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Assessment of copper intensity in selected tissues of two different classes of ruminants in Punjab, Pakistan

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This paper deals with the imbalances in the supply of micro-elements from dietary sources, which can influence significantly the animal health and susceptibility to disease. The present work was carried out to determine the copper concentration of two genders of cows and buffaloes at two districts of Punjab, Pakistan. The various samples were collected from soil, forages and from different classes of animals, then analyzed after wet digestion for concentration of copper. Analysis of variance of data for copper concentration in liver, kidneys, ribs, cerebrum, blood-serum, lungs, bones and hair showed significant effect ($P < 0.05$) of the locality and age of the female cows and female buffaloes, and also this metal had statistical significance ($P < 0.05$) in liver, cerebrum, lungs, bones and hair of male cows but kidney and ribs of male cows showed non-significant effect ($P > 0.05$). In forage of district Jhang, the mean ± standard error (SE) of copper concentration (22.18 ± 0.45 mg/kg) was lower while at Toba Tek Singh, its concentration (23.86 ± 0.31) was higher as compared to corresponding concentration (22.72 ± 0.27 mg/kg) of control farms, respectively. Similarly, at district Jhang in male cows, female cows, male buffaloes and female buffaloes, the copper concentrations in kidney, ribs and bones, respectively were found to be higher than the corresponding concentrations of control groups, which show the translocation of copper from soil to plants and then to animal tissues, which are the best indicators of availability of the minerals. It can be concluded that copper has a particular significance in the experimental areas with respect to beef consumptions by human being.

Key words: Tissue, serum, buffloes, cattles, plant, soil, animal, mineral.

INTRODUCTION

It is a pre-requisite for productivity and the health of ruminants to provide them an appropriate quantity of minerals (Khan et al., 2006). The cattle get minerals by grazing forage plants but imbalanced mineral intake adversely affect their rate of reproduction and productivity (Khan et al., 2007). It is observed that copper is an essential component of many metalloproteins or metalloenzymes that is, superoxide dismutase, lysyl

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oxidase, ceruloplasmin, metallothionein and cytochrome c oxidase, so it is responsible for various oxidative processes (Minate and Carfagnini, 2002). The toxicity of copper causes oxidative damage, renal failure, distortion of erythrocytes and eventually sudden death due to the rapid emission of copper from different sites of animal liver (Underwoodand Suttle, 1999). Copper is a cofactor of many cuproenzymes, and recent research has identified several proteins involved in its metabolism (Harris, 2000). Ruminants are particularly susceptible to copper toxicity, whereas monogastric species are usually quite tolerant. Copper toxicity in cattle is associated with excessive intake of copper in the diet or contamination of pastures by soils, slurry, mining or industrial emissions and waste (Lopez-Alonso et al., 2002; Miranda et al., 2005). It was also observed that the concentrations of Cu in the liver were correlated with the Cu content in the soil (Lopez-Alonso et al., 2003; Miranda et al., 2006).

It was observed that there were no significant difference between male and female buffaloes and cows, but male animals have lower copper concentration in blood-serum than that of female animals. In polluted areas, copper levels increased with age, in the liver of buffaloes and cows. There is a competition between binding sites of metallothionein, which become a cause of antagonistic interaction of zinc (Zn), copper (Cu) and cadmium (Cd). Cu play an important role in increasing the rate of heart beat, bone production and connective tissue development etc. Heart diseases, anemia, and change of hair color, diarrhea, infertility and also low antibodies production, was mostly observed in ruminants, which have copper deficiency. The excess of copper accumulation shows toxicities, which may cause many severe diseases, such as collapse, abdominal pain, nausea, vomiting and paralysis. Hypercupremic occur due to increasing copper concentration, as a result of which decreases the rate of reproduction and growth and also cause muscular dystrophy. The adverse effect was experienced in buffaloes and cows due to calving, which is also called reproductive disorders (El-Wishy, 2007).

