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Evaluation of tear and serum trace elements (copper, selenium, and cobalt) in sheep

Duygu TARHAN[1], Sinem ÜLGEN[2], Fatma ATEŞ ALKAN[1,*], Dilek OLGUN ERDİKMEN[3], Çağla PARKAN YARAMIŞ[4], Mehmet Erman OR[2], Ümit Bora BARUTÇU[1]

[1]Department of Biophysics, Cerrahpaşa Faculty of Medicine, İstanbul University, İstanbul, Turkey
[2]Department of Internal Medicine, Faculty of Veterinary Science, İstanbul University, İstanbul, Turkey
[3]Department of Surgery, Faculty of Veterinary Science, İstanbul University, İstanbul, Turkey
[4]Vocational School of Veterinary Faculty, Equine and Equine Training Program, İstanbul University, İstanbul, Turkey

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Abstract: Metabolic diseases such as enzootic ataxia, white muscle disease, and white liver disease are among the most important problems of livestock for sheep. The aim of this study was to analyze whether tears can be used as an alternative diagnostic method in determination of diseases that result from lack or excess of copper, selenium, and cobalt. Samples of serum and tears taken from 45 healthy sheep of the Kivircik breed aged between 2 and 4 years were analyzed. The concentrations of copper, selenium, and cobalt were measured in serum and tears as 118.0 ± 36.0, 58.0 ± 15.0, and 0.5 ± 0.3 µg/dL and 16.0 ± 8.0, 7.0 ± 4.0, and 2.0 ± 1.0 µg/dL, respectively, by using inductively coupled plasma optical emission spectrometry. The concentrations of copper and selenium in serum samples were significantly higher than those of tear samples ($P < 0.001$). However, cobalt concentrations in serum were found to be lower than those in tears ($P < 0.001$). As a result, the authors suggest that determining cobalt concentrations in tears may be a valuable alternative diagnostic method in the diagnosis of metabolic diseases of sheep, rather than determining the copper and selenium concentrations, which might not be as reliable.

Key words: Sheep, tear, trace elements, metabolic diseases

1. Introduction

There is a gradual decline in sheep farming as a result of the existing diseases and lack of livestock production. Metabolic diseases such as enzootic ataxia, white muscle disease (WMD), and white liver disease (WLD) are currently the most important livestock diseases.

Most of the elements commonly found in nature are present as electrolytes in the intracellular and extracellular fluids and they are constituents in the tissues and organs of living organisms (1,2). Elements are classified as macrominerals and trace elements according to their required quantities in the body. The distribution of trace elements throughout the body is at the microgram level. These play structural roles in enzymes, vitamins, and hormones and take part in chemical reactions in the body (1). Trace elements are important due to the fact that animals are unable to synthesize them. Therefore, these elements should be acquired via diet, water, and other sources (3,4).

Trace elements in the body exist both in the tear fluid as well as in the blood (5,6). Tear fluid forms a tear film over the conjunctiva and the cornea of the eye and has several important functions for the health of the ocular surface (7). The tear film covering the eye surface is a fundamental component to preserve homeostasis and the wellbeing of the eye surface. The structure of tear film is constituted of three layers: the inner mucous layer, the middle aqueous layer, and the outermost lipid layer. The mucous layer mainly consists of the caliciform cells of the conjunctiva. The aqueous layer, which is the biggest part of the tear film, is composed of a watery solution of inorganic salts, enzymes, glycoproteins, proteins, glucose, and urea. The most important components in tears are proteins (8). The lipid layer is made up of waxes and cholesterol esters (6–11). Tear fluid forming the tear film covering the cornea and the conjunctiva of the eye has several important functions and is responsible for the health of the eye surface (7). The tear fluid has non-Newtonian properties because of the presence of large protein and mucin molecules in the tear film. Tears would become non-Newtonian if all lipids were separated from tears (10,12).

Information available by using tear-analyzing trace elements is extremely limited due to the low concentration of trace elements in tears with limited quantity and

* Correspondence: fatmaate@istanbul.edu.tr

the difficulty of obtaining tear samples. Nonetheless, the variations in the concentration of trace elements within the tear fluid could be a marker to evaluate some diseases resulting from the lack of trace elements. The concentrations of trace elements such as copper (Cu), selenium (Se), and cobalt (Co) can be analyzed in tear fluid due to the fact that trace elements bind to proteins in the human ocular tear film (6).

The aim of our study was to analyze the trace elements using an inductively coupled plasma optical emission spectrometry (ICP-OES) device to learn whether tears can be used as an alternative diagnostic method in the determination of diseases such as WLD, WMD, and enzootic ataxia, which occur as a result of copper, selenium, and cobalt deficiencies.

