

## Determination of the Levels of Zinc, Copper, Calcium, Phosphorus and Magnesium of Chios Ewes in the Aydın Region

Funda KARGIN, Kamil SEYREK, Ayşegül BİLDİK, Serap AYPAK  
Department of Biochemistry, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın - TURKEY

Received: 12.03.2003

**Abstract:** This study was undertaken to investigate the Cu, Zn, Ca, P and Mg levels of blood serum and the Cu and Zn levels of wool from clinically healthy Chios ewes grazed in meadows in the Aydın region. A total of 100 blood and wool samples from various villages in different districts of Aydın (Germencik, Yenipazar, Çine, Koçarlı and the central district) were studied. The Zn and Cu values were determined by an atomic absorption spectrophotometer. Serum Ca, P and Mg were measured by photometer using commercially available kits.

The average Cu, Ca, P and Mg values of sera were near to the normal values. However, the Zn levels of sera and wool were lower than the normal values. Likewise, the amounts of Cu in wool were below the normal levels. The levels of Cu, Zn, Ca, Mg and P in serum and the values of Cu and Zn in wool showed highly significant differences among the districts ( $P < 0.001$ ). There were no significant correlations between serum and wool values of Cu and Zn ( $P > 0.05$ ). In the light of these findings, the rations of animals grazing in meadows are recommended to be reinforced with Zn and Cu, at least in the winter.

**Key Words:** Chios ewes, copper, serum, wool, zinc

### Aydın Bölgesindeki Sakız Koyunlarının Çinko, Bakır, Kalsiyum, Fosfor ve Magnezyum Değerlerinin Tayini

**Özet:** Bu çalışmada Aydın bölgesindeki meralarda otlayan, klinik olarak sağlıklı görünen sakız koyunlarının serumlarındaki Cu, Zn, Ca, P ve Mg seviyeleri ile yünlerindeki Cu ve Zn düzeylerinin araştırılması amaçlandı. Çalışmanın materyalini Aydın'ın ilçelerinden (Germencik, Yenipazar, Çine, Koçarlı ve Aydın merkez) toplanan toplam yüzer adet serum ve yün örneği oluşturdu. Zn ve Cu değerleri atomik absorpsiyon spektrofotometresinde, Ca, P ve Mg değerleri ise ticari kitleler kullanılarak fotometrede tespit edildi.

Ortalama serum Cu, Ca, P, ve Mg düzeyleri normal değerlere yakın olarak bulunurken yündeki Cu ve Zn düzeyleri ile serumdaki Zn düzeyi normal değerlerin altında tespit edildi. Cu, Zn ve diğer mineraller yönünden ilçeler arasında istatistiksel olarak önemli farklılıkların bulunduğu tespit edildi ( $P < 0,001$ ). Serum ile yünlerdeki bakır ve çinko değerleri arasında istatistiksel olarak önemli bir fark bulunmadı ( $P > 0,05$ ). Bu bilgiler ışığında, Aydın bölgesi meralarında otlayan koyunların rasyonlarına en azından kış mevsiminde Cu ve Zn takviyesinin yapılmasının yararlı olacağı kanısındayız.

**Anahtar Sözcükler:** Sakız koyunu, bakır, serum, yün, çinko

### Introduction

The essential nature of trace elements is widely accepted. The indispensable feature of the most prominent of these trace elements rests on their role as functional or structural components of crucial metalloenzymes and metalloproteins (1). Disorders of essential elements arise from inadequate intake, genetic defects, excessive exposure, or impaired elimination. Severe deficiency is rare, but aspecific symptoms and reduced protection against risk factors have been ascribed to suboptimal intake of trace elements (2,3).

Zn is an important constituent of several enzymes mainly carbonic anhydrase and alkaline phosphatase, in the organic matrix (4-8). The major part of the total body Zn is in the bones and competes with Cu for absorption from the intestinal tract. The relation between Cu and Zn is very much similar to that between calcium and phosphorus (9,10). Clinical symptoms attributed to chronic Zn deficiency include impaired immunofunction, increased susceptibility to infections, poor wound healing, persistent skin disorders and poor growth (11-13).

It is known that Cu plays a role in the biochemical pathways of mammals, and is a building block of many

enzymes that have key functions in the metabolism (14-16). Copper levels in tissues and body fluids depend on diet, state of health, age and sex (17).