Copper plays a number of important biological roles in animals through several Cu-dependent enzymes (Xin et al., 1991). Copper deficiency in ruminants occurs either as a primary or as a secondary deficiency. Most of the copper deficiencies in livestock which occur naturally are conditioned by the presence of dietary factors that interfere with the absorption or utilization of copper by the animal (Underwood and Suttle, 1999). These dietary factors, such as iron, molybdenum or sulfur, interfere with the absorption and metabolism of copper (Suttle, 1991). In the ruminants, molybdenum combines with reduced sulfur to form tetrathiomolybdate that binds copper to prevent its absorption, while other thiomolybdates and molybdates are absorbed into blood and bind endogenous copper to render it unavailable for metabolic purposes (Mason, 1982).

Copper deficiency has been reported in grazing live-stock in some parts of Iran (Nouri, 1998; Nouri et al., 2005). In the west and east Azerbaijan and Kurdistan provinces, high molybdenum levels were responsible for copper deficiency among ruminants. Swayback in lambs and kids in the Khuzestan province of Iran has been observed as frequently (Nouri et al., 2005) reported enzootic ataxia in lambs in this region. There is no information with regard to copper deficiency in goats in Khuzestan province; therefore the aim of this study was to determine serum and liver copper concentrations of goats in some parts of the area and to evaluate the effects of antagonistic minerals for the determination of primary or secondary copper deficiency. Trace element deficiencies are more common in combination than single element deficiency (Kankofer, 2000).

Low level of copper, iron, and selenium (Grys and Kubinski, 1979; Sobiech and Kuleta, 2002; Faixova et al., 2007; Bickhardt et al., 1999) and low levels of selenium and zinc (Gupta et al., 2004; Han and Kim, 2005) have been reported in cases of retention of fetal membranes as compared to control values in cows. Significant low level of zinc has also been reported in the plasma of cows with calving difficulty (Dufty et al., 1977) and in buffaloes with uterine prolepses (Kelkar et al., 1989; Bhatti et al., 2006). Trace elements of metals such as manganese (Mn), copper (Cu), iron (Fe), selenium (Se) and zinc (Zn) are essential in animal nutrition and are needed in very small amounts for essential metabolic reactions in the body. Their deficiencies are often associated with alterations in many metabolic processes and cause various kinds of diseases.

Deficiency of these trace elements causes severe economic loss due to increased susceptibility to oxidative stress, growth retardation in young animals, anemia (Bureau et al., 2003), decrease in feed efficiency and fertility (Grenier et al., 2003), enhance the virulence of the infectious agent (Failla, 2008) and decrease immune system function (Rink and Ibs, 2003). On the other hand, normal thyroid status is dependent on the presence of many trace elements such as Se, Fe, Cu and Zn, for both the synthesis and metabolism of thyroid hormones. Although the role of some of these elements such as iron, zinc and copper in the thyroid are less well defined, sub or super optimal dietary intakes of these elements can adversely affect thyroid hormone metabolism (Nazifi et al., 2009).

The purpose of present study was to determine the mineral status (copper) of animals, we can determine the mineral requirements for ruminants and economic benefit for mineral supplementation, so that we can easily adopt various strategies to overcome the problems of mineral imbalances in soil and plants consumed by animals.

MATERIALS AND METHODS

This study was designed to determine the level of micro-mineral copper in liver, kidney, ribs, cerebrum, blood-serum, lungs, bones

and hair of cows and buffaloes at district Jhang, Toba and controlled farm (Ever Green livestock farm Bukhar road District Jhang), and also to determined its level in the fodder and soil of the above given locations. Samples from free grazing cattle were obtained from abattoirs situated in widely spread localities in Punjab, Pakistan. The animals were not selected according to sex or age but on the acknowledged assumption that they were aged from 2 to 5 years, and taking of the parts that reach the final consumers. The samples were packed in polyethylene bags and conveyed to the laboratory. Upon reception, gross fat was removed and stored at -10°C in sealed plastic container until required. Samples of various organs were collected from 60 animals, consisting of 12 cows and 12 buffaloes (district Jhang), 12 cows and 12 buffaloes (district Toba) and 6 cows and 6 buffaloes from experimental and controlled farms, respectively.

