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Estimation of Zinc in Small ruminants, Soil and Plants in the Northern state -Sudan

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ABSTRACT

In the present investigation, zinc levels have been determined in sixteen soil samples and fifty three plant samples in two regions of the Northern State of the Sudan.Three hundred sera and forty eight liver samples of sheep and goats raised in those regions were also collected and analyzed for their Zn content.

Soil zinc levels at the Northern and Southern region of the State were 2.81±1.914ppm and 1.276±0.27.73 ppm, respectively. Zinc level in plants in the same parts of the State, were 22.465±1.73ppm and 24.953±2.258ppm, respectively.

The levels of zinc in sheep and goats sera were 0.531±0.022 ppm and0.537±0.02ppm, respectively. While hepatic zinc in sheep and goats were 10.325±0.478ppm and10.471±0.544 ppm, respectively. Alkaline phosphatase level as indicator of zinc status was also assessed. 27.18%of sheep and 52.6% of goats showed levels of Alkaline phosphatase lower than the normal levels. Alkaline phosphatase level in sheep was found to be 99.2±17.263 U/L lower than the level in goats (106.97±27.232 U/L).

INTRODUCTION

Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes that are essential for life (Soetan *et al.*, 2010). Zinc is a constituent of numerous metalo-enzymes, required for normal protein synthesis and metabolism in animals (Church and Pond, 1988**),** where it is

encountered in enzymes and proteins activities, that are linked to vitamin-A synthesis, carbon dioxide (CO_2) transport, collagen fibre degradation, free radical destruction, membrane stability of red blood cells, metabolism of essential fatty acids, carbohydrate metabolism, protein synthesis, metabolism of nucleic acids, and others (Rubio *et al*., 2007). Zinc is involved in reproduction, where it is encountered in the gonadal cell growth and division (MacDonald, 2000) and embryo implantation (Robinson et al., 2006), consequently its deficiency is directly correlated with reproductive failure. There are many causes of Zinc deficiency; primary due to inadequate levels in the ration or secondary due to the presence of substance that interfere with its absorption or metabolism (Wikse *et al.*, 1992).Theclinical signs of zinc deficiency include; alopecia, thickening or keratinization of epithelial cells, growth retardation, swelling of the coronet, hock and knee joints, rough coat and congestion of the eye mucous membrane (El-Atar, 1979).

The Zn level could be measured directly by determining its concentration in tissues, or indirectly by the assaying the alkaline phosphatase enzyme in the serum. Alkaline phosphatase enzyme is a zinc containing enzyme and its measurement is an indicator of zinc status in ruminants (Roth and Roth and Kirchgessner, 1980). Although there were some reports concerning Zn deficiency in sheep and goats in some States in the Sudan(Mahamoud *et al.*, 1983; Bakhiet *et al.*, 2007), reports on zinc status in Northern Sudan are lacking.

The objective of the present study is to determine the Zinc concentration in tissues (blood and liver) of sheep and goats, plants and soil, in the Northern State, Sudan.

MATERIALS and METHODS

Study area and samples collection:

The Northern and Southern districts of the Northern Stateof the Sudanwere selected as the study area. A number of 300 whole blood and 48 liver samples were collected from both Sheep and goats .Theblood specimens were collected by jugular vein puncture into clean vacutainer tubes, and the serum was separated by centrifugation of the blood at 3000 rpm for 5 minutes and kept at -20°C. While the liverspecimenswere collected from the slaughter houses by clean sterile stainless blades and placed in sterile plastic bags. Liver specimens were immediately frozen at -20°C until chemical analysis. A total of 53 plant samples including alfalfa, and different other plants available in the visited farms were collected and placed in open paper bags for aeration. The plant samples were air dried and were ground in a mechanic mill. A number of 16 soil samples from a depth of 15 cm were collected from different localitiesafter avoiding plant roots and humus and placed in plastic bags. The soil samples were air dried ground and sieved.

