



Investigating the Serum Levels of Copper and Ceruloplasmin in Sheep of Kazerun City, Iran

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Abstract

Background and aim: In many areas of the world, copper deficiency may occur in livestock. This is not only dependent on the total concentration of copper in the diet but also on the effective factors in the absorption and acquisition of copper. This study aimed to investigate the serum levels of copper and ceruloplasmin in sheep in Kazerun City, Iran.

Materials and Methods: This study measured serum levels of copper and ceruloplasmin of sheep in Kazerun city in four regions of Konartakhteh, Kuhchenar, Baladeh, and Central. Sixty sheep were randomly selected (15 sheep in each region), and their blood was sampled. After separating the serum, an atomic absorption device was used to check the serum concentration of copper and ceruloplasmin. Data were analyzed using SPSS software and Pearson and Chi-square correlation tests.

Results: The results showed that the highest and lowest average copper serum level was 65.25 ± 0.3 $\mu\text{g/dL}$ in the Konartakhteh and 40.38 ± 0.5 $\mu\text{g/dL}$ in the Central area. The highest and lowest average serum levels of ceruloplasmin were 9 ± 0.01 mg/dL in the Baladeh and 8.5 ± 0.03 mg/dL in the Central region. Also, the overall average results showed that the average copper serum level was 55.97 ± 19.57 $\mu\text{g/dL}$ and the average ceruloplasmin was 8.875 ± 0.5 mg/dL , that the copper serum level was in a subclinical deficiency status. The ceruloplasmin level was in a favorable state. The statistical analysis of the correlation between the serum levels of copper and ceruloplasmin in sheep showed a correlation of $r=0.892$ and a significance level of $P=0.05$. A statistical correlation was observed between the serum levels of copper and ceruloplasmin and respectively, the highest and lowest serum levels of copper were 94 and 30.55 $\mu\text{g/dL}$, and the highest and lowest serum levels of ceruloplasmin were 30.55 and 7.8 mg/dL .

Conclusion: In sheep from different regions of Kazerun city, the serum level of copper was subclinical deficient, and the serum level of ceruloplasmin was in a favorable condition. It seems that adding copper supplements to the ration of sheep in these areas is necessary.

Keywords: Sheep, serum copper, ceruloplasmin, Kazerun city

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Introduction

Today, according to the growing trend of the world population, providing the basic needs of people, the most important of which is food, is of great importance. In order to implement profitable animal husbandry in any region, it is important to investigate the metabolic status of that region (Radostits *et al.*, 2010). Copper in the form of various enzymes in the cell respiratory chain in the mitochondria of all cells, including red blood cells, is effective in the formation of hair pigment and the oxidative defense system, and its deficiency causes irreparable damage to animal husbandry, including Problems such as reduced fertility, reduced production of milk, wool, meat, and increased sensitivity to infectious diseases, genetic abnormalities, abortion, paralysis of hind limbs, etc. (Rezaei saber & Rezaei, 2007). Copper is one of the rare and essential elements of animals and plants. Most of the copper can be found in the liver, bones, muscles and skin, but the amount of copper in the liver is higher than in other tissues (Burkhead & Collins, 2021). Copper deficiency mainly manifests in ruminants and with clinical symptoms such as growth reduction, lameness, ataxia (Hill *et al.*, 2019).

Copper deficiency is considered one of the most important mineral deficiencies in livestock and it is in two primary and secondary forms (Alidadi, 2001). Primary copper deficiency occurs when the amount of copper in the diet is low, and secondary copper deficiency occurs when Absorption and consumption of copper by tissues will be disturbed. Impaired absorption of copper can be due to the increase of molybdenum, zinc, iron, lead and calcium carbonate in the diet. Cattle are more sensitive to copper deficiency than sheep (Cheng *et al.*, 2000; Gursel *et al.*, 2010). When the concentration of forage copper in most pastures is less than 3 mg per kilogram of dry matter, the symptoms of copper deficiency in livestock are fully evident, and at a concentration of 3 to 5 mg of copper per kilogram of dry matter, the animal is exposed to deficiency. Levels higher than 5 mg per kilogram of dry matter are sufficient for livestock unless copper nutritional antagonists cause secondary deficiency. The most important nutritional antagonists of copper are high concentrations of molybdenum, sulfur and zinc in the diet. Molybdenum through the formation of copper thiomolybdate, sulfur through the formation of copper

sulfide, and zinc through the stimulation of metallothionein protein production cause disturbances in copper absorption (Aliarabi *et al.*, 2011; Gartner *et al.*, 1969).

