

EFFECT OF SEASONAL VARIATION ON ZINC CONTENT OF SOIL, FORAGE, WATER, FEED, AND SMALL RUMINANTS GRAZING THE NATIVE AND IMPROVED PASTURE DURING DIFFERENT SEASONS IN THE SEMI-ARID REGION OF PAKISTAN

Z. I. Khan¹, E. E. Valeem^{2*}, A. Hussain¹, M. Ashraf¹, M. Y. Ashraf³, L.R. McDowell⁴ M. S. Akhtar¹ and M. A. Mirza⁵

¹Department of Botany, University of Agriculture, Faisalabad-Pakistan.

²Govt. Degree Sci. & Com. College Gulshan-e-Iqbal, Karachi-75300, Pakistan.

³Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad, Pakistan.

⁴Animal Science Department University of Florida P.O. Box 110910 Gainesville, F.L. 32611-0691

⁵Department of Animal Nutrition, University of Agriculture, Faisalabad-Pakistan.

ABSTRACT

An investigation was conducted to evaluate the zinc status of three different classes of grazing goats on the basis of zinc concentrations in pasture and animal samples as affected by season and class of animals in central Punjab, Pakistan. Soil, water, plasma, urine, and fecal zinc levels of lactating and male goats did not show seasonal differences while forage, feed and milk zinc concentrations in lactating and faeces in non lactating animals had seasonal variations with greater concentrations in winter than in summer except feed which was higher in summer than that in winter. Mean soil, forage and plasma zinc concentrations were adequate for plants and animals requirements during both seasons. Milk concentration of zinc showed seasonal variation with being consistently higher in winter than in summer. In both seasons, most milk samples had lower/higher zinc concentrations than reported reference values of goat milk. The overall zinc status of these goats based on pasture and animal samples may be considered adequate mainly due to supplemental feed provided containing different amount of zinc, since forage zinc concentration were deficient particularly in summer season.

Keywords: Zinc, Seasonal variation, soil, plant, animal, goat, Pakistan

INTRODUCTION

Mineral imbalances in soil and forages have been associated with low reproductive rates and animal production among grazing ruminants (McDowell, 1985, 1992; McDowell *et al.*, 1984).

Mineral composition of plants is affected by soil-plant factors, including pH, drainage, fertilization, forage species, forage maturity, and interaction among minerals (Gomide, 1978; Reid and Horvath, 1980). Analysis of forage can provide insights into the likely nutrient status of animal; however, factors such as soil contamination, variation in availability of ingested minerals, and selective grazing may limit the value of analysed mineral concentrations. In Pakistan, pasture is the only source of feed for grazing animals during the year and mineral supplements are not usually provided, especially for small ruminants, consequently mineral deficiencies occur in these animals. Among minerals zinc is one of the essential micro elements for many body functions (Hambidge *et al.*, 1986; Brody, 1999).

Zinc is an essential element in the nutrition of animals and plants. Zinc is required in the genetic make-up of every cell and is an absolute requirement for all biologic reproduction. Zinc is needed in all DNA and RNA syntheses and is required at every step of the cell cycle. DNA is about 5000 times less susceptible to damage by Zn ion than is RNA, suggesting its roles in the predominant evolutionary selection of DNA, rather than RNA, as the bearer of the primary genetic information (Butzow and Eichhorn, 1975; Ho and Ames, 2002). In prebiotic chemistry of Earth billions of years ago, zinc most likely was the first effective nonenzymatic polymerase. Zinc remains an essential component of all DNA and RNA polymerases examined today (Kornberg, 1982). "Zinc fingers" are finger-like protrusions extending from transcription factors or gene-regulating proteins and fastening to the wide, major groove of a DNA molecule (Rhodes and Klug, 1993; Brody, 1999; Maret, 2002).

About 2 grams of zinc is distributed throughout the body (average 10 to 200 mmg/gram) of an adult animals (Zapsalis and Beck, 1985). Absorption of dietary zinc occurs over the duodenal and jejunal regions of the gastrointestinal tract (Cousins, 1996). Active transport of zinc into portal blood is mediated by metallothionein. Zinc competes with other metals for absorption, and absorption is believed greatly retarded by ingestion of fiber and phytates (Zapsalis and Beck, 1985; Oberleas and Harland, 1977).

Plasma zinc is complexed to organic ligands. Zinc-albumin complexes account for about 50 percent of the zinc, and the metal is readily exchangeable throughout the peripheral circulation (Bremmer, 1993). About 7 to 8 percent is loosely bound to amino acid constituents in plasma. The remaining 40 percentage of plasma zinc is largely bound to

macroglobulins and unavailable for nutritional purposes. Serum and plasma zinc concentrations in adults range from 80 to 150 mmg/dL, although circadian diurnal fluctuations occur in concentration (Zapsalis and Beck, 1985). Circadian diurnal variation peaks at 9:30 AM and reaches a low at 8 PM with differences of 19 mmg/Dl (Markowitz *et al.*, 1985). Rather than an enterohepatic circulation, zinc experiences a similar enteropancreatic recycling (Zapsalis and Beck, 1985).

Zinc is an integral component of about 200 metalloenzymes, including carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, glutamic dehydrogenase, latic dehydrogenase, and alkaline phosphatase as well as hormones, such as thymulin, testosterone, prolactin, and somatomedin. (Zapsalis and Beck, 1985; Cousins, 1996; Castillo-Duran and Weisstaub, 2002; McDowell, 2003).

