact daily Zn intake per animal.

Materials and Methods

Study area: The study was conducted at Livestock Experimental Station using a flock of Thalli Breed of lactating, non-lactating and male sheep grazing similar pastures on a farm at Livestock Experimental Station, Rakh Khaire Wala located in Southern Punjab, Pakistan, during summer and winter of the year corresponding to wet and dry seasons. Pastures were predominantly, grasses, legumes, tree leaves and crop wastes. In addition, all animals on the farm had access at all times to a free-choice complete mineral mixture. This sheep ranch comprises 400 ha and receives annual precipitation of 250-750 mm restricted to July and August. The soils are sandy and vertisols. The ranch has about 7000 animals of which 2000 are breeding sheep. This ranch is low-lying semiarid region of south western Punjab between latitudes 23^{o}-36^{o} N and longitudes 60^{o}-75^{o} E. Average temperature during the experimental year was between 38 ± 5^{o}C during summer and 15 ± 7^{o}C during winter: relative humidity 48±5% during summer and 80±8% during winter. The livestock farm was characterized by two pastures, denoted as feeding sites, one pasture was intensively managed with fertilized soils, irrigated with canal water and with grazing reserves characterized by the availability of sown forage species including *Panicum, Andropogon, Penisetum, Setaria, Medicago sativa, Trifolium alexandrium, Hordeum vulgare, Cichorium intybus* and *Cynodon* genera Vernal grass, Imported velvet grass, Tall fescue, Orchard grass, Molasses grass, Elephant grass, Pangola grass and Jaragua grass. The other pasture is with unfertilized soils, barren and uncultivated area with natural weeds like vegetation and low intensity cropping largely accessible to grazing animals. This pasture was overgrazed with extensive replacement of perennial grasses by annual grasses, forbs and bush encroachment by *Accacia* spp., *Zyzyphus mucronata, Trachipogon* spp., *Cyperus rotandus, Tribulus terrestris, Chenpodium morale, Lathyrus odoratus, Alhagi* spp., *Salvadora* spp., *Calotropis* spp., and some wild spp., and additional free choice mineral mixture of different proportion. Each pasture maintained three animals /ha /year under a rotational grazing system.

Animals: Ten animals, each of clinically healthy lactating, non-lactating and male sheep were selected for study purpose. Average body weights were 40-45 kg with 2-3 years of age. These animals were with variable degrees of cross breeding. The lactating sheep were in their second lactation. All the experimental animals were same throughout the study period.

Housing and management: The lactating, non-lactating and male sheep were housed in well-ventilated building, divided by the solid wood walls into pens, provided with straw bedding, 30 cm thickness and supplied with fresh clean water containing buckets, feed troughs and salt blocks for free licking access to goats. The animals were provided a good opportunity to feed on mineral mixture. All hygienic measures are good in the farm.

Feeding and nutrition of animals: This work was carried out under two different feeding systems which are stable-diet (indoors) and grazing (outdoors) conditions according to the season of the year. The animals fed on the stable diets formulated to cover their nutrient requirements according to the NRC (Anon., 1995). However, both the animal classes were gradually transferred to the grass pasture for grazing. The time of grazing was 2.5-5.0 h. The animals fed on the grass pasture *adlibitum* and supplemented with free-choice mineral mixture.

Sample collection and preparation: Soil, forage, feed, water and animal samples were taken from the farm. These collections were made eight times fortnightly during the year (four times both during the summer and winter seasons). Composite soil and forage samples were collected at three sites from each pasture. The five sub-samples of soil and forages were taken from the beginning, middle and end of each pasture.

Each composite soil sample that 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 of soil collection. 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 glass-distilled water to remove any soil, which was present. Soil and forage samples were placed in clean cloth bags for air drying.

Blood plasma, milk, faeces and urine samples from lactating, plasma, faeces and urine from non-lactating while plasma and faeces from male sheep 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, drawn by vacuum into evacuated tubes containing lithium heparin as an anticoagulant. Plasma was separated by centrifugation and was harvested into polyethylene tubes and frozen at -20^{o} C for subsequent zinc analysis. Fecal samples were collected from the rectum of the animals manually and urine samples collected *via* manual stimulation of the vulva of female animals and a 10 ml aliquot was transferred to a polyethylene tube, acidified with 0.3 ml concentrated HCl, and frozen for subsequent analysis. The faecal samples were kept in open bags and allowed to dry in the sun under constant atmospheric moisture (<30 %). Milk samples were collected in 125 ml nalgene

bottles using the first drawn milk. All lactating animals were sampled shortly after administration of 1 ml oxytocin injection to stimulate milk let down. Milk samples were taken in plastic vials and stored frozen until analysis (Fick *et al*., 1979).