Ceruloplasmin is one of the main circulating plasma proteins that acts as a copper carrier and since it is an acute phase protein, it is synthesized in response to inflammation and tissue damage in the liver. Among livestock, sheep are more sensitive to deficiency and excessive amounts and copper poisoning, which always causes large economic losses. Copper deficiency in sheep causes disorders such as decreased wool quality, anemia, bone disorders and decreased fertility, and following copper deficiency in sheep, complications such as enzootic ataxia are observed in infant lambs (Radostits *et al.*, 2010; Smith, 2009). The level of ceruloplasmin has been introduced as an indicator of body copper status (Radostits *et al.*, 2010; Pourjafar *et al.*, 2008). Ceruloplasmin has an oxidase activity and contains copper and has the ability to bind iron ions and is actually ferrooxidase. Cases such as weaning, transportation, pregnancy and some parasitic infections increase serum ceruloplasmin concentration (Smith, 2009; Sirajwala *et al.*, 2007).

Ceruloplasmin is one of the most important extravascular antioxidants and protects cells against free radicals, prevents lipid peroxidation and prevents damage to the red blood cell membrane caused by iron and copper ions. Pregnancy, in turn, is accompanied by an increase in the oxidative stress of fat oxidation (Zhang *et al.*, 2012). Copper is vital for the extraction of iron in the production of hemoglobin, therefore copper deficiency affects hematopoiesis and leads to anemia. Plasma copper is positively correlated with the humoral immune response of various diseases. Copper is one of the vital components of ceruloplasmin. During inflammatory processes, ceruloplasmin increases, which increases copper levels (Blakley & Hamilton, 1985; Gartner *et al.*, 1969). Therefore, the purpose of this study was to measure the serum levels of copper and ceruloplasmin in sheep of Kazerun city.

Materials and Methods

In this study, the serum levels of copper and ceruloplasmin of sheep in Kazerun city were measured in four regions: Konartakhteh, Kuhchenar, Baladeh and Central. For this purpose, 60 sheep were

randomly selected (15 sheep in each region) and blood was taken by a vacuum venoject. Blood was collected from the vena cava of sheep by a sterile vacuum venoject (Greiner, Germany). In order to separate the serum samples, they were placed in the laboratory using a centrifuge for 10 minutes at 1500 rpm, and after separation, the serums were transferred into eppendorf tubes with the help of a sampler. Half a ml of each blood sample was poured into a separate and clean test tube, then 0.5 ml of a prepared mixture of perchloric acid (Merck, Germany) and nitric acid (Merck, Germany) was added to each sample in a ratio of 3:7. Then, in order to digest the serum and prepare the elements, it was placed in a hot water bath at 100°C for two consecutive days for 16 hours, and after the digestion process, the volume was reduced in order to know the dilution factor, with distilled water without fixed iodine. Before the solutions enter the atomic absorption device (UNICAM939, England) the device was adjusted by standard solutions related to copper element. Then the digested samples were entered into the device and their absorption rate for copper atom at the wavelength of 324.8 nm was determined. Copper concentration was calculated based on the standard curve and reported in ppm. Also, checking ceruloplasmin activity is similar to measuring serum copper, with the difference that 0.5 ml of serum was mixed with 0.5 ml of perchloric acid and nitric acid. Plasma ceruloplasmin was measured by spectrophotometric method, by measuring its oxidation activity. Seroplasmin at pH=5.4 can oxidize para-phenylenediamine and produce colored products. Its amount is proportional to the