Many Natural occurring diets contain adequate amounts of Zn, but only small amounts of the element seem to be available to the animal. Therefore, factors must be present that reduce Zn absorption or impair its utilization in the animal. The metabolism of Zn may be influenced by interaction with other elements like cadmium, Ca, Cu, Fe, Se, and Mn (Ivan and Grieve, 1975). The presence of chelating materials, phytic acid, levels of certain minerals, particularly Ca, and many unknown factors affect Zn absorption in monogastric animals. However, in most instances, the factors that many affect the requirements of ruminants are unknown. In normally fed ruminants, there is at present lack of direct evidence that Ca, phytic acid, or other factors in plant proteins decrease Zn absorption (Miller, 1970; Hambidge *et al.*, 1986; Baker and Ammerman, 1995; Cousins, 1996; Brody, 1999).

The minimum Zn requirement of ruminants varies with the chemical form, the combination in which the element occurs with other components of the diet, and with the criteria of adequacy employed. The suggested Zn requirement for cattle is 20-50 ppm (NRC, 1984, 1978; ARC, 1980).

The Zn requirement would be dependent on the criterion of adequacy employed. For example, the minimum Zn requirements for spermatogenesis and testicular development in young male sheep are significantly higher than they are for body growth. Testicular growth and spermatogenesis in ram lambs were markedly subnormal at 17ppm dietary Zn and entirely normal at intakes of 32 ppm. If body growth is taken as the criterion of adequacy, Zn requirements would clearly be lower than for similar animals kept for breeding (O'Dell, 1979; NRC, 1998; DRI, 2001).

Early effects of Zn deficiency include reduced feed intake, reduced growth rate and feed efficiency, followed by skin disorders. Parakeratosis of the skin is perhaps the most obvious clinical sign of severely Zn-deficient cattle and goats (Miller, 1979). In calves, the scrotum, head, and the area around the nostrils, neck, and legs most often are parakeratotic. In lactating cows, teats may show considerable parakeratosis. The effects, including lesions, of a Zn deficiency in goats and sheep are similar to those in cattle (Miller, 1979). Zinc-deficient calves grow more slowly and are lethargic, their wounds heal very slowly, if at all, and they are highly susceptible to nonspecific secondary infections (Miller, 1970). Testicular growth and development are often retarded in a Zn deficiency. Inhibited testicular growth has been observed in Venezuela in cattle consuming low-Zn rations (J. Perdomo, personal communication) (NRC, 1995; Gardner *et al.*, 2002).

Zinc deficiency symptoms are nonspecific, perhaps in part because of their need in so many enzymes and their critical roles in both protein synthesis and molecular genetics. Many enzymes may become nonfunctional in the absence of zinc, even though the presence of the enzyme remains undisturbed. The integrity of cell membranes, including the integrity of red and white blood cells, depends upon loosely bound ionic zinc. Moreover, zinc deficiency is a cause of 33 percent of all olfactory disorders. In many respects, the total picture of zinc deficiency is reminiscent of essential amino acid deficits (Zapsalis and Beck, 1985).

Zinc deficiency stunts growth and causes serious metabolic disturbances. Inadequate intake in people and animals results in serious immunodeficiency, increased numbers of infections, increased severity of infections, stunted growth, and delayed sexual maturation. As deficits become worsened, skin and orificial lesions develop only to be subjected to an unchallenged bacterial invasion, yet lesions do not mount a significant inflammatory response (Zapsalis and Beck, 1985). Therefore, severe zinc deficiency produces a patently obvious immunodeficiency in the cell-mediated (T-cell) immune system. Advanced deficiency culminates in diarrhea, severe wasting, and ultimately death. This scenario is topical of at least 12 animal species including man (Zapsalis and Beck, 1985).

Large areas of Zn-deficient soils exist in many countries in which significant responses have been demonstrated in the yield of pastures and crops to applications of Zn-containing fertilizers (Underwood, 1981). Zinc deficiency, under experimental conditions, has been produced by many workers. However, a deficiency under natural conditions was once thought unlikely. Since few reports of clinical signs of Zn deficiency in grazing animals appeared, it was generally assumed that the herbage of Zn-deficient soils carries enough Zn for the needs of animals (McDowell, 2003).

Although severe clinical Zn deficiencies in ruminants have been described in different regions of the world, this likely is of much less economic importance than borderline subclinical deficiencies. The possibility of a widespread,

mild or borderline deficiency of economic importance should be investigated. Marginal Zn deficiency in grazing sheep and cattle, characterized by subnormal growth, fertility, and serum Zn values but without other clinical signs, is more widespread than was earlier believed (Underwood, 1981). The first effects of a mild Zn deficiency would be expected to be decreased feed intake, growth, feed efficiency, and milk production, resistance to infection and stress, and lower reproductive efficiency (Miller, 1979). A borderline Zn deficiency, which is more likely to occur, would be difficult to diagnose clinically (McDowell, 1985, 2003).

Biochemical changes with the most promising diagnostic value are decline in Zn concentration of plasma, hair, and bone, with a tissue decline of alkaline phosphatase. Kirchgerner and Roth (1981) reported the Zn-binding capacity of serum and the activity of alkaline phosphatase as good indicators of Zn status. Under survey conditions for determining the likelihood of deficiencies for large numbers of ruminants, Zn concentrations in plasma (<0.6-0.8 $\mu\text{g/ml}$) and forage (<40 ppm) would be indicators (McDowell *et al.*, 1984; McDowell, 1985; Cousins, 1996; Chester, 1997).