Samples Processing:

A volume of 2 ml from each serum sample was pipette into a polyethylene tube and diluted with 18 ml deionized water and stored at -20°C until analysis by Atomic Absorption Spectrophotometer. Five grams (5g) ofliver samplewere placed in a 250 ml kjeldahl flask, digested with, 10 ml HNO_3 and 5 ml H_2SO_4, then the digest was adjusted to 100 ml with distilledwater to make the standard

solution, and finally stored at room temperature until analysis. Two grams (2g) of the respective plant samples were placed in a furnace and ashed at 500 °C overnight. The ash was dissolved in 5-mL of 20% HCl, filtered through Whatman filter paper, then diluted to 50 mL with deionized water and well mixed (Adrian, 1973), and finally stored at room temperature until analysis. Five grams (5g) soil were placed in an Erlenmeyer flask, 20 mL of extraction solution (0.05N HCl + 0.025N H_2SO_4) were added, then placed onashaker for 15 minutes. The mixture was filtered through a Whatman filter paper before dilution to 50-mL volume with the formerextraction solution (Isaac and Kerber, 1971), and finally stored at room temperature until analysis.

Determination of Zinc concentration:

A stock standard Zinc solution containing 1,000 ppm/ml, was prepared by dissolving 4.3983 gm of Zinc sulfate in a small volume of nitric acid, and then compensated to 1,000 ml with deionized water. The working standards were prepared by diluting 0.5 ml from the stock solution to 100 ml with deionized water to give 100 ppm/ml, from which a volume of one ml was diluted with 500 ml deionized water in a serial dilution to give 0.01, 0.02, 0.03, 0.04, and 0.05 ppm/ml. The samples were subjected to automated, flame atomic absorption spectrophotometer(Phoneix, model No. 986, Biotech, UK) for the determination of Zinc concentration compared to the standard solution.

Determination of Alkaline phosphatase (ALP):

Alkaline phosphatase was determined according to the method described byRec. GSCC(DGKC)(1972).

Statistical analysis:

The results were analyzed using the Statistical Package for Social Sciences (SPSS 2010) using Mann-Whitney and Independent t-test.

RESULTS

The soil zinc levels at the (Northern and Southern region) of the state were 2.81±1.914 ppm and 1.276±0.27.73 ppm, respectively, whereas, the Zinc level in plants in the same parts of the state, were 22.465±1.73ppm and 24.953±2.258ppm, respectively, as shown in Table (1). Variation in the concentration of soil zinc between the two localities was not significant but tended to be higher in the Northern locality. The mean values of zinc in plant samples were inadequate. The level of zinc in plants was higher in Southern locality in spite of soil zinc level.

Table 1: **The mean concentration values of Zn (ppm) in grasses and soil at different location**

Location		Grass	Soil
North Region	Number	13	3
	(Mean ± SD)	22.465±1.73	2.81±1.914
	Range	(14.0-34.75)	(0.57-6.62)
South Region	Number	40	13
	(Mean ± SD)	24.953±2.258	1.276±0.27
	Range	(9.6-75.7)	(0.14-3.02)

Means on the same column having similar superscripts are notsignificantly different at $p \leq 0.05$.

The levels of zinc in sheep and goat sera were 0.531±0.022 ppm and 0.537±0.02 ppm, respectively; whereas the hepatic zinc in sheep and goats was 10.325±0.478ppm and10.471±0.544 ppm, respectively, as illustrated in Table (2).The concentration of zinc in the serum of sheep and goats at different localities is shown in Table (3).The zinc level in the Southern locality was higher than in the Northern locality.

The ALP levels in sheep and goats were 99.2±17.263U/L, and 106.97±27.232 U/L respectively (Table 4).

Table 2: **The mean concentration (ppm) of Zn in serum and liver in sheepand goats**

Species		Serum	Liver
Sheep	Number	189	33
	(Mean ±SD)	0.531±0.022	10.325±0. 478
	Range	(0.138-4.169)	(4.1-17.375)
Goats	Number	111	15
	(Mean ±SD)	0.537±0.02	10.471±0. 544
	Range	(0.181-1.822)	(7.1-15.075)

Means on the same column having similar superscripts are not significantly different at $p \leq 0.05$.