Sample collection

The work was conducted in different regions of two districts of Punjab, Jhang and Toba. Soil, fodder and animal samples were collected from fields of Jhang, Toba and controlled farm. The soil samples were obtained from those fields where fodders were cultivated. Soil samples were collected from each selected field and controlled farm from different surfaces up to 15 to 20 cm depth at three different points by using a stainless steel sampling augur, the samples were air dried, ground and mixed. 10 g of air dried soil was added in 125 ml conical flask along with 40 ml mehlich-extracting solution (0.05 N HCl + 0.025 N H_2SO_4), then shaken for 15 min in a reciprocal shaker. The extract was then filtered through Whatmann filter paper No. 2 and stored in labeled clean plastic bottles for further laboratory analysis.

The samples of various fodders, which were being fed as such to the buffaloes, were collected from both districts from the owners of animals. A total of 300 fodder samples were collected. Fodder frequently grazed by cows and buffaloes are turnips, sugar cane tops, berseen, maize, local weeds, barley, paddy straw, wheat straw, tree leaves, grass and sorghum etc. Forage samples were collected at 15 cm from the ground, by hand plucked method, which is grazing behavior of animals. The samples were washed with 1% HCl followed by 3 to 4 washing. With double distilled water, and air dried and then oven dried at 65°C, these samples were ground in a powder form, then 1 g of the dried forage sample was added in a 50 ml conical flask, added 5 ml concentrated HNO_3 along with 5 ml $HClO_4$, then these samples were digested on hot plate at high temperature till volume reduced to 1 to 2 ml. The material was allowed to cool down, the contents were filtered and diluted unto 50 ml with double distilled water and stored in clean, air tight labeled bottled for copper mineral analysis.

A total of 480 animal tissue samples (blood-serum, cerebrum, liver, kidney, lungs, ribs, bones and hair) which are considered excellent mineral status indicating organs, were collected in triplicate from each of 30 cows and buffaloes (both male and female, aging between 2 to 13 years) after knowing their origin, from different fields and control farm at two districts, viz, Jhang and Toba Tek Singh, Punjab, Pakistan. Blood samples were collected from the jugular vein of animals into 20 ml sterilized plastic tubes heparinized with 6 drops of heparin as anticoagulant. The heparinized blood samples were stored at -20°C and were allowed to stand for approximately 3 h in a slanting position at room temperature. These samples we centrifuged for 10 min at 3000 rpm by Micro centrifuge, to get blood-serum. After labeling, the serum sample was stored in refrigerator in contaminant free tubes. After collection of blood samples, the above mentioned organ samples (60 g) of the same animal were also collected. These organ samples were dried in an oven after placing them in labeled paper envelop for several hours till complete dryness. Dried sample was ground to a fine powder so that they can easily be digested. Atomic absorption spectrophotometer requires clear, organic matter free and transparent solution. For this purpose, wet digestion was carried out.

Digestion of samples

For wet digestion, 2 ml of blood sample and 2 g of each of various organs samples were taken in a conical flask and 20 ml of acid mixture in the ratio of 1:5:2 (H_2SO_4: HNO_3: $HClO_4$) added. The solution was heated first at low temperature and then at high temperature on a hot plate until the whitish fumes come out (that is, all the organic matter dissolved) and 2 to 3 ml of clear, transparent solution left behind in the flask. Then after cooling, de-ionized water was added to make 60 ml of solution and this solution filtered through whatmann filter paper #42 to get transparent filtrate, which was preserved in labeled sample bottles. These digested and diluted samples were used for the estimation of copper element (Kamada et al., 2000). Standard solution of Cu micro mineral was prepared by using available standard salt (copper sulphate) of copper to get standard curve of absorbance value of this mineral. Finally, the copper concentration in each of the sample was calculated. For blood-serum and organs of animal copper, concentrations were calculated in mg/L and mg/kg, respectively.

Analytical procedure

The above samples of soil, forage, animal organs and blood-serum were diluted as required and analyzed for copper metal by using atomic absorption spectrophotometer, according to the method reported by Anwar et al. (2004).

Statistical processing

The values obtained from the studied indicators were processed by using the general linear model STATISTICA (StatSoft Inc., 7.1) procedure. The differences among means were ranked using Duncan's new multiple range test (Duncan, 1955).