The vegetation of many 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 and other crops residues are unknown in Pakistan. The purpose of this investigation was to identify zinc composition of soil and dietary components during two seasons of the year, keeping in view the importance of this element in animals, as well as determine grazing animals requirement of zinc.

MATERIALS AND METHODS

Soil, forage, feed, water, and animal samples were taken from the farm "Livestock Experimental Station" located in southern Punjab, owned by the Govt. of Punjab, Pakistan. 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 the pasture. The five sub samples of soil and forages were taken from the beginning, middle, and end of the pasture.

Each composite soil samples which was derived from five sub-samples taken at a depth of 20cm 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 goats on the farm. Forages were collected after careful observation of goats 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 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.

For sampling purpose animals were divided into 3 classes, lactating/non-lactating and male animals respectively, with 10 animals per class. Blood plasma, milk, faeces and urine samples from lactating, plasma, faeces, and urine from non-lactating and plasma and faeces from male goats 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 heparine as an anticoagulant, and plasma was separated by centrifugation and was harvested in to polyethylene tubes and frozen at -20°C for subsequent analysis for zinc. 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 10ml aliquot was transferred to a polyethylene tubes, acidified with 0.3ml concentrated HCl, and frozen for subsequent analysis (Tucker *et al.*, 1990). The fecal samples were kept in open bags and allowed to dry in sun to constant atmospheric moisture (<30%). Milk samples were collected in 125ml nalgene bottles using the first drawn milk. All lactating animals were sampled shortly after administration of 1ml 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 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°C for 48h.

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 and Kidder, 1983) extraction procedure: 5g of soil were added to 20ml of 0.05 M HCl in 0.025 M 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

fit through a 1-mm mesh. To prepare samples for estimation of zinc representative dried and ground samples of about 2g each of forages, feed, and faeces were digested by nitric acid and perchloric acid (3:1) at 250°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, AOAC, 1990; Neathary *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 were taken into crucible after thawing. To pre digest, the samples were pretreated with 50% HNO₃ over an electric heater until smoking ceases to char the majority of organic matter. These samples then were ashed for 6 hours at 550°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 50ml. 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 (Mpofo *et al.*, 1999; Nockels *et al.*, 1993; AOAC, 1990; Fick *et al.*, 1979).

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 Zn by atomic absorption spectrophotometry (Perkin-Elmer Model 5000).

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

Soil, forage, and plasma zinc concentrations were compared to established 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 each nutrient.

RESULTS

PASTURE SAMPLES

Soil

There was no seasonal effect on soil Zn²⁺ concentration, whereas the effect of sampling period was found to be significant (Table 1). Soil Zn²⁺ was found to be maximum at the 2nd fortnight during winter and at the 3rd fortnight during summer, whereas, at the remaining fortnights the soil Zn²⁺ did not differ significantly. (Fig. 1a).

Forage plants

Both seasons and sampling time had significant effects in changing the forage Zn²⁺ concentration (Table 1). Forage Zn²⁺ level was markedly higher during winter than that during summer. A decreasing trend in forage Zn²⁺ level was observed with time during both seasons (Fig. 1b).

Water

The concentration of Zn²⁺ in water was not affected by the seasons or the fortnights (Table 1). At the initial three fortnights, a progressive decrease in water Zn²⁺ level was observed during both seasons followed by an increase in water Zn²⁺ at fortnight 4 (Fig. 1c).

Feed

Significant seasonal and non-significant fortnights effects were found in changing the feed Zn²⁺ level (Table 1). As is evident from Fig-1d, there was a decreasing trend in feed Zn²⁺ in the early summer up to fortnights 3. In contrast, during winter, a non consistent variation in feed Zn²⁺ was observed at different fortnights. Overall, feed Zn²⁺ was markedly higher in summer than that in winter.

ANIMAL SAMPLES

LACTATING GOATS

Plasma

No seasonal or sampling interval effects were found in changing the plasma Zn²⁺ concentration (Table-2a). The plasma Zn²⁺ concentration remained almost uniform at different fortnights during both seasons (Fig-2a). Overall, lactating animals had higher Zn²⁺ in plasma during summer than that during winter.

Table 1. Analysis of variance of data for Zn^{2+} concentration in soil, forage plants, water and feed at different fortnights during winter and summer seasons at goat tanch.

Source of variation S.O.V	Degree of freedom df	M e a n s q u a r e s			
		Soil	Forage plants	Water	Feed
Season (S)	1	1.06 ^{ns}	19250.16 ^{***}	0.0004 ^{ns}	3097.60 ^{**}
Error	8	1.85	23.19	0.005	68.25
Fortnight (FN)	3	1.59 [*]	292.62 ^{***}	0.0082 ^{ns}	94.70 ^{ns}
Sx FN	3	1.98 ^{**}	43.36 ^{**}	0.0032 ^{ns}	306.20 [*]
Error	24	0.39	5.74	0.0030	77.53

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

Table 2. Analysis of variance of data for Zn^{2+} concentration in blood plasma, faeces, urine and milk of lactating goats at different fortnights during winter and summer seasons.