Table 3: The mean concentration (ppm) of serum Zn in sheep and goats at different localities

Location		Sheep	Goats
North Region	Number	44	42
	(Mean ± SD)	0.5±0.031	0.559±0.026
	Range	(0.139-1.059)	(0.231-0.91)
South Region	Number	145	69
	(Mean ± SD)	0.54±0.028	0.525±0.029
	Range	(0.138-4.169)	(0.181-1.822)

Means on the same column having similar superscripts are not significantly different at $p \leq 0.05$.

Table 4:**The concentration of alkaline phosphatase (ALP) in the blood of sheep and goats**

Species		ALP (U/L)
Sheep	Number	40
	(Mean ± SD)	99.2±17.263
	Range	(8-597)
Goat	Number	38
	(Mean ± SD)	106.97±27.232
	Range	(14-951)

Means on the same column having similar superscripts are not significantly different at $p \leq 0.05$

DISCUSSION

Sheep and goats play a significant role in the food chain and overall livelihoods of rural households (Lebbie, 2004).These animals are reared for various reasons including meat, milk and hide production.The mean values of sera Zn concentrations in sheep and goats were below critical levels (0.6-0.8ppm) as reported by McDowell and Arthington, (2005) and were similar in the two species, but lower than the values reported by Ishraga (1999) and Tag Eldin (1997) in other parts in the Sudan. Mills *et al*. (1967) and Rhadostitis (1995) reported that normal serum Zn level in lambs was 0.8-1.2 µg/mL and Ahmed *et al*. (2002) in his study found that normal serum Zn level in goat was a round1.12 µg/ml. Also Mills *et al*. (1967) reported a decline from 0.8-1.2 µg/mL to < 0.4 µg/mL in severely Zn deficient lambs. All animals in the current study were dependent on alfalfa as their main food. Analysis of the plants revealed that their Zn contents were below the estimated Zn requirements of farm animals. This justifies the low Zn observed in sheep and goats in this study. NRC (1985) recommended a minimum Zn content of 45 ppm for farm animals. The primary deficiency was further complicated by the fact that alfalfa contains high level of Ca (1.64%). Calcium is known to block Zn uptake by animals even in sufficient diet (Singer *et al*., 2000). The primary reason for Zn deficiency in alfalfa was the low Zn content in the soil in the Northern state where 75% of soil samples were below critical level, (<2ppm) as suggested by Sanchez (1976).No significant difference in Zn concentration was observed between the two localities.The mineral concentration in the liver reflects the dietary status of animals (Webb *et al*., 2001).The liver is the primary storage for many of the essential minerals, which can augment diagnosis of mineral deficiency and adequacy in animals (Hall, 2005). In the present study the mean hepatic concentrations of Zn found in sheep and goats were below the critical level 84.0(ppm) as suggested by McDowell and Conrad, (1977), and below previous reports (Bakhiet *et al*, 2007) in Eastern region of Sudan, who reported a fall within the lower range recorded for ruminants.The mean value of Zn in plants was inadequate depending on critical levels of Zn <40 ppm (McDwell and Conrad 1977). Zinc deficiencies were indicated in 92% of all samples reflecting the soil zinc deficiencies (69.2%). Lamand (1984) stated that primary Zn deficiency due to dietary Zn is rare but does occur in ruminants. Many factors influence the availability of Zn from soils. The most important factors in this respect being the nitrogen and phosphorous concentrations in the soil (Ray *et al*., 1997). As fertilization with phosphorus and nitrogen increases this will increase the risk of Zn deficiency. The mean values of Zn concentration in soil samples were below the critical level of Zn <2 ppm as suggested by Sanchez (1976).

A percentage of 27.18% and 52.6% of the investigated samples for Alkaline phosphatase were below reference values (68-387 U/L for sheep and 93-387 U/L for goats;) in sheep and goats, respectively which could be a good indicator for zinc deficiencies investigated in the present study Roth and Kirchgessner (1980) reported that ALP is notable for its rapid loss of activity following zinc deficiency. As

reported in the literature that high levels of ALP can be normal in growing animals because of the active state of osteogensis .There was no significant difference between sheep and goats but tended to be higher in goats as in the reference values. Also different location had no significant effect on the activity of ALP and the difference in results may be due to the factor of age.

It is recommended to investigate other minerals to determine their effects on Zn availability or absorption.

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