Magnesium is an important mineral in the human body and is mostly deposited in the skeleton. Absorption and transport of magnesium are affected by calcium and phosphorus. Magnesium is one of the 4 bulk metals in the human body. It is a co-factor for about 300 cellular enzymes (18).

Calcium has several important functions in the formation of bone and dental tissues, in the releasing of hormones, contraction of muscles and glycogen metabolism (12).

Phosphorus has various important functions in cells, such as the phosphate bonds in ATP as sources of energy for the contraction of muscles, neurological functions and electrolyte transport, among others (12).

The aim of the present study is 3-fold. The first is to determine the serum levels of Zn, Cu, Ca, P and Mg as well as the wool Cu and Zn values of Chios ewes grazed in the meadows of the Aydin region and to determine whether there is any deficiency in these elements. Another aim was to determine the differences in those elements among the districts the Aydin region.

## Materials and Methods

In this study, a total of 100 units of blood and wool samples, belonging to clinically healthy Chios ewes aged between 1 and 2 and grazed in meadows in the Aydin region were used. Collection of material was performed in the spring. Five districts (Germencik, Yenipazar, Çine, Koçarlı and the central district) were selected for the study. Blood samples were collected from the jugular vein and centrifuged at 3000 rpm for 5 min. Supernatants were separated to determine Zn, Cu, Ca, Mg and P levels. To measure the serum Zn and Cu levels the supernatants were diluted 20 times in a solution of 0.1 N HCl and levels were determined as described previously (19) using an atomic absorption spectrophotometer (Variant Spectra AA 220). Levels of Ca, P and Mg were determined by Microlab 2000 (Merck) using commercially available kits (Biomedical Systems, Barcelona, Spain). The analyses were carried out according to the manufacturer's recommendations.

Wool was collected from each ewe and stored at +4 °C until use. Wool samples were washed 4 times in a

solution containing 1% Triton x 1000. Following the washes they were dried over a period of 2 h at 100 °C: 100 mg from each sample was weighed and incubated in 1/5 nitroperchloric acid at 60 °C for 6-7 h. Dissolved hair samples were diluted with 1% Triton x 100. The measurement was carried out with an atomic absorption spectrophotometer (20).

Statistical analysis was carried out using the SPSS 10.0 program. ANOVA was used for comparison of districts and Pearson's correlation coefficient test was used for the correlation analysis. A value of  $P < 0.05$  was considered statistically significant. Variables are reported as mean  $\pm$  SE.

## Results

As shown in Table 1, in Chios ewes from different districts the mean Cu levels of serum were within normal values (between 116.1  $\mu\text{g}/\text{dl}$  and 147.3  $\mu\text{g}/\text{dl}$ ), whereas the level of wool Cu was remarkably below the normal values (between 2.1  $\mu\text{g}/\text{g}$  and 3.06  $\mu\text{g}/\text{g}$ ). Except for one district (Germencik) Zn values in both wool and serum were below the reference levels (between 43.7 and 89.3  $\mu\text{g}/\text{dl}$  in serum and 54.2 and 74.7  $\mu\text{g}/\text{g}$  in wool). The average values of Ca, P and Mg were near to normal values and they showed significant differences between districts (Table 2). No correlation was found between the serum and wool values of Cu and Zn ( $P > 0.05$ ).

## Discussion

Epidemiological studies have in the past been very fruitful in linking low levels of trace elements such as Cu and Zn in the environment or in the tissues of subjects to particular disease states. Even if the concentrations of an element in the blood are currently at or near normal, prior recent exposure can be documented by determining the concentration of the suspected element in wool at various distances from the scalp. Therefore wool offers a good way of discerning long-term variations in trace element concentration by providing a better assessment of normal trace element concentrations (21,22).

In this study Cu values of sera from Chios ewes from different districts were between 116.1  $\mu\text{g}/\text{dl}$  and 147.3  $\mu\text{g}/\text{dl}$ , while wool Cu levels were between 2.1  $\mu\text{g}/\text{g}$  and 3.06  $\mu\text{g}/\text{g}$ . The levels of Cu in serum were consistent with

Table 1. The average levels of copper and zinc in serum and in wool of Chios ewes.