Source of variation S.O.V	Degree of freedom df	M e a n s q u a r e s			
		Plasma	Faeces	Urine	Milk
Season (S)	1	1.02 ^{ns}	1513.80 ^{ns}	0.00003 ^{ns}	19.71 ^{***}
Error	18	3.30	6751.73	0.0002	0.39
Fortnight (FN)	3	0.09 ^{ns}	2039.95 ^{***}	0.000008 ^{ns}	0.43 ^{ns}
S x FN	3	0.024 ^{ns}	32.90 ^{ns}	0.00003 ^{ns}	0.20 ^{ns}
Error	54	0.21	38.97	0.00012	0.26

Table 3. Analysis of variance of data for Zn^{2+} concentration in blood plasma, faeces, and urine of non-lactating goats and that of plasma and faeces of male goats at different fortnights during winter and summer seasons.

Source of variation S.O.V	Degree of freedom df	M e a n s q u a r e s				
		Non-lactating goats			Male goats	
		Plasma	Faeces	Urine	Plasma	Faeces
Season (S)	1	0.44 ^{ns}	43.51 ^{ns}	1.09 ^{ns}	1.96 ^{ns}	475.31 ^{ns}
Error	18	1.11	3488.37	2.47	2.49	5102.47
Fortnight (FN)	3	0.11 ^{ns}	516.98 ^{**}	0.04 ^{***}	0.31 ^{ns}	609.55 ^{***}
S x FN	3	0.03 ^{ns}	19.61 ^{ns}	0.09 ^{***}	0.31 ^{ns}	40.95 ^{**}
Error	54	0.06	94.93	0.004	0.35	9.64

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

Faeces

There was a non-significant effect of seasons on fecal Zn^{2+} but that of fortnights was significant (Table 2a). A consistent decrease in fecal Zn^{2+} was observed at all fortnights with time during both seasons (Fig. 2b).

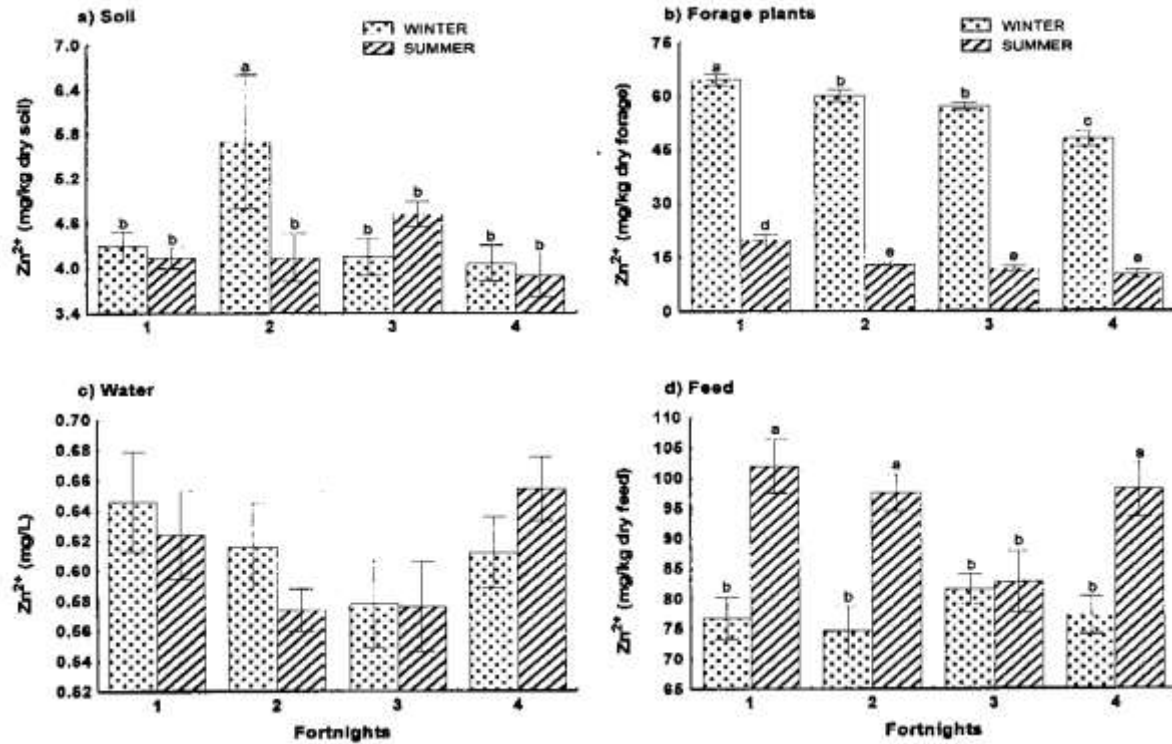


Fig.1. Zn²⁺ concentration in (a) soil, (b) forage plants, (c) water and (d) feed at different fortnights during winter and summer seasons at goat ranch.