RESULTS

Mineral values in soil, forages, animal organs and their blood-samples obtained in present study were compared with those of controlled farm and also with reference values already present in literature to determine the various categories of levels of mineral imbalances. The results pertaining to concentration of copper in various tissues of cows and buffaloes (liver, kidneys, ribs, cerebrum, blood- serum lungs, bones and hair) are shown and their levels are presented as mean ± SE values, and augmented with bar charts and tables. Analysis of variance of data for copper concentration in liver, cerebrum, blood-serum, lungs, bones and hair showed significant results ($p < 0.05$) of the district and age of the male cows, but the reverse was true for kidney and ribs, in which copper concentration was non significant ($p > 0.05$) (Table 1).

The mean ± SE copper concentrations (21.28 ± 1.23, 28.06 ± 0.62, 27.03 ± 2.63 mg/kg, 21.43 ± 0.94 mg/l, 22 ± 1.33, 19.38 ± 1.52 and 11.03 ± 0.38 mg/kg) and its

Table 1. Analysis of variance of data for Cu concentration in various organs of male cows of different age groups at two districts of Punjab.

Source of variation (SOV)	Degree of freedom (df)	Mean square (male cows)							
		Liver	Kidney	Ribs	Cerebrum	Blood-serum	Lungs	Bones	Hair
Districts (D)	1	308.003*	8.01^{ns}	0.04^{ns}	114.74*	2800*	542.84*	93.41*	2839.8*
Error	4	0.645	1.37	0.56	0.699	6	1.48	0.66	1.5
Ages (A)	5	37.432*	179.94*	356.35*	18.373*	346.67*	238.61*	146.05*	1764.5*
D×A	5	39.048*	208.09*	438.1*	58.142*	187.93*	280.58*	140.5*	1821.2*
Error	20	0.578	0.73	0.87	0.758	2.12	0.75	0.55	0.3

* = Significant Level at 0.05 levels; ns = non-significant levels.

concentrations (27.13 ± 1.30, 29 ± 3.85, 25.21 ± 1.97 mg/kg, 22.51 ± 3.02 mg/l, 29.77 ± 4.05, 16.16 ± 2.80, and 16.2 ± 1.53 mg/kg) in liver, kidney, ribs, blood-serum, lungs, bones and hair of male cows of both districts Jhang and Toba, were higher as compared to the corresponding concentrations (5.86 ± 0.37, 6.03 ± 0.48, 9.29 ± 0.48, 5.8 ± 0.86 mg/l, 4.71 ± 0.49, 7.35 ± 0.57 and 6.79 ± 0.53 mg/kg) of control groups, respectively (Figure 1a). Analysis of variance of data for copper concentration in liver, kidney, ribs, cerebrum, blood-serum, lungs, bones and hair showed significant result ($p < 0.05$) of the district and age of the female cows (Table 2). The mean ± SE copper concentrations (18.69 ± 1.21, 24.75 ± 1.38, 21.44 ± 1.84 mg/kg, 20.54 ± 1.06 mg/l, 21.73 ± 1.32, 20.72 ± 2.67 and 11.3 ± 0.53 mg/kg) and its concentrations (29.39 ± 2.09, 26.7 ± 1.89, 25.58 ± 2.93 mg/kg, 28.8 ± 2.56 mg/l, 21.53 ± 2.03, 15.07 ± 2.58 and 14.53 ± 2.06 mg/kg) in liver, kidney, ribs, blood-serum, lungs, bones and hair of female cows of both districts Jhang and Toba were higher as compared to the corresponding concentrations (5.99 ± 0.86, 7.5 ± 0.49, 7.9 ± 0.64 mg/kg, 4.72 ± 0.54 mg/l, 3.28 ± 0.55, 6.32 ± 0.56 and 7.00 ± 1.06 mg/kg) of control groups, respectively and lower for cerebrum (Figure 1b).