Counties	Cu in serum µg/dl	Zn in serum µg/dl	Cu in wool µg/g	Zn in wool µg/g
Yenipazar	147.3 ± 15 <sup>a</sup>	69.4 ± 8.9 <sup>cd</sup>	2.1 ± 0.34 <sup>a</sup>	59.9 ± 7.8
Çine	116.1 ± 13 <sup>b</sup>	53.9 ± 14 <sup>bc</sup>	2.46 ± 0.43 <sup>b</sup>	60.4 ± 9.1
Koçarlı	138.4 ± 15 <sup>b</sup>	43.7 ± 5.4 <sup>ab</sup>	2.43 ± 0.46 <sup>b</sup>	74.7 ± 8.4
Germencik	118.2 ± 6.1 <sup>b</sup>	89.3 ± 14 <sup>d</sup>	3.06 ± 0.41 <sup>c</sup>	64.1 ± 7.1
Central district	116.7 ± 6.3 <sup>b</sup>	46.3 ± 5.6 <sup>b</sup>	2.28 ± 0.7 <sup>a</sup>	54.2 ± 6.6
F	6.756 <sup>***</sup>	7.943 <sup>***</sup>	29.823 <sup>***</sup>	1.226 <sup>NS</sup>

a,b,c,d : Differences between the values involving different letters on the same column are significant, \*\*\* = P<0.001, NS = Non significant.

Table 2. The average serum levels of calcium, phosphor and magnesium of Chios ewes.

Counties	Ca mg/dl	P mg/dl	Mg mg/dl
Yenipazar	13.65 ± 0.54 <sup>c</sup>	7.18 ± 0.26 <sup>a</sup>	2.12 ± 0.15 <sup>b</sup>
Çine	10.87 ± 0.40 <sup>b</sup>	6.06 ± 0.3 <sup>b</sup>	1.25 ± 0.078 <sup>a</sup>
Koçarlı	11.28 ± 0.51 <sup>b</sup>	5.96 ± 0.21 <sup>b</sup>	2.22 ± 0.21 <sup>b</sup>
Germencik	8.28 ± 0.25 <sup>a</sup>	5.27 ± 0.23 <sup>b</sup>	2.35 ± 0.02 <sup>b</sup>
Central district	9.16 ± 0.27 <sup>a</sup>	5.36 ± 0.26 <sup>b</sup>	2.2 ± 0.077 <sup>b</sup>
F	25.92 <sup>***</sup>	6.96 <sup>***</sup>	12.15 <sup>***</sup>

a,b,c,d : Differences between the values involving different letters on the same column are significant, \*\*\* = P<0.001.

the previous findings (14,23), which were reported as normal for healthy sheep, whereas the values of wool Cu were below the normal values. These controversial results may be explained by the fact that concentrations of Cu found in the wool do not reflect the status of Cu in the body at the time the sample was collected, but at

some prior time. Many investigators (8,24,25) reported that the levels of serum Zn in sheep should be between 80 and 120 µg/dl. In contrast to these findings our results were between 43.7 and 89.3 µg/dl. Furthermore, wool Zn values were measured between 54.2 and 74.7 µg/g. The values obtained in this study were remarkably lower than those of previous studies (4,14,26).

The serum levels of Ca, P and Mg were within the reference values in all the districts (25).

In summary, it has been shown that the values of Zn in Chios ewes grazing around the Aydın region were lower than normal values. It has also been shown that the average wool Cu levels of the animals used in this study were remarkably lower than the reference values. In the light of these data, it is thought that there is a Cu and Zn insufficiency in the rations given to the animals in the winter and these rations are recommended to be reinforced with Zn and Cu, at least in the winter.

## References

- Keen, C.L., Graham, T.W.: Copper. In: Biochemistry of Domestic Animals. 4<sup>th</sup> ed. Ed. Kaneko, J.J., New York, Academic Press Inc., 1989; 757-765.
- Patriarcha, M., Menditto, A., Di Felice, G., Petrucci, F., Caroli, S., Merli, M., Valente, C.: Recent developments in trace element analysis in the prevention, diagnosis, and treatment of diseases. Microchem. J. 1998; 59: 194-202.
- Paşa, S., Kargin F, Bildik, A., Seyrek, K., Özbek, Y., Özensoy, S.: Serum and hair levels of zinc and other elements in dogs with visceral leishmaniasis. Biol. Trace Elem. Res. 2003; 94: 141-147.
- Aksoy, G, Şahin, T., Çimtay, İ.: Effects of zinc oxide administration on body weight gain and some biochemical parameters in lambs. Turk. J. Vet. Anim. Sci. 2002; 26: 85-90.
- Gabor, S.: Trace elements and lipid peroxidation. Trace elements in health and disease. Ç.Ü. Pub. Trace. 89: Adana 1991; 273-277.
- Keen, C.L., Graham, T.W.: Zinc. In: Biochemistry of Domestic Animals. 4<sup>th</sup> ed. Ed. Kaneko, J.J., New York, Academic Press Inc., 1989; 776-795.