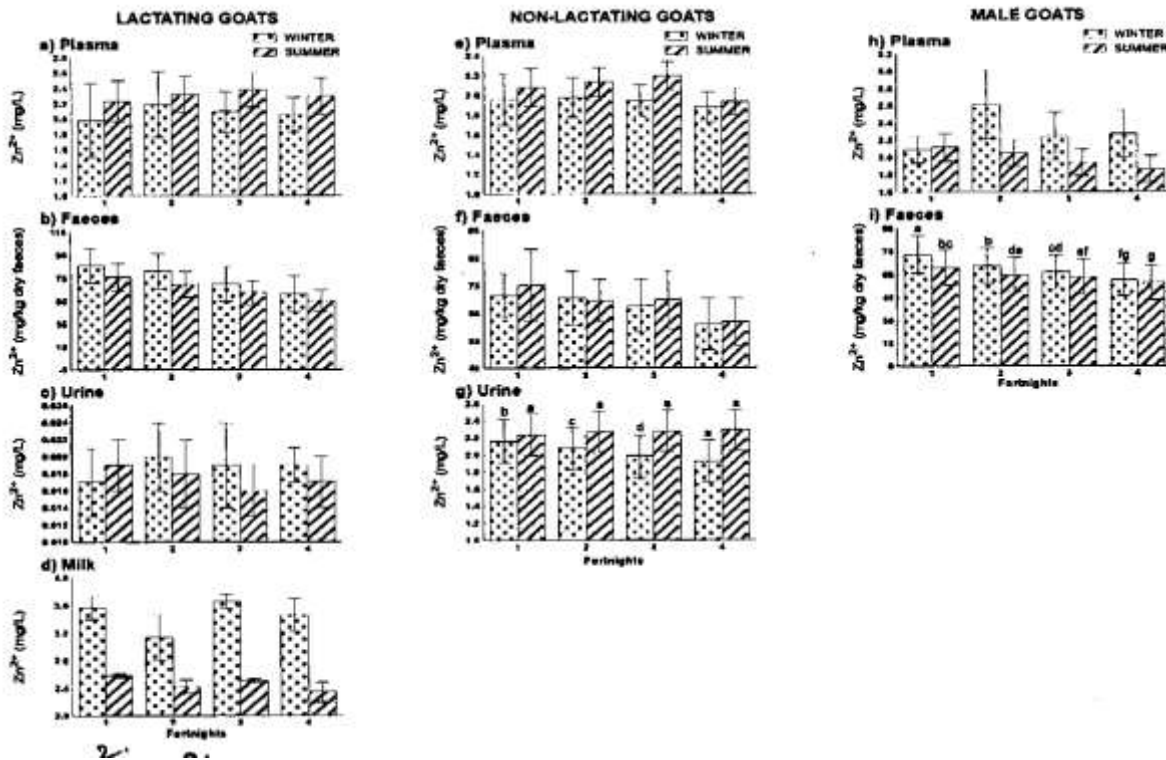


Fig.2. Zn²⁺ concentration in different sample types of lactating, non-lactating and male goats at different fortnights during winter and summer seasons.

Urine

The urine Zn^{2+} concentration was not affected by the seasons or fortnights (Table 2a). A slightly higher amount of Zn^{2+} excretion through urine was observed in winter than that in summer (Fig. 2c). During summer, there was a consistent decrease in Zn^{2+} level up to fortnight 3 followed by slight increase at fortnight.

Milk

A significant seasonal effect and non-significant of sampling period was observed on milk Zn^{2+} concentration (Table 2a). Zn^{2+} level in milk was markedly higher in winter than that in summer. During both seasons changes in Zn^{2+} level were inconsistent at different fortnights (Fig. 2d).

NON-LACTATING GOATS

Plasma

Both seasons or fortnights were found to play no role in changing the plasma Zn^{2+} concentration (Table 2b). Overall, plasma contained higher amounts of Zn^{2+} during summer than that during winter (Fig. 2e).

Faeces

Fecal Zn^{2+} concentration was affected significantly by sampling interval but not by seasons (Table 2b). In winter, fecal Zn^{2+} remained unchanged at the first three fortnights followed by a slight decrease at the 4th fortnight (Fig. 2f). An almost similar pattern of change in fecal Zn^{2+} was found during summer.

Urine

Non-significant seasonal effect but significant of that of fortnights was found on the urine Zn^{2+} concentration (Table 2b). A consistent decrease in Zn^{2+} level was observed with time of sampling during winter (Fig. 2g), whereas during summer urine Zn^{2+} concentration remained uniform at all fortnights. Generally, higher excretion of Zn^{2+} through urine was found in summer than that in winter.

MALE GOATS

Plasma

No seasonal or fortnight effects on plasma Zn^{2+} concentration were found (Table 2b) Amount of Zn^{2+} in plasma was higher in winter than that in summer. In winter, a sharp increase in plasma Zn^{2+} level was observed from 1st to 2nd fortnight followed by a sharp decrease up to fortnight 3 with almost no significant change at the last fortnight (Fig. 2h). In summer, the plasma Zn^{2+} level decreased consistently with time of sampling.

Faeces

No significant seasonal affect but significant that of sampling period was found in changing the fecal Zn^{2+} concentration (Table 2b). A consistent decrease in fecal Zn^{2+} level was observed with time of sampling during winter (Fig. 2i). The same was true for fecal Zn^{2+} level during summer.

DISCUSSION

In the present study soil Zn^{2+} contents across all samples during both seasons were almost similar and these values of soil Zn^{2+} were above the critical level for normal plant growth (Rhue and Kidder ,1983). Similar values above critical levels have already been reported by Prabowo *et al.* (1990) in Indonesia and Tiffany *et al.* (2001) in North Florida. Slightly higher soil Zn^{2+} in winter than in the summer season as found in this study is in agreement with the findings of Velasquez-Periera *et al.* (1997). In contrast, Pastrana *et al.* (1991) found higher soil Zn^{2+} concentration in summer than that in winter. Extractable Zn^{2+} has been found to be affected by low pH and cultivation (Aubert and Pinta, 1977). Zn^{2+} may be more soluble and susceptible to leaching in low pH soils and high rainfall areas.