Analysis of variance of data for copper concentration in liver, kidney, ribs, cerebrum, blood-serum and lungs, showed significant result ($p < 0.05$) of the district and age of the male buffaloes, but reverse was true for bones and hair, in which copper concentrations were non significant ($p > 0.05$) (Table 3). The mean ± SE copper concentrations (10.62 ± 0.84, 22.06 ± 1.17, 24.34 ± 0.70 and 11.42 ± 0.59 mg/kg) and its concentrations1(6.83 ± 1.39, 20.86 ± 0.99, 26.64 ± 1.19 and 11.34 ± 0.90 mg/kg) in liver, kidney, ribs, and bones of male buffaloes of both districts of Jhang and Toba were higher as compared to the corresponding concentrations (23.99 ± 2.64, 27.3 ± 1.20, 29.54 ± 2.05 and 19.46 ± 3.35 mg/kg) of control groups, respectively but reverse was true for liver, kidney, ribs and bones (Figure 2a).

Analysis of variance of data for copper concentration in liver, kidney, ribs, cerebrum, blood-serum, lungs, bones and hair showed significant result ($p < 0.05$) of the district and age of the female buffaloes (Table 4). The mean ± SE copper concentrations (10.02 ± 0.97 mg/kg, 20.19 ± 1.17 mg/l, 24.01 ± 0.76, 13.89 ± 0.82 and 12.44 ± 0.85 mg/kg) in cerebrum, blood-serum, lungs, bones and hair of female buffaloes of district Jhang were lower as compared to the corresponding concentrations (23.36 ± 2.42 mg/kg, 25.33 ± 1.28 mg/l, 27.03 ± 1.40, 15.26 ± 0.71 and 16.02 ± 1.64 mg/kg) of control groups, respectively and the reverse was true for liver, kidney and ribs and its concentrations (23.58 ± 1.66, 73.66 ± 1.58, 27.3 ± 1.15 and 18.84 ± 1.16 mg/kg) in kidney, ribs, lungs and bones of female buffaloes of district Toba were higher as compared to the corresponding concentrations (17.13 ± 1.01, 33.38 ± 0.50, 27.03 ± 1.40 and 15.26 ± 0.71 mg/kg) of control groups, respectively and the reverse was true for liver, cerebrum, blood-serum and hair (Figure 2b). The mean ± SE copper levels for the fodder samples from the fields of Jhang and Toba were 22.18 ± 0.45 and 22.86 ± 0.31 mg/kg, respectively, and for these samples from the control farm were 22.72 ± 0.27 mg/kg. Copper concentration in fodders was slightly lower at districts Jhang and slightly higher at Toba as compared to those of control farm, respectively (Figure 3a). The mean ± SE copper levels for the soil samples from the fields of Jhang and Toba were 2.84 ± 0.11 and 3.62 ± 0.20 mg/kg, respectively, and for the soil samples from the control farm were 1.82 ± 0.20 mg/kg. Copper concentration was higher in soil of both districts Jhang and Toba as compared to that of control farm (Figure 3b).

DISCUSSION

In the present study, the mean ± SE copper levels in liver, kidney, ribs, blood-serum, lungs, bones and hair of both male and female cows of both districts were higher as compared to those of control groups but slightly lower than those of earlier reported reference values, but reverse was true for the cerebrum. Erdogen et al. (2002) also studied lower copper mineral contents in blood of cattle. They concluded that copper mixtures should be continuously added to the fodders of cattle. The slight difference of copper levels in various groups of cows and buffaloes was due to the variation of digestibility of copper from their diets, fluctuation in the climatic conditions, soil chemistry or nature of forages.

Table 2. Analysis of variance of data for Cu concentration in various organs of female cows of different age groups at two districts of Punjab.

Source of variation (SOV)	Degree of freedom (df)	Mean square (female cows)							
		Liver	Kidney	Ribs	Cerebrum	Blood- serum	Lungs	Bones	Hair
Districts (D)	1	1030.4*	34.047*	11339*	12.935*	3772.9*	117.43*	50.08*	2031.8*
Error	4	1.25	1.252	0.7	0.361	4.84	1.71	1.57	0.7
Ages (A)	5	92.92*	73.638*	654*	28.978*	311.57*	278.75*	290.95*	1853.7*
D×A	5	44.9*	62.851*	500.9*	16.182*	246.98*	299.85*	645.02*	1647.5*
Error	20	0.47	0.51	0.5	0.423	3.45	0.51	0.53	0.5

* = Significant Level at 0.05 levels; ns = non-significant levels.