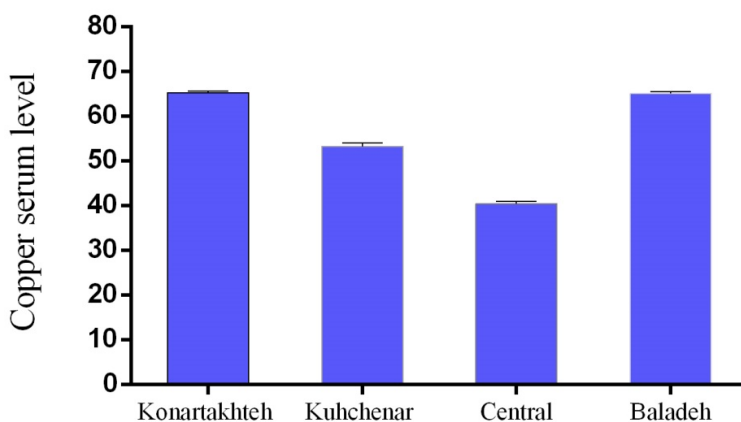
concentration of ceruloplasmin or serum. Also, a certain amount of para-phenylenediamine is oxidized by a non-enzymatic method, which should be done simultaneously in ceruloplasmin once in the presence of sodium azide and once without the presence of this substance. Sodium azide inhibits the enzyme paraphenylene diamine, in other words, it inhibits the activity of ceruloplasmin and causes only the non-enzymatic oxidation of paraphenylene diamine to continue. The resulting difference in light absorption intensity in this type of test is proportional to ceruloplasmin concentration (Pourjafar & Saei Dehkordi, 2007).

Statistical analysis

In this study, Graphpad software (Version 6) was used to draw graphs. SPSS version 20 software and Pearson correlation test and chi-square method were used for statistical analysis. A significant level of $P < 0.05$ was considered.

Results

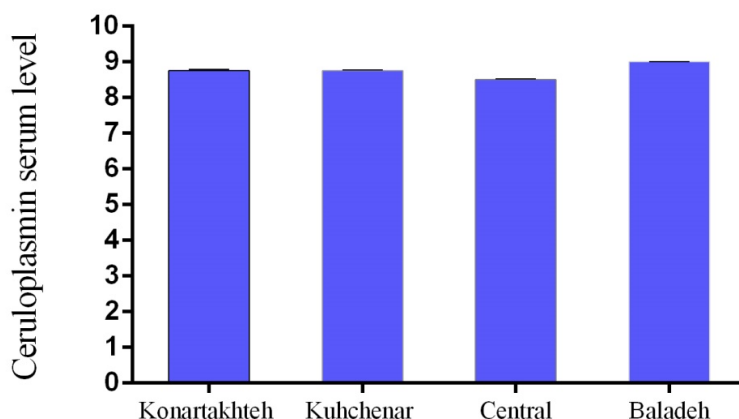
The results of the investigation of copper serum levels in different regions of Kazerun city showed that the highest average copper serum levels in the Kanaratakhte area were 65.25 ± 0.3 micrograms/dl and the lowest average copper concentration in the central area was 40.38 ± 0.5 . It was micrograms per deciliter. Also, the average copper serum levels in Kohchenar area were 53.25 ± 0.7 in Baladeh area and 65 ± 0.4 µg/dL (Chart 1). The average serum levels of copper in sheep in different regions of Kazerun city was 55.97 ± 19.57 , which was determined below the standard level (120-70 µg/dL).



Graph 1. Results of average copper serum levels (µg/dL) in different regions of Kazerun city.

The results of examining the average serum levels of ceruloplasmin in different regions of Kazerun also showed that the highest average level of ceruloplasmin in the Baladeh region was 9 ± 0.01 mg/dL and the lowest average level of ceruloplasmin in the central region was 5.0 ± 0.03 . It was 8 mg/dL.