Forage Zn^{2+} concentration showed seasonal variation, with high concentration during the winter season and above the requirement of ruminants, but in summer all samples were deficient or below the critical level (McDowell *et al.*, 1993). Similar seasonal differences in forage Zn^{2+} levels were reported by Velasquez-Pereira *et al.* (1997) in Nicaragua. Forage Zn^{2+} varied considerably depending on various ecosystem, characteristics, plant species, and stage of maturity. However, Kabata-Pendias and Pendias (1992) reported that Zn^{2+} concentration of certain forages from different countries do not differ significantly.

There are some controversial reports on Zn^{2+} concentration in plants at the adult stage. For example; Underwood (1981) reported that as plants mature, their Zn^{2+} concentration decreases. In contrast, high concentration of Zn^{2+} has been found in old leaves of plants (Kabata-Pendias and Pendias, 1992).

High and low Zn^{2+} contents in the feed during summer and winter were within the range of requirements of ruminants and water Zn^{2+} level was found to be uniform during both seasons. Plasma Zn^{2+} concentration did not differ during different seasons in all animal classes. However, in all classes, it was above the critical level (McDowell *et al.*, 1993). There were slight differences among animals, perhaps because of physiological status, age and growth rate of animals. It is also known that absorption and utilization of Zn^{2+} is affected by many elements, which include Cd, Ca, Fe, Mg, Mn, Mo and Se (Ivan and Grieve, 1975). Similar levels of plasma Zn^{2+} have also been reported by Mpofu *et al.* (1999) in Zimbabwe and by Velasquez-Periera *et al.* (1997) from the plasma of cattle in Nicaragua. Milk Zn^{2+} concentrations recorded in this study showed seasonal influence. These were slightly below the normal limits found in literature (Underwood, 1981), which should not be considered as large aberration. The milk Zn^{2+} concentrations found in this study are similar to those reported by Cuesta *et al.* (1993) in those of grazing cattle in north Florida.

Fecal and urine Zn^{2+} contents were not affected by the seasons in different group of animals. However, lactating animals were found to excrete more Zn^{2+} during winter than that during summer as compared to other groups. However, urine Zn^{2+} concentration of non-lactating goats was considerably higher than that excreted by lactating goats.

Some factors affecting plasma Zn^{2+} concentrations are collection procedures and stress. Corah and Ives (1991) reported that plasma Zn^{2+} concentration is increased by hemolysis and decreased by stress. Wegner *et al.* (1973) observed that animals under hyperthermic stress showed a decline in plasma/serum Zn^{2+} concentration, and this low Zn^{2+} level remained after 192 h of post stress. Graham (1991) reported that there is not an accurate biochemical index of Zn^{2+} status due to the influence of diseases on enzymes such as carbonic anhydrases and alkaline phosphatase. Similar results, as found in this study where forage Zn^{2+} concentrations were below the requirement for grazing animals, were also found in Argentina (Balbuena *et al.*, 1989). The small intestine is the main site of Zn^{2+} absorption. Absorption is affected by dietary level, amounts and proportions of several other elements and dietary components (Underwood, 1977). A high percentage of all Zn^{2+} ingested, is excreted through faeces by entrance via pancreatic secretions and very little by way of urine (Miller, 1970). Therefore high Zn^{2+} content of feed and forage in winter only as found in this study does not mean that is sufficient to the requirements of animals. Many naturally occurring diets contain adequate amounts of Zn^{2+} , but only small amounts of the element seemed to be available to the animals. Therefore, factors must be present that reduce Zn^{2+} absorption or impair its utilization in the animals.

It is concluded that the Zn^{2+} contents of feed were found effective in raising plasma Zn^{2+} level in lactating and non-lactating goats while in male goats plasma Zn^{2+} was not affected by such supplement during summer. As the Zn^{2+} content of forage was low during this season therefore, elevation of plasma Zn^{2+} in lactating and non-lactating goats may possibly be due to supplementation of Zn^{2+} contained in feed to complement the low forage Zn^{2+} concentration during summer.

REFERENCES

- A.O.A.C. (1990). *Official Methods of Analysis of the Association of Analytical Chemists*. 14th Ed. Arlington, Virginia, U.S.A.
- A.R.C. (1980). "The Nutrient Requirements of Farm Livestock, No. 2 Ruminants." London.
- Aubert, H. and M. Pinta. (1977). *Trace Elements in Soils*. Elsevier Scientific Publishing Co., NY.
- Baker, D.H. and C.B. Ammerman (1995). In: *Bioavailability of Nutrients of Animals* (C.B. Ammerman, D.H. Baker, and A.J. Lewis eds), p. 367, Academic Press, San Diego.
- Balbuena, O., L.R McDowell, H.O. Toledo, J.H Conrad, N.S. Wilkinson and Y. F.G. Martin (1989). Estudio de la nutrición mineral de los bovinos para carne del este de las provincias de Chaco y Formosa (Argentina). 5. Cobalts y Seleno. *Vet. Arg.*, 61:25.
- Bremner, I. (1993). In: *Proc. 8th International Symp. Trace Elements in Man and Animals* (TEMA-8). Verlag Media Touristic, Gersdorf, p. 507, Germany.
- Brody, T. (1999). In: *Nutritional Biochemistry*. Academic Press, New York. p. 803.
- Butzow, J.L. and G.L. Eichhorn (1975). Different susceptibility of DNA and RNA to cleavage by metal ions. *Nature* (London), 254: 358-359.
- Castillo-Duran, C. and G.N. Weisstaub (2002). In: *Trace Elements in Man and Animals* (TEMA-11). (J.R. Prohaska and J.C.King, eds.). Univ. California, Berkeley, CA. p. 40.