2. Materials and methods

2.1. Animals

In this study, serum and tear samples taken from 45 healthy sheep of the Kivircik breed, aged between 2 and 4 years old, were analyzed. The animal materials in our research were obtained from the farm at the **İstanbul** University Faculty of Veterinary Science. In this study, the sheep were selected regardless of sex.

2.2. Sample collection

Forty-five healthy Kivircik sheep were included in the study. Animals were held in a dry stone sheepfold and, in warm weather, sheep were shorn depending on the climate. They were fed with hay. Samples of blood and tears were collected once from each animal. The animals were held tightly in a standing position and the head was raised up to an angle of about 30° to allow the tear to accumulate at the lateral canthus. With one hand, the lower eyelid was gently rolled out using thumb pressure and the conjunctiva was exposed. Capillary tubes were used for tear sampling. The tubes were held between the thumb and index finger of the free hand. Tubes were kept at a degree of 30° or 45°, downwards, to eyelids of both eyes (Figure 1). The tip of the capillary tube was placed close to the tear pool that accumulated at the lateral canthus and slowly advanced toward the pool of tears. Capillary action drew the tears into the tube. Latex medical gloves were used to prevent contamination. Care was taken to prevent contact of tubes with eyelid margins, cornea, or facial hair. The tears collected into 80 IU/mL microhematocrit tubes (NRIS) were transferred to Eppendorf tubes. Blood was taken from the jugular vein under sterile conditions into sterile tubes (5 mL; Venglect). Blood tubes were centrifuged at 1500 × *g* for 5 min for obtaining serum samples. All tubes were stored at 4 °C until analysis.

2.3. Serum and tear measurements

The concentrations of Cu, Se, and Co elements for serum and tears were analyzed by ICP-OES (Thermo Scientific iCAP 6000). In the study, 324.754 nm, 196.090 nm, and

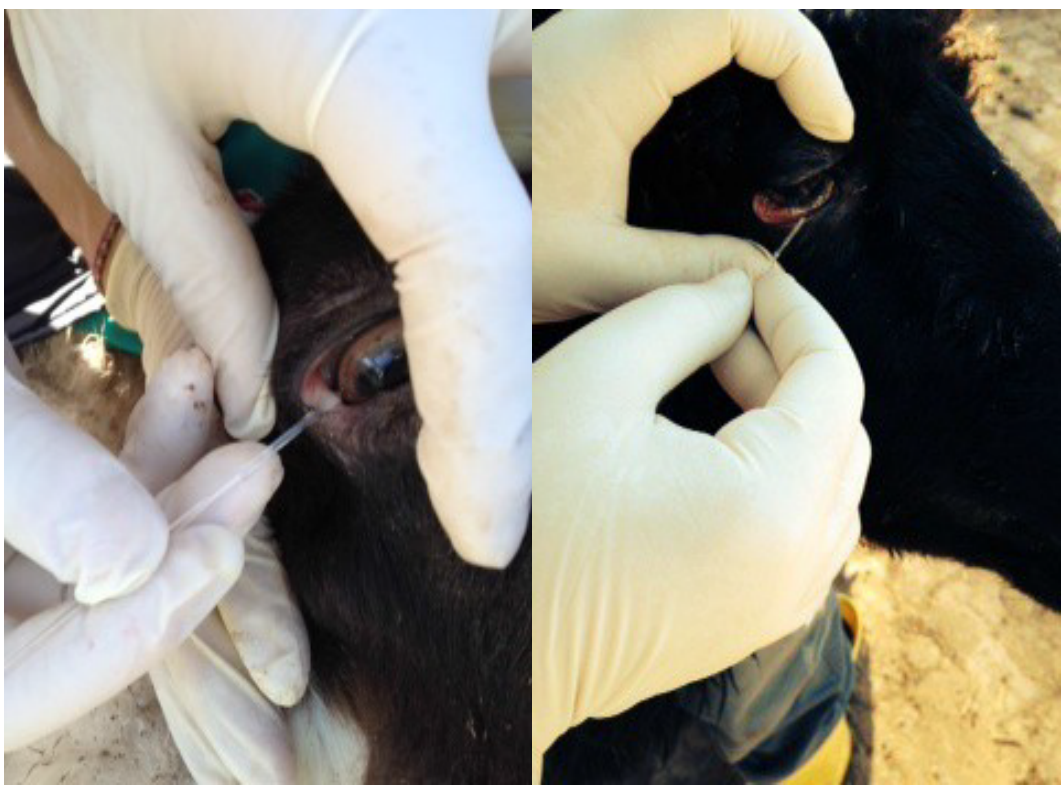

Figure 1. Taking the tear sample from sheep.