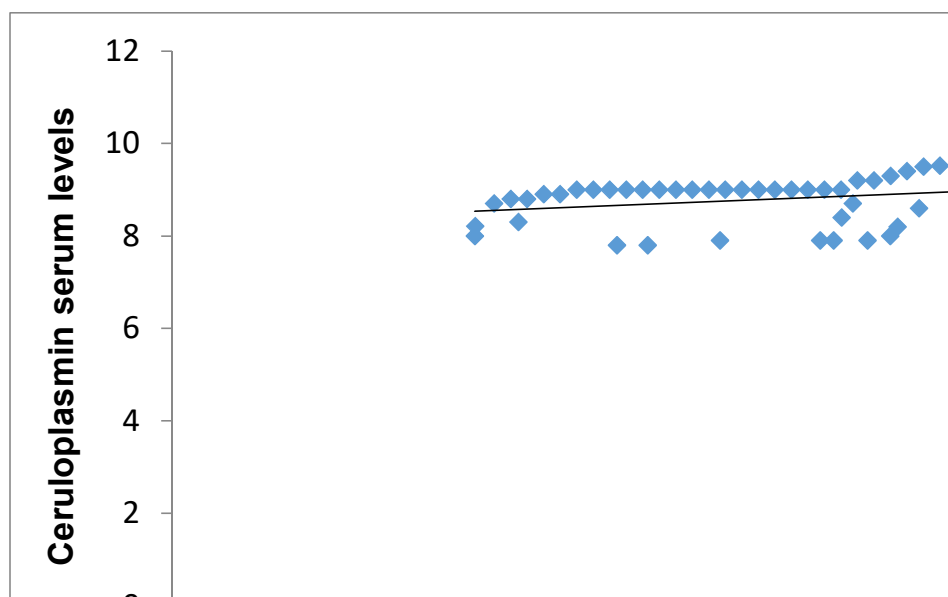
Also, the average serum levels of ceruloplasmin were 8.75 ± 0.02 in Kohchenar region and 8.75 ± 0.04 mg/dL in Konartakhteh area (Graph 2). The average serum levels of ceruloplasmin in sheep from different regions of Kazerun city were 8.87 ± 0.5 within the standard range (4.5-10 mg/dL).



Graph 2. The results of average ceruloplasmin serum levels (mg/dL) in different regions of Kazerun city.

The results of the overall average in sheep from different regions of Kazerun showed that the average copper serum levels were 55.97 ± 19.57 μ g/dL with deficiency status and the average ceruloplasmin level was 8.87 ± 0.5 mg/dL with favorable status. Also, the results of the statistical analysis of the correlation between serum copper concentration and

ceruloplasmin levels in sheep in different regions of Kazerun city showed a correlation of $r=0.892$ and a significant level of $P=0.05$, so between serum levels of copper and serum levels a statistically significant correlation was observed with ceruloplasmin (Graph 3).



Graph 3. The results of correlation between serum copper concentration and ceruloplasmin level.

Also, the highest copper serum levels were 94 µg/dL and the lowest copper serum levels were 30.55 µg/dL. The highest ceruloplasmin serum levels were

30.55 mg/dL and the lowest ceruloplasmin serum levels were 7.8 (Table 1).

	Maximum	Minimum	Correlation rate	Significance level
Copper	94	30.55	R=0.892	0.05
Ceruloplasmin	30.55	7.8		

Table 1. The results of statistical analysis of copper and ceruloplasmin serum levels.

Discussion

In many parts of the world copper deficiency may occur in grazing animals. This is not only dependent on the total concentration of copper in the diet, but also on the effective factors in the absorption and availability of copper (Rasooli *et al.*, 2014). Copper is one of the scarce mineral elements that is present in many important processes of the body and plays a vital role. Enzymes of ceruloplasmin, cytochrome *c* oxidase, copper-zinc superoxide dismutase and several other enzymes in mammalian tissues depend on copper. The role of copper in the activity of these enzymes has made this element play an important role in physiological functions such as hematopoiesis, immune system health, protection against oxidants and other functions. Copper deficiency causes clinical symptoms such as anemia, bone disorders, connective tissue disorders, imbalance in newborns, cardiovascular disorders, loss of body epidermal pigments, loss of wool and hair quality, diarrhea, infertility, and sensitivity to infections and diseases. Compared to other domestic animals such as goats, cows, pigs and chickens, sheep are more sensitive to excess amounts of copper, and if the amount of copper in the diet reaches more than 15 mg/kg, there is a possibility of copper poisoning (Vansaun, 2000; Smith, 2009). Despite the high sensitivity of sheep to copper excess, the presence of sulfur and molybdenum elements and the antagonistic relationship of these elements with copper reduces the absorption and occurrence of deficiency in sheep.