- Chesters, J.K. (1997). In: *Handbook of Nutritionally Essential Mineral Elements* (B.L. O'Dell and R. A. Sunde, eds.). Marcel Dekker, Inc., New York. p. 185.
- Corah, L.R., and S. Ives (1991). The effects of essential trace minerals on reproduction in beef cattle. *Vet. Clin. N. Am. Food Anim. Practice*, 7: 153.
- Cousins, R.B. (1996). In: *Present Knowledge in Nutrition*, 7th ed. International Life Science Institute-Nutrition Foundation, (L. J. Filer and E. E. Ziegler, eds.), p. 293. Washington, D.C.
- Cuesta, P.A. L.R. McDowell, W.E. Kunkle, F. Bullock, A. Drew. N.S. Wilkinson and F.G. Martin (1993). Mineral concentration in liver and milk of grazing beef cattle as related to season and location. *Int. J. Anim. Sci.*, 8: 189-193.
- DRI (Dietary Reference Intakes) (2001). *Panel on Micronutrients of Food and Nutrition Board*. National Academy Press, Washington, D.C.
- Duncan, D.B. (1955). Multiple range and multiple F-test. *Biometrics*, 11:1-42.
- Fick, K.R., L.R. McDowell, P.H. Miles, N.S. Wilkinson, J.D. Funk and J.H. Conrad (1979). *Methods of mineral analysis for plant and animal tissues*, 2nd ed. Dept. Anim. Sci., Univ. Florida, Gainesville.
- Gardner, J.M., C.A. Powell and S.M. Grantham-McGregor (2002). In: *Trace Elements in Man and Animals – 11 (TEMA-11)* (J.R. Prohaska and J.C.King, eds.). Univ. California, Berkeley, CA. p.37.
- Gomide, J.A. (1978). Mineral Composition of Grasses and Tropical leguminous forages, In: *Latin American Symposium on Mineral Nutrition with grazing ruminants*. (J.M. Conrad and L.R. McDowell (eds.)). Uni. of Florida, Gainesville, F.L. pp. 32-40.
- Graham, T.W. (1991). Trace element deficiencies in cattle. *Vet. Clin. North America: Food Anim. Pract.*, 7: 153-215.
- Hambidge, K.M., C.E. Casey and N.F. Krebs (1986). In: *Trace Elements in Human and Animal Nutrition*, Vol. 2 (W. Mertz, ed.), P. 1. Academic Press, New York.
- Hesse, P.R. (1972). *A textbook of soil analyses*. Chemical Publishing Co., NY.
- Ho, E. and B.N. Ames (2002). In: *Trace Elements in Man and Animals (TEMA-11)* (J.R. Prohaska and J.C.King, eds.). Univ. California, Berkeley, CA.p.102.
- Ivan, M. and C.M. Grieve (1975). Effect of copper and manganese supplementation of high concentration digestibility, growth and tissue content of Holstein Calves. *J. Dairy Sci.*, 58: 410-417.
- Kabata-Pendias, A. and H. Pendias (1992). *Trace Elements in Soils and Plants*. CRC Press Inc., Boca Raton, FL.
- Kirchgeßner, M., and J.P. Roth, 1981. In: *Proc. Trace Elem. Metab. Man Anim. (TEMA-4)* (J. McC. Howell and J.M. Gawthorne, eds.). Australian Academy of Science, Canberra, Australia. p.32.
- Koh, T.S. and G.T. Judson (1986). Trace elements in sheep grazing near a lead-zinc smelting complex at port pirie, South Australia. *Bull. Environ. Contaim Toxicol.*, 37: 87-95.
- Kornberg, A. (1982). Origin of DNA on earth. In: *Supplement to DNA replication*. San Francisco. W.H. Freeman; S. Maret, W. (2002). In: *Trace Elements in Man and Animals (TEMA-11)* (J.R. Prohaska and J.C. King, eds.). Univ. California, Berkeley, CA.p.30.
- Markowitz M.E. J.F. Rosen and M. Mizruchi (1985). Circadian variations in serum zinc (Zn) concentrations: correlation with blood ionized calcium, serum total calcium and phosphate in humans. *American Journal of Clinica Nutrition*, 41: 689-696.
- McDowell, L.R. (1985). In: *Nutrition of Grazing Ruminants in Warm Climates*. Academic Press New York, pp-443.
- McDowell, L.R. (1992). *Minerals in Animal and Human Nutrition*. Academic Press, San Diego, Calif.
- McDowell, L.R. (2003). *Minerals in Animal and Human Nutrition* (2nd Ed.). Uni. Florida Gainesville.
- McDowell, L.R., J.H. Conrad and F.G. Hembry (1993). *Mineral for Grazing Ruminants in Tropical Regions*. Univ. of Florida, Gainesville.
- McDowell, L.R., J.H. Conrad and G.L. Ellis (1984). Mineral deficiencies and imbalances, and their diagnosis. In: *Symposium on Herbivore Nutrition in Sub-Tropics and Tropics-Problems and Prospects* (F.M.C. Gilchrist and R.I. Mackie, eds.). Craighall, South Africa. pp. 67-88.
- Miller, W.J. (1970). Zinc nutrition of cattle: A review. *J. Dairy Sci.*, 53: 1123.
- Miller, W.J. (1979). *Dairy Cattle Feeding and Nutrition*. Academic Press, New York.
- Miller, W.J., and P.E. Stake, 1974. In: *Proceedings Georgia Nutrition Conference for Feed Industry*. pp. 25-43. Univ. of Georgia, Atlanta.
- Mpofu, I.D.T., L.R. Ndlova and N.H. Casey (1999). The copper, cobalt, iron, selenium and zinc status of cattle in the Sanyati and Chinamhora small holder grazing area of Zimbabwe. *Asian-Aust. J. Anim. Sci.*, 12: 579-584.
- N. R. C. (1978). *Nutrient Requirements of Dairy Cattle*, Nutrient Requirements of Domestic Animals, No. 3. Natl. Acad. Sci., Washington, D.C.