228.616 nm wavelengths were used to analyze Cu, Se, and Co elements, respectively. The standards used in the study for the analysis of each element are shown in Table 1. In Figure 2, the emission graphs of Cu, Se and Co as a function of concentration are shown. Serum samples were obtained after blood tubes were centrifuged and tear samples were diluted with deionized water in a fresh pretreated tube using an automatic pipette before analysis. Deionized water was used for a blank throughout.

2.4. Statistical analysis

Statistical analysis was carried out with SPSS 17.0. The concentrations of Cu, Se, and Co determined in different samples underwent a statistic-descriptive analysis for all parameters. Significant values in samples were then assessed with the unpaired Mann–Whitney U test. All results are expressed as mean ± standard deviation (SD). $P < 0.05$ was regarded as significant.

Table 1. Table of used standards for copper, selenium, and cobalt (ppm = µg/mL).

Standard name	Stated (ppm)
Std 1	0.05
Std 2	0.10
Std 3	0.50
Std 4	1.00

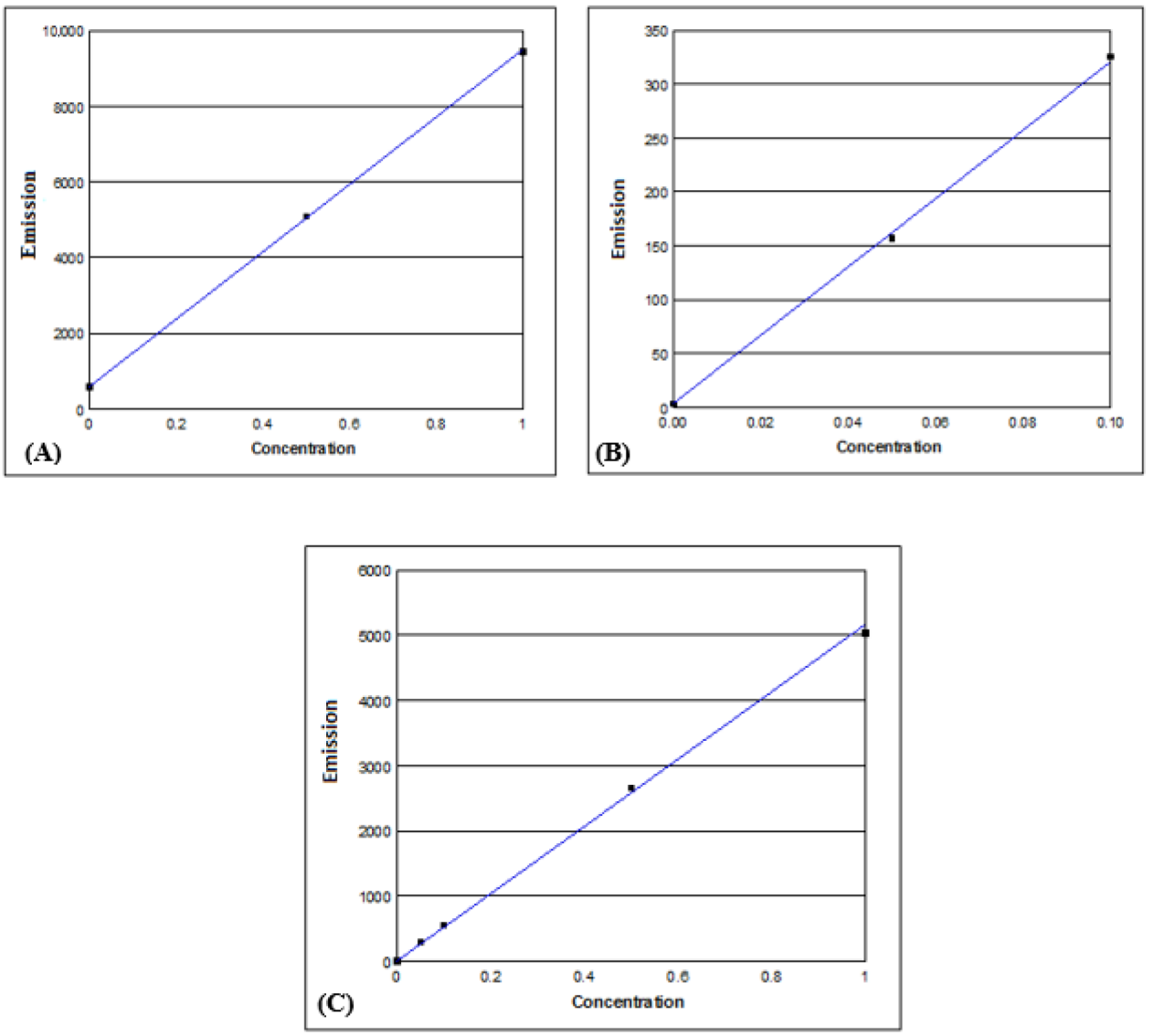

Figure 2. The emission graphs of copper (A), selenium (B), and cobalt (C) for all standards as a function of concentration.

3. Results

As a result of analysis, the concentrations of Cu, Se, and Co in serum and tears were measured as 118.0 ± 36.0, 58.0 ± 15.0, and 0.5 ± 0.3 µg/dL and 16.0 ± 8.0, 7.0 ± 4.0, and 2.0 ± 1.0 µg/dL, respectively (mean ± SD). Cu concentrations in tear samples ranged between 8 µg/dL and 32 µg/dL (mean 16 µg/dL; median 13.5 µg/dL; SD 8 µg/dL) and Cu concentrations in serum ranged between 36 µg/dL and 235 µg/dL (mean 118 µg/dL; median 115 µg/dL; SD 36 µg/dL). Se concentrations in the tear samples ranged between 1 µg/dL and 13 µg/dL (mean 7 µg/dL; median 8 µg/dL; SD 4 µg/dL) and that of serum samples ranged between 15 µg/dL and 88 µg/dL (mean 58 µg/dL; median 58.5 µg/dL; SD 15 µg/dL). Co concentration in tear samples ranged between 1 µg/dL and 4.4 µg/dL (mean 2 µg/dL; median 1.3 µg/dL; SD 1 µg/dL) and that of serum samples ranged between 0.2 µg/dL and 1.6 µg/dL (mean 0.5 µg/dL; median 0.5 µg/dL; SD 0.3 µg/dL).