According to the latest statistics of the World Food and Agriculture Organization, in terms of sheep population, Iran includes more than 53 million, it is one of the top countries in the world, but unfortunately, in terms of economic efficiency, it is not very important in this matter. It provides the importance of raising this livestock and consequently the importance of increasing productivity in this matter. According to the available statistics, there are

about 11 million livestock units in summer pastures and more than 41 million livestock units in Qashlagi pastures. Grazing in excess of pasture capacity has caused a decrease in the average weight of livestock carcasses in recent years. Considering the above, nutritional problems and nutritional and metabolic diseases become important in such conditions (Vansaun, 2000; Smith, 2009).

Ceruloplasmin is a glycoprotein that contains 95% of the total copper in the blood circulation and the presence of this molecule is necessary for iron consumption and hemoglobin formation. Studies on the half-life of the Ceruloplasmin molecule show that copper is hardly removed from it and cannot be a source of primary transport. Ceruloplasmin is a copper-containing enzyme through which copper exerts its physiological function. The major pathogenesis of Cu deficiency injuries associated with tissue incomplete oxidation is due to defects in this enzyme system (Egbe-Nwiyi *et al.*, 2000). In a study conducted by Alidadi to investigate copper deficiency in Urmia pasture sheep, it was emphasized on the subclinical copper deficiency in the examined sheep (Alidadi, 2001). The results of this research are similar to the results of the present study. In the study of Rasooli *et al.*, on the sheep of Yasouj city, the results showed that the mean copper serum levels in spring, summer, autumn and winter were 1.27, 1.72, 1.91 and 1.21 micrograms per milliliter, respectively. The results of this research show the absence of copper deficiency in the sheep studied in Yasouj (Rasooli *et al.*, 2014). The results of this research show a higher concentration of copper compared to the present study.

Rastmanesh *et al* investigated the relationship between the amounts of copper, sulfur, iron, molybdenum and zinc in soil and fodder with the serum levels of copper and zinc in sheep in Sosangard city and concluded that compared to natural levels, the serum levels of copper and zinc In the sheep

under investigation, they were at the level of borderline deficiency or below the normal level (Rastmanesh *et al.*, 2018). The results of this research are similar to the results of the present study.

Rezaei Saber and Rezaei investigated the seasonal changes of blood copper in 200 sheep in Mahabad city of West Azerbaijan province, the results indicated a lack of copper serum levels in all seasons except spring (Rezaei Saber & Rezaei, 2007). The results of this research are consistent with the results of the present study. In the study by Pourjafar and Saei Dehkordi, which was conducted in order to measure the serum level of ceruloplasmin and copper, liver and kidney of sheep and check the correlation of the mentioned parameters in Shahrekord slaughterhouse, the results of the research showed that the minimum and maximum level of ceruloplasmin was 14 to 29 milliliters, grams per deciliter, serum copper is 0.6 to 2.2 micrograms per milliliter, liver copper is 87 to 570 ppm, and kidney copper is 10 to 36 mg per kilogram of dry matter (Shamsaddinini Bafti, 2016).

In the study by Pourjafar and Saei Dehkordi, which was conducted in order to measure the serum level of ceruloplasmin and copper, liver and kidney of sheep and check the correlation of the mentioned parameters in Shahrekord slaughterhouse, the results of the research showed that the minimum and maximum level of ceruloplasmin was 14 to 29 mg. In deciliter, serum copper is 0.6 to 2.2 micrograms per milliliter, liver copper is 87 to 570 ppm, and kidney copper is 10 to 36 mg per kilogram of dry matter. Also, the correlation coefficients between ceruloplasmin and serum copper, ceruloplasmin and liver copper, serum copper and liver copper, ceruloplasmin and kidney copper, serum copper and kidney copper, liver copper and kidney copper are 0.941, 0.572, 0.533 respectively. 0.488, 0.426 and 0.288 were determined. The results of the investigation showed that the amount of serum ceruloplasmin and copper in the serum, liver and kidney of sheep was within the normal range and the risk of copper deficiency in the sheep of the region was low (Pourjafar & Saei Dehkordi, 2007).