- N. R. C. (1984). *Nutrient Requirements of Beef Cattle*, 6th Rev. ed. Nutrient Requirements of Domestic Animals, No.4. Natl Acad. Sci. Washington, D.C.
- N. R. C. (1995). *Nutrient Requirements of Domestic Animals, Nutrient Requirements of Laboratory Animals*. National Academy of Science-National Research Council, Washington, D.C.
- N. R. C. (1998). *Nutrient Requirements of Domestic Animals.* " *Nutrient Requirements of Swine*, 10th Ed. National Academy of Science-National Research Council, Washington, D.C.
- Neathery, M.W., N.A. Crowe, W.J. Miller, C.T. Crowe, J.L. Varnado, and D.M. Blackmon (1990). Influence of dietary aluminum and phosphorus on zinc metabolism in dairy calves. *J. Anim. Sci.*, 68: 4326-4333.
- Nockels, C.F. J. Deboniss and J. Torrent (1993). Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. *J. Anim. Sci.*, 71: 2539-2545.
- O'Dell, B. L. (1979). In: *Copper and Zinc in Animal Nutrition*. Literature Review Committee, National Feed Ingredients Association, West Des Moines, IA.
- Oberleas, D. and B.F. Harland (1977). Nutritional agents which affect metabolic zinc status. In: *Zinc Metabolism: Current Aspects in Health and Disease*. New York: Alan R. Liss, Inc.; 11-24.
- Pastrana, R., L.R. McDowell, J.H. Conrad and N.S. Wilkinson (1991). Mineral status of sheep in the Paramo region of Colombia. II. Trace minerals. *Small Rumin. Res.*, 5: 23-34.
- Prabowo, A., L.R. McDowell, N.S. Wilkinson, C. J. Wilcox and J. H. Cornad (1990). Mineral status of grazing cattle in South Sulawesi, Indonesia; I. Macrominerals. *Am. J. Anim. Sci.*, 4: 111-120.
- Reid, R.L. and D.J. Horvath (1980). Soil chemistry and mineral problems in farm livestock: a review. *Anim. Feed Sci. Tech.*, 5: 95-167.
- Rhodes, D. and A Klug (1993). Zinc Fingers. *Scientific American*, 268: 56-65.
- Rhue, R.D. and G. Kidder (1983). *Analytical procedures used by the IFAS extension soil laboratory and the interpretation of results*. Soil Sci. Dept., Univ. Florida, Gainesville.
- Sanchez, P.A. (1976). *Properties and Management of Soils in Tropics*. John Wiley and Sons, NY. pp. 97-101.
- Steel, R.G.D. and J.H. Torrie (1980). *Principles and procedures of statistics. A Biometrical approach* 2nd Ed. McGraw Hill Book Co. New York.
- Tiffany, M.E., L.R. McDowell, G.A. O'Connor, H. Nguyen, F.G. Martin, N.S. Wilkinson and N.A. Katzowitz (2001). Effects of residual and reapplied biosolids on forage and soil concentrations over a grazing season in north Florida. II. Microminerals. *Commun. Soil Sci. Plant Anal.*, 32: 2211-2226.
- Tucker, W.B; J.F. Hogue, D.F. Waterman, T.S. Swenson, Z-Xin, R.W. Hemken, J.A. Jackson, G.D. Adams, and L.J. Spicer (1991). Role of sulfur and chloride in the dietary cation-anion balance equation for lactating cattle. *J. Anim. Sci.*, 69:1205-1213.
- Underwood, E.J. (1977). *Trace Elements in Human and Animal Nutrition*. Academic Press, New York.
- Underwood, E.J. (1981). *The Mineral Nutrition of Livestock*. Commonwealth Agricultural Bureaux, London.
- Velasquez-Pereira, J.B., L.R. McDowell, J.H. Conrad, N.S. Wilkinson and F.G. Martin (1997). Mineral status of soils, forages, and cattle in Nicaragua. I. Micro-minerals. *Rev. Fac. Agron.*, 14:73-89.
- Wegner, T.N., D.E. Ray, C.D. Lox and G.H. Stott (1973). Effect of stress on serum zinc and plasma corticoids in dairy cattle. *J. Dairy Sci.*, 56: 685.
- Zapsalis, C and R.A. Beck (1985). *Food Chemistry and Nutritional Biochemistry*. New-York: John Willey and Sons; p. 1006-1009.

(Accepted for publication November 2004)