Feed samples consumed by the animals being fed at farm were collected in five replicates for assay of zinc at each sampling period in cloth bags and were air-dried. Water samples were taken in borosilicate vials from pans fortnightly during both sampling seasons alongwith other samples in five replicates. The samples of forages, feed and faeces were dried in an oven at 60^{o}C for 48 h. Air and oven dried soil samples were pulverized in a ceramic mortar to pass through a 2mm sieve and were analyzed for Zn concentrations using a Mehlich-1 (Hesse, 1972; Rhue & Kidder, 1983) extraction procedure: 5 g of soil were added to 20ml of 0.05 N HCl in 0.025 N H_2SO_4 and final volume was analyzed.

Water and urine samples were filtered into sterilized plastic beakers and 1ml aliquots were used to prepare serial dilutions for analysis. Air and oven-dried samples of forage, feed and faeces were ground with a Wiley mill to pass through a 1 mm mesh. To prepare samples for estimation of zinc, representative dried and ground samples of about 2 g each of forages, feed and faeces 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 & Judson, 1986, Anon., 1990; Neathery *et al*., 1990). Direct dry or wet ashing of plasma and milk 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 and milk sample was taken into crucible after thawing. To pre digest, the samples were pre-treated 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 h 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 labelled plastic tubes suitable to fit the auto sampler of atomic absorption spectrophotometer (Perkin-Elmer Model 5000,City of Banska Stiavnica, Slovakia).

Statistical analysis: The data were analysed using a split-plot design (Steel & Torrie, 1980). Differences among means were ranked using Duncan's New Multiple Range Test (Duncan, 1955).

Results

The seasonal or sampling interval effects on soil, forage, water and feed Zn concentrations are shown in Fig. 1.

Soil: There was not a significant effect of seasons on soil Zn concentration, but in contrast, fortnights affected significantly. There was a sharp depression in Zn level up to 3rd fortnight in summer and up to last fortnight in winter. The Zn level in soil at the last two fortnights of summer and at the first two fortnights of winter was statistically equal. However, the soil Zn was slightly higher in winter than that in summer, particularly at the end of the season (Fig. 1a).

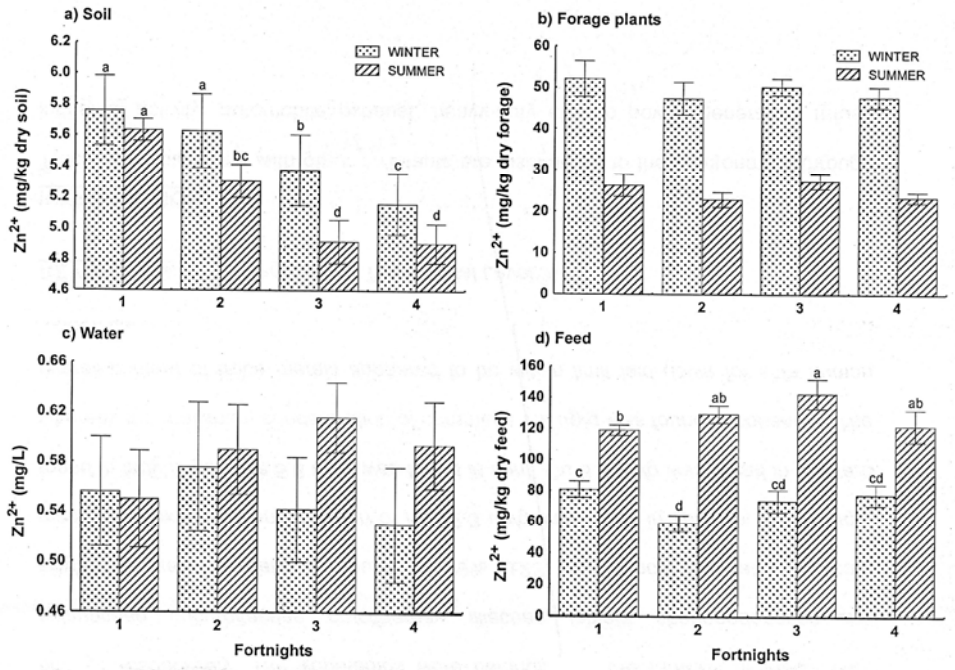

Fig. 1. Zn^{2+} concentration in (a) soil, (b) forage plants, (c) water and (d) feed at different fortnights during winter and summer seasons (sheep farm).
(Means with the same letters do not differ significantly at $p \leq 0.05$)

Forage plants: The forage Zn concentrations varied significantly during different seasons and sampling intervals. The forage Zn in winter at all fortnights of sampling was higher than that in summer (Fig. 1b). However, the pattern of increase or decrease in forage Zn level at different fortnights was non-consistent.