Table 3. Analysis of variance of data for Cu concentration in various organs of male buffaloes of different age groups at two districts of Punjab.

Source of variation (SOV)	Degree of freedom (df)	Mean square (male buffaloes)							
		Liver	Kidney	Ribs	Cerebrum	Blood- serum	Lungs	Bones	Hair
Districts (D)	1	6.674*	13.888*	2150.6*	347.02*	13.02*	47.702*	0.094^{ns}	0.047^{ns}
Error	4	0.167	0.535	3.71	1.257	0.896	0.447	2.081	1.104
Ages (A)	5	57.946*	51.079*	586.49*	11.497*	25.123*	26.833*	37.146*	20.115*
D×A	5	54.522*	36.01*	740.33*	65.591*	43.892*	28.411*	13.014*	13.387*
Error	20	0.765	0.941	3.63	0.771	0.542	0.728	0.441	0.267

* = Significant Level at 0.05 levels; ns = non-significant levels.

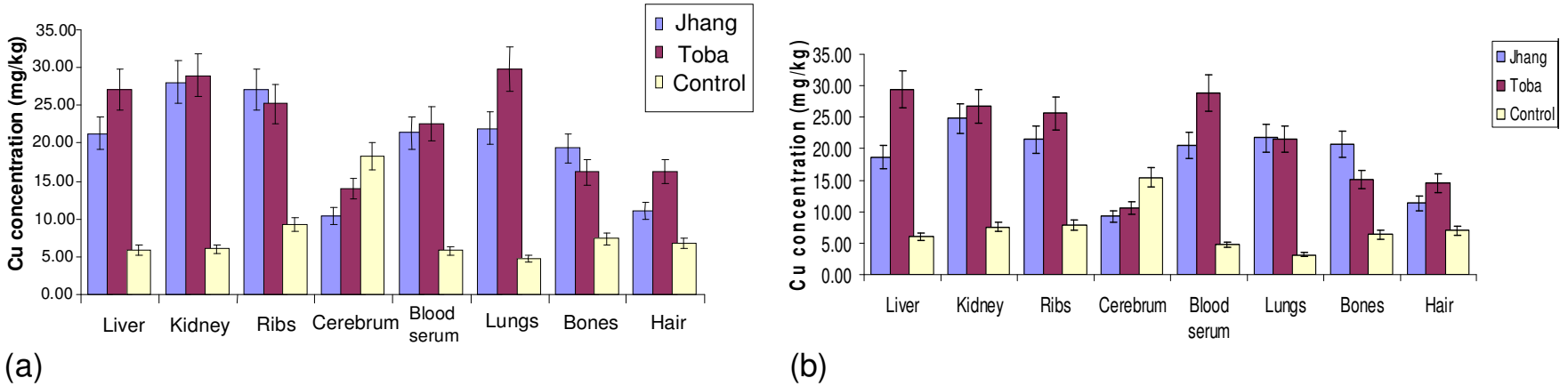

Figure 1. Fluctuation in levels of Cu in liver, kidney, ribs, cerebrum, blood-serum, lungs. bones and hair of (a) male cows and (b) female cows.

Table 4. Analysis of variance of data for Cu concentration in various organs of female buffaloes of different age groups at two districts of Punjab.

Source of variation (SOV)	Degree of freedom (df)	Mean square (female buffaloes)							
		Liver	Kidney	Ribs	Cerebrum	Blood- serum	Lungs	Bones	Hair
Districts (D)	1	11.696*	30.104*	1038.8*	620.43*	59.008*	97.904*	8.102*	4.114*
Error	4	0.453	0.518	2.28	0.423	0.844	0.883	0.16	0.33
Ages (A)	5	72.586*	135.67*	506.32*	47.236*	39.555*	38.147*	37.227*	30*
D×A	5	72.078*	18.637*	648.42*	89.934*	79.301*	16.964*	21.94*	26.834*
Error	20	0.765	0.417	232.24	0.554	0.658	0.673	0.69	0.372

* = Significant Level at 0.05 levels; ns = non-significant levels; ns = non-significant levels.