The results for Cu, Se, and Co concentrations obtained from the tear and serum samples are summarized in Figure 3.

Cu and Se concentrations in serum were found to be statistically significantly higher when compared to the concentrations in tear fluid ($P < 0.001$) (Table 2). However, Co concentrations in serum were found lower than that of tear fluid ($P < 0.01$) (Table 2).

4. Discussion

Tears play an important role in the normal maintenance and functioning of the eye. The tear fluid, which is composed of a watery structure covered by an outermost lipid layer, has several serum proteins that pass from the tear fluid (13,14). Thus, serum proteins and the compositions of several serum proteins in tears resemble each other. Major components of human tear proteins were compiled and listed by Ohashi et al. (9). These include proteins such as albumin, transferrin, immunoglobin, lactoferrin, cystatin, lipocalin, epidermal growth factor, mammaglobin B, proline-rich protein, and aquaporin 5 (AQP5). The major part of total tear protein consists of lysozyme, lactoferrin, secretory immunoglobin A, serum albumin, lipocalin, and lipophilin (15). Shamsi et al. (16), comparing the tear proteins in the tear samples of humans, cows, sheep, and camels, showed similar major tear proteins in their study.

Trace elements are carried by proteins. Cu, Se, and Co bind to albumin in the serum (17–19). They can also join the structure of different proteins in the serum such as selenocysteine and α_2-macroglobulin.

The lack of Cu, Se, and Co leads to disorders such as enzootic ataxia, WMD, and WLD, respectively (3,4). WLD, characterized by increased accumulation of fat in the liver due to lack of cobalt and vitamin B_{12}, is one of most important diseases of sheep (20,21). WMD, which