The results of this research showed a higher level of serum copper concentration and ceruloplasmin level compared to the present study. In a study conducted by Nikbin *et al.*, to investigate copper deficiency in Mazandaran sheep in the four seasons of

the year, they reported that sheep had borderline copper deficiency in spring and winter, so that this deficiency was greater in spring and they concluded that in the study area, livestock have a subclinical borderline deficiency (Nikbin *et al.*, 2016). The results of this research are consistent with the results of the present study. One of the limitations of this study was the simultaneous assessment of ceruloplasmin and copper serum levels in a cross-sectional study. It is suggested that the simultaneous assessment of serum copper and ceruloplasmin levels as well as liver and kidney copper levels should be done in a longer period of time.

Conclusion

Based on the results of this research, it can be concluded that the sheep of Kazerun city are suffering from subclinical copper deficiency and the result of this deficiency can cause adverse nutritional effects and metabolic diseases. Therefore, it seems necessary to use copper supplements in the diet of these animals.

Conflict of interest

There are no conflicts of interest between the authors.

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بررسی سطوح سرمی مس و سرولوپلاسمین در گوسفندان شهرستان کازرون، ایران

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چکیده

زمینه و هدف: در بسیاری از نقاط جهان ممکن است کمبود مس در دام ها رخ دهد. این امر نه تنها به غلظت تام مس در جیره غذایی بلکه به فاکتور های موثر در جذب و دستیابی به مس نیز وابسته است. هدف از انجام این مطالعه بررسی سطوح سرمی مس و سرولوپلاسمین در گوسفندان شهرستان کازرون، ایران بود.
مواد و روش ها: این مطالعه به منظور اندازه گیری سطوح سرمی مس و سرولوپلاسمین گوسفندان شهرستان کازرون در چهار منطقه کنارتخته، کوهچنار، بالاده و مرکزی انجام شد. ۶۰ راس گوسفند به صورت تصادفی انتخاب (هر منطقه ۱۵ راس گوسفند) شدند و خون گیری از آنها انجام گرفت. پس از جداسازی سرم، به منظور بررسی میزان غلظت سرمی مس و سرولوپلاسمین از دستگاه جذب اتمی استفاده شد. داده ها با استفاده از نرم افزار SPSS و آزمونهای همبستگی پیرسون (Pearson) و کای دو (chi-square) تجزیه و تحلیل شدند.

یافته ها: نتایج نشان داد به ترتیب بیشترین و کمترین میانگین سطح سرمی مس در منطقه کنارتخته $65/25 \pm 0/3$ میکروگرم بر دسی لیتر و منطقه مرکزی $40/38 \pm 0/5$ میکروگرم بر دسی لیتر بود. بالاترین و کمترین میانگین سطح سرمی سرولوپلاسمین در منطقه ی بالاده $9 \pm 0/01$ میلی گرم بر دسی لیتر و منطقه ی مرکزی $8/5 \pm 0/03$ میلی گرم بر دسی لیتر بود. همچنین نتایج میانگین کلی نشان داد، میانگین سطح سرمی مس $55/97 \pm 19/57$ میکروگرم بر دسی لیتر و میانگین سرولوپلاسمین $8/875 \pm 0/5$ میلی گرم بر دسی لیتر بودند که سطح سرمی مس در وضعیت کمبود تحت بالینی و سطح سرولوپلاسمین در وضعیت مطلوبی بود. آنالیز های آماری حاصل از بررسی همبستگی بین سطوح سرمی مس و سرولوپلاسمین در گوسفندان میزان همبستگی $r=0/892$ و سطح معناداری $P=0/05$ را نشان داد، بین سطوح سرمی مس و سرولوپلاسمین همبستگی آماری مشاهده شد و به ترتیب بیشترین و کمترین سطح سرمی مس ۹۴ و $30/55$ میکروگرم بر دسی لیتر و بیشترین و کمترین سطح سرمی سرولوپلاسمین $30/55$ و $7/8$ میلی گرم بر دسی لیتر بود.

نتیجه گیری: در گوسفندان مناطق مختلف شهرستان کازرون سطح سرمی مس دارای کمبود تحت بالینی و سطح سرمی سرولوپلاسمین وضعیت مطلوبی داشتند. بنظر می رسد اضافه کردن مکملهای مس به جیره گوسفندان این مناطق ضروری است.

واژه های کلیدی: گوسفند، مس، سرولوپلاسمین، شهرستان کازرون

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