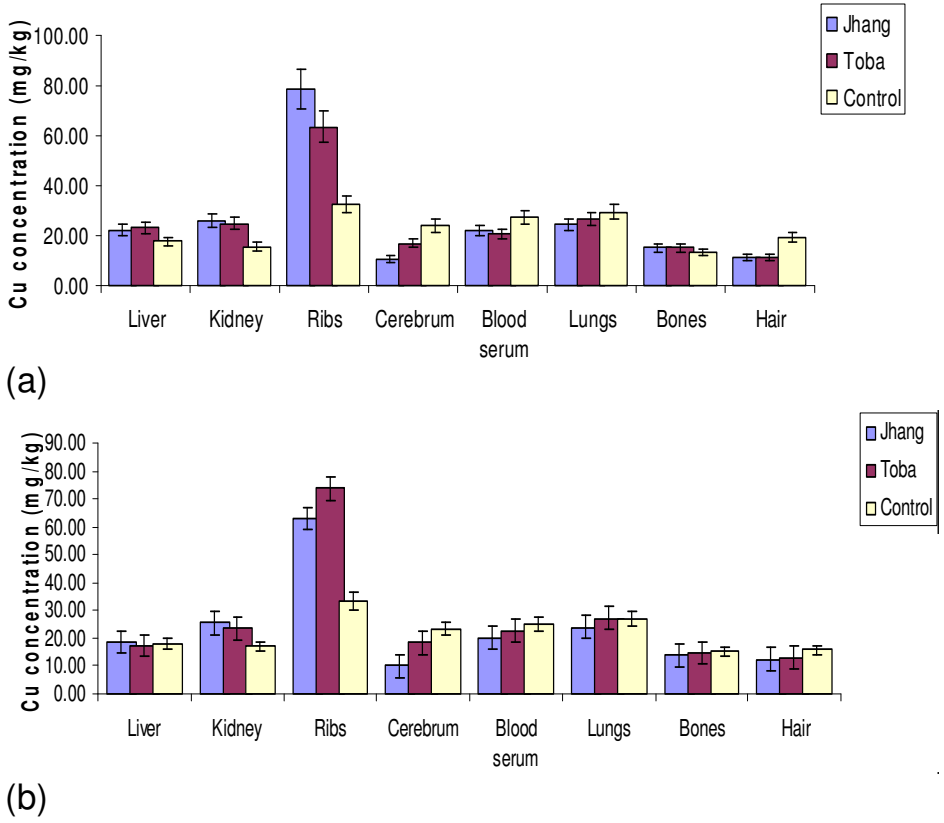

Figure 2. Fluctuation in levels of Cu in liver, kidney, ribs, cerebrum, blood-serum, lungs, bones and hair of (a) male buffaloes and (b) female buffaloes.