Water: There was no significant effect of seasons or sampling time on Zn level of water offered to sheep at farm. However, great fluctuations in water Zn were observed at different fortnights during both the seasons (Fig. 1c). Overall, water Zn was higher in winter than that during summer.

Feed: Feed Zn concentration was affected by the seasons, but not by the fortnights. The feed Zn levels at different fortnights during both seasons varied inconsistently. However, considerably higher concentration of Zn in feed was recorded in summer than that in winter (Fig. 1d).

Animal samples: The seasonal or sampling interval effects on the plasma, faeces, urine and milk Zn concentrations are shown in Fig. 2.

Lactating sheep

Plasma: The plasma Zn concentration was significantly affected by the seasons, but sampling periods had no effect on it. During summer the amount of Zn in plasma was

lower as compared to that in winter and it remained almost unaffected by the sampling time in both the seasons (Fig. 2a).

Faeces: No significant variation was found in Zn level excreted *via* faeces in two different seasons or at different sampling intervals. The concentration of Zn in faeces was slightly higher in summer than that in winter. The low value of faecal Zn was observed at the 4th fortnight during both seasons (Fig. 2b).

Urine: The concentrations of Zn excreted through urine in summer and winter did not differ significantly, but varied significantly at different sampling intervals within each season. Highest excretion of Zn *via* urine was observed at the last sampling interval during both seasons (Fig. 2c).

Milk: Zn level in milk was affected significantly by seasons but sampling time did not show its effect. Thus, the Zn level in milk at different fortnights was maintained uniform during both the seasons. Milk during winter contained markedly higher concentration of Zn than that during summer (Fig. 2d).

Non-lactating sheep: The seasonal or sampling interval effects on the plasma, faeces and urine Zn concentrations are shown in Fig. 2.

Plasma: Seasons and fortnights had no significant effect in changing Zn level in plasma. However, plasma Zn level was slightly higher in summer than that in winter. (Fig. 2e).

Faeces: Seasons and sampling periods did not show significant effects on faecal Zn level. However, Zn concentration in faeces was slightly higher in summer than that in winter, but the variation in faecal Zn level at different fortnights was non-consistent (Fig. 2f).

Urine: Owing to sharp changes in urine Zn concentration observed during sampling periods in summer and winter, the effect of the seasons or fortnights on urine Zn level was marked. In winter, a gradual increase in Zn level was observed except at the 4th interval where it was almost equal to that at the 3rd fortnight. In contrast, in summer a tendency of abrupt decrease though inconsistent, was found with time (Fig. 2g).

Male sheep: The seasonal or sampling interval effects on the plasma and faeces Zn concentrations are shown in Fig. 2.

Plasma: Zn concentration of plasma was not affected by the seasons or sampling periods. A slightly higher level of Zn in plasma was observed in winter than that in summer (Fig. 2h).

Faeces: From the analysis of variance of data for faecal Zn, it is evident that excretion of Zn through faeces was not under the control of seasons or sampling times but under certain other factors. A gradual increase in Zn level during summer and a decrease in winter were found with time (Fig. 2i).