7. Hays, V.W., Swenson, M.J.: Minerals and Bones. Ed. M.J. Swenson, M.J. In: Physiology of Domestic Animals, 10<sup>th</sup> ed, Cornell University Press, London. 1984; pp. 449-466.
8. Ozan, S.: The Relation Between Fleece Losses and Zinc, Copper Levels in Blood Serum of Merino Sheep in the Karacabey State Farm Flock. Selçuk Üniv. Vet. Fak. Derg. 1985; 1: 133-142.
9. Söderhjelm, L: Some afflictions on trace elements in paediatrics. Acta Ped. Scand. 1985; 74: 17-25.
10. Wachnik, A.: The physiological role of copper and the problems of copper nutritional deficiency. Die Nahrung. 1988; 32: 755-765.
11. Ergün, A.: Zinc metabolism and deficiency in domestic animals, Ankara Üniv. Vet. Fak. Derg. 1983; 30: 308-316.
12. Kaneko, J.J., Harvey, J.W., Bruss, M.L.: Clinical Biochemistry of Domestic Animals. 5<sup>th</sup> ed. Academic Press, London., 1977; 619-702.
13. Rodriguez, A., Soto, G., Torres, S: Zinc and copper in hair and plasma of children with chronic diarrhea. Acta Pediatr. Scand. 1985; 74: 770-777.
14. Kurt, D., Denli, O., Kanay, Z., Güzel, C., Ceylan, K: An investigation of the copper (Cu), zinc (Zn) and selenium (Se) levels of blood serum and the Cu and Zn levels of wool of Akkaraman ewes in the Diyarbakır region. Turk. J. Vet. Anim. Sci. 2001; 25: 431-436.
15. Murray, R.K., Mayers, P.A., Graner, D.K., Roodweel, V.W.: Harper's Biochem. Appleton & Lange, New York. 1995; 137-143.
16. Yarze, J.Y., Martin, P., Munoz, S.J., Friedman, L.S.: Wilson's disease. Current Status. Am. J. Med. 1992; 92: 643-654.
17. Ashton, W.M.: Trace elements in enzyme systems with special reference to deficiency of copper and cobalt in some animal diseases. Outlook Agricul. 1970; 6: 95-101.
18. Devlin, T.M.: Textbook of Biochemistry with Clinical Correlations. Wiles-Liss, Inc., USA, 1997; 1129-1136.
19. Camas, H., Bildik, A., Gülsever, F.: Investigation of some trace elements (Cu, Mo, Zn, Co, Mn) and sulphate in soil, grass and sheep's blood. Y.Y.Ü. Vet. Fak. Derg. 1999; 10: 87-91.
20. Okamoto, K., Morita, M., Quan, H., Uehiro, T., Fuwa, K: Preparation and certification of human hair powder reference material. Clin. Chem. 1985; 31: 1592-1597.
21. Laker, M.: On determining trace element levels in man: the use of blood and hair. Lancet. 1982; 2: 260-262.
22. Rao, K.S., Balaji, T., Rao, T.P., Babu, Y., Naidu, G.R.K.: Determination of iron, cobalt, nickel, manganese, zinc, copper, cadmium and lead in human hair by inductively coupled plasma atomic emission spectrometry. Spectrochimica Acta Part B. 2002; 57: 1333-1338.
23. Bires, J., Kovac, G., Vrzgula, L: Mineral profile of serum in experimental copper intoxication of sheep from industrial emissions. Vet. Hum. Tox. 1991; 33: 431-435.
24. Georgievskii, V.I.: The Physiological Role of Microelements. Mineral Nutrition of Animals Eds: Georgievskii, V.I., Annenkov, B.N., Samokhin, V.T. Butterworths, London, UK. 1982; 72-171.
25. Rushton, B.: Veterinary Laboratory Data. London. B.V.A. Publications. 1984; pp: 1-55.
26. Eryavuz, A., Durgun, Z., Keskin, E.: Effects of rations supplemented with zinc on some rumen and blood parameters, mohair production and quality in faunated and defaunated angora goats. Turk. J. Vet. Anim. Sci. 2002; 26: 753-760.