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Research Article

Determination of Macro and Trace Element Levels of Serum