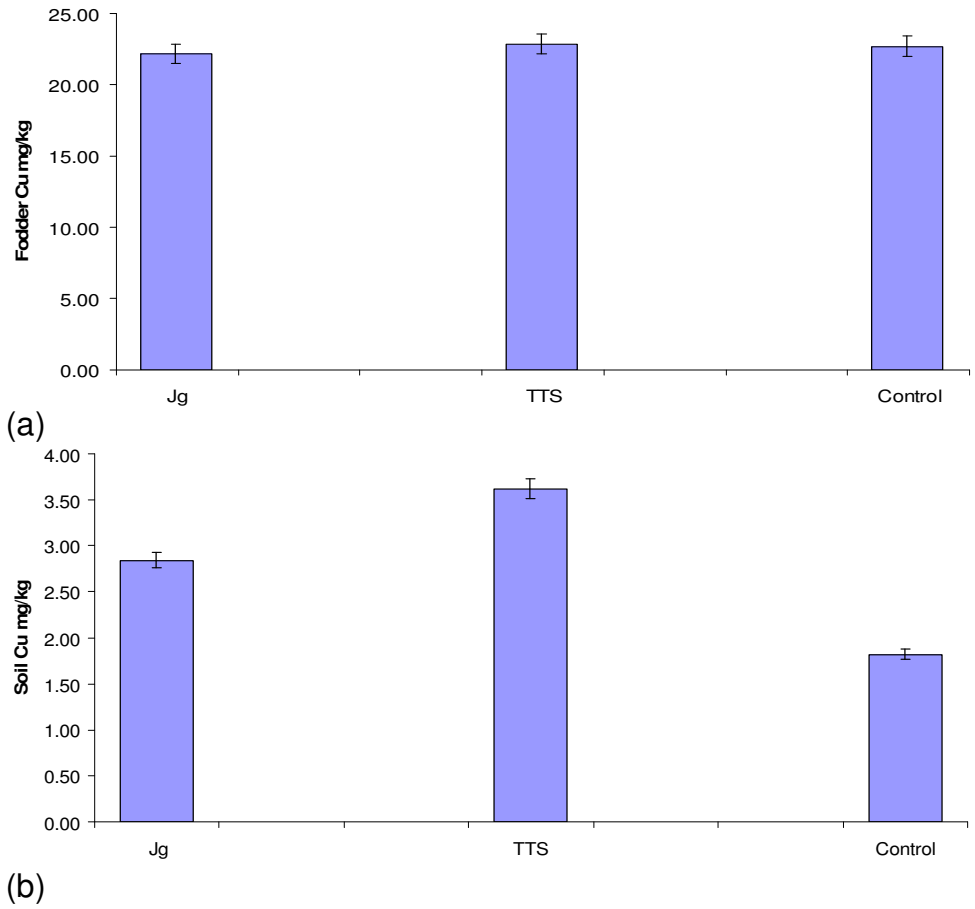

Figure 3. Mean ± SE Cu levels in the (a) fodders and (b) soil grown at Jhang, Toba and controlled farms.

In present study, the copper levels in liver, kidney and ribs and bones were higher but lower in blood-serum, cerebrum, lungs and hair of male buffaloes in experimental sites of both districts, and also significantly lower in cerebrum, blood-serum, lungs, bones and hair of female buffaloes of Jhang. Muehlenbein et al. (2001) also reported the lower levels of copper in blood-serum of buffaloes, but these results showed no agreement with those reported by Kankoffer (2000). The cause of copper deficiency was due to high intake of molybdenum and sulphur which showed antagonistic behavior among these minerals. In this study, the higher levels of copper in liver, kidney and ribs of male buffaloes than those of earlier reported reference values indicate more accumulation of organic materials in the soil.

The analysis of fodders of both districts and control form under study indicated that the fodders of district Toba were slightly deficient in copper minerals. Akhtar et al. (2009) also reported slightly lower copper levels in fodders of fields as compared to control farm. In blood-serum and other animal tissues of all classes of animals at given experimental sites, it showed copper deficiency except liver, kidney and ribs. Copper element deficiency was more common in combination than single element deficiency, which became a cause of retention of fetal membrane (Kankoffer, 2000). Muhlenbein et al. (2001) reported that the cows showed higher copper concentration by 24 mg/kg than that of control groups when these cows were supplied with supplementations of organic materials. Copperdeficiency adversely affects the immune system by decreasing number of antibody producing cells (Chew, 2000). Copper availability of plants from soil may be affected by soil pH. Actually, its availability in plants increased by decreasing soil pH, so its availability in plants was also highly dependent on the amount and kind of organic matter in the soil. The study on cows and buffaloes had rather equal serum copper while more serum zinc, which was about 5 times higher (Nazifi et al., 2009).

Conclusion

The findings of this work showed that the distribution of minerals in different organs of ruminants depended upon the rate of absorption through gastro intestinal tract of

grazing animals. By knowing the mineral status of animals, we can determine the mineral requirements for ruminants and economic benefit for mineral supplementation, so that we can easily adopt various strategies to overcome the problems of mineral imbalances in soil and plants consumed by animals. It is recommended to provide additional copper in a concentrated mixture form to the animal nutrition for the correction of copper deficiency. Copper deficiency in animals can be prevented by using intramuscular injection which is a slowly absorbed form of copper. On the basis of the information obtained from soil, pasture forages, blood-serum and various organs, it is possible that low levels of zinc and copper in soil and forage could potentially limit ruminant reproduction. It is concluded that mineral supplement should continually be supplied to the grazing ruminants to improve the mineral status for maximizing the production potential of livestock at the sites of these districts of Punjab.

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