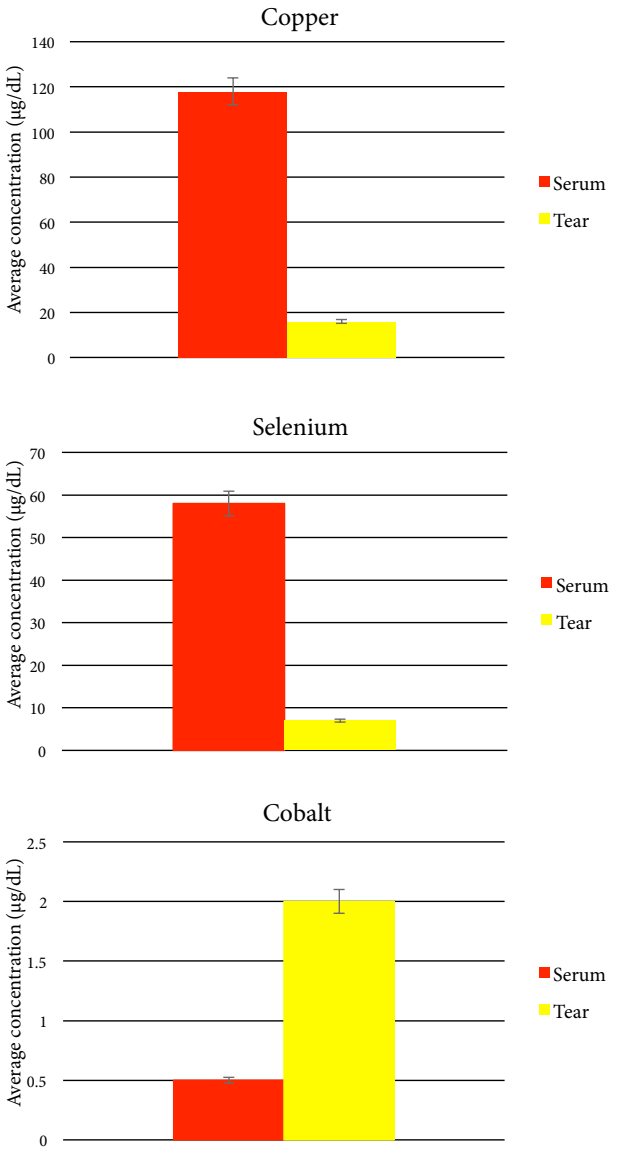

Figure 3. The average concentrations of copper, selenium, and cobalt in the serum and tears (mean ± SD).

Table 2. Copper (Cu), selenium (Se), and cobalt (Co) values of serum and tears.

Trace elements	Serum (n = 45)	Tears (n = 45)
Cu (µg/dL)	118 ± 36	16 ± 8***
Se (µg/dL)	58 ± 15	7 ± 4***
Co (µg/dL)	0.5 ± 0.3	2 ± 1**

Values are expressed as means ± standard deviations.
**P < 0.01, ***P < 0.001 compared with the serum.

is a disease of muscles different from the pale muscles of animals with anemia, is a nutritional disease characterized by degeneration of muscles, such as the diaphragm and skeletal muscle, due to the lack of selenium and vitamin E (3,22). Enzootic ataxia is also characterized by hypochromic macrocytic anemia, growth retardation, reduced production yield, deterioration in wool quality, diarrhea, and neurological symptoms as a result of primary or secondary copper deficiency (23). Primary copper deficiency develops in copper-deficient pastures in the area of livestock. Secondary copper deficiency causes symptoms of enzootic ataxia disease. It may occur despite the normal copper concentration of soil and plants in the area. This is due to the fact that the absorption of copper into the body is inhibited by elements antagonistic to copper, such as molybdenum, sulfur, and iron (24,25).

Semeraro et al. (6) analyzed the tear fluids taken from 42 women and 18 men in urban and agricultural areas using an ICP-MS device in their study that examined the trace elements in the tear film. They concluded that it was possible to analyze higher concentrations of chromium, arsenic, copper, zinc, selenium, and cobalt elements in the tear fluid (6).

Schoster et al. (5) determined that the copper trace element existed in the tear fluid of 6 healthy adult sheep. The concentrations of Cu in the tear samples were measured by atomic absorption electrothermal atomization (with graphite furnace) spectrometry.

Cu, Se, and Co concentrations in the tear fluid were detected in this study. The authors of this article point out that trace elements can only be measured if the amounts of tear samples suffice for analysis. Other trace elements can also be analyzed in tear fluid for diagnosis of metabolic diseases occurring as a result of trace element deficiencies and/or due to the level of their concentrations.

In our study, Cu and Se concentrations in the serum were significantly higher compared to those of tear samples. However, Co concentrations in the serum were lower than those of tear samples. The difference between the Co concentrations of the serum and tear fluid was less than the difference between Cu and Se concentrations. Thus, there was a positive correlation between the Co concentrations of the serum and the tear fluid.

In conclusion, our results demonstrated that the trace element concentrations in tears could be measured. Additionally, it might be postulated that copper and selenium concentrations in tears would not be reliable as an alternative diagnostic method. However, cobalt concentrations in tears may be useful for diagnosis and follow-up of all sheep. In our opinion, further studies should be carried out to analyze cobalt concentrations in tears.

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