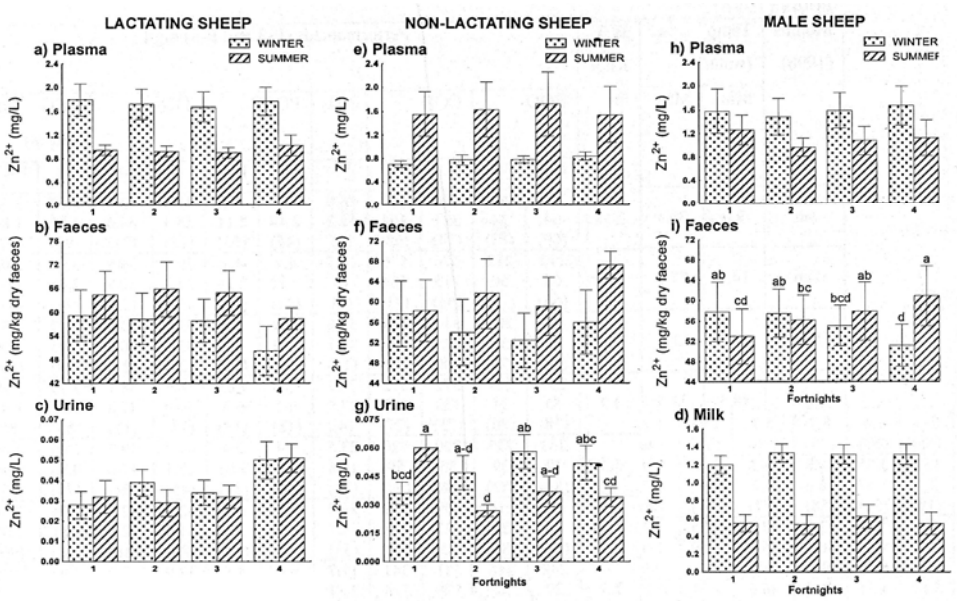

Fig. 2. Zn^{2+} concentration in different sample types of lactating, non-lactating, and male sheep at different fortnights during winter and summer seasons.
(Means with the same letters do not differ significantly at $p \leq 0.05$)

Discussion

The study has been performed in a semi arid region of Pakistan, the climate of which will certainly influence the mineral composition of soils and forages as well as the animal's metabolism. Therefore, results obtained only concerned grazing animals rearing in such region and under such sub tropical or hot semi arid conditions.

Soil, forage and plasma zinc concentrations were compared to establish critical values to determine the various categories of deficient levels. The critical level for soils indicates the zinc 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 (Khan *et al*., 2005).

Extractable soil Zn concentrations were adequate (1mg/kg) (Rhue & Kidder, 1983) for normal plant growth during both seasons of the year. These values fall within the range of those reported by Tiffany *et al*., (2001) and Tejada *et al*., (1985, 1987). These were higher than those found in north Florida by Cuesta *et al*., (1993) in central Florida by Espinoza *et al*., (1991) and in Venezuela by Rojas *et al*., (1993). These extractable soil Zn concentrations above the critical level provide adequate Zn for plant growth (Rhue & Kidder, 1983).

Forage Zn concentration was also found above the requirements of ruminants (30 mg/kg) (Anon., 1995) but only in winter. However, the level in summer was considered

slightly deficient for growing and lactating animals (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. The feed and water Zn levels were found to have contributed major part of the ruminant requirements during summer and winter, respectively. The feed Zn content was in excess of the requirements for sheep, but below the maximum tolerable levels.

A number of factors including soil, plant species, pasture management and climate may affect the likelihood of Zn deficiency in ruminants. 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). Over all, total Zn level contained in all sources was higher in summer than that in winter. In the summer season the plasma Zn level was higher only in non-lactating sheep than that of other two groups. Plasma Zn showed a response to the dietary Zn content during summer in non-lactating sheep. While the higher concentrations in diet Zn in summer was ineffective in increasing the plasma Zn levels in lactating and male sheep. Physiological status also seemed to have a significant effect on plasma Zn levels. In lactating sheep during winter, which corresponded to early lactation, plasma Zn level was higher than non-lactating and male sheep. The milk Zn concentration was higher in early lactation period than late lactation. The levels of faecal and urine Zn were not affected by the seasons, but the Zn content of faeces indicated the pasture Zn levels in all classes of animals being slightly higher in summer than that in winter. Urine Zn level seemed to be affected by the physiological status of an animal. The possible explanation of the high amount of faecal Zn excreted in lactating sheep during summer may be given that there is low plasma Zn in this season. A significant association between Zn concentration of pasture and in the faeces from all groups of animals was observed in the study.

Signs of Zn deficiency are non-specific, therefore Zn status should be considered in cases of unexplained reproductive problems in ewes (Apgar & Fitizgerald, 1985; Khan *et al*., 2005). Plasma Zn is a reasonable criterion however, values are susceptible to animal stress during sampling and can fluctuate rapidly (Underwood, 1981). According to Conrad (1978) the plasma Zn is rapidly and markedly reduced with severely deficient diets, although concentrations were not greatly influenced by marginally deficient forage Zn as found in this study. The role of feed Zn concentration seemed to have elevated the plasma Zn level in different group of sheep. Based on soil, forage, feed, water and animal samples analyses, it was concluded that no sample was deficient except forage in summer. Therefore, a mixture containing Zn should be continually supplemented particularly during summer when forage Zn contents were on borderline levels.

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(Received of Publication 10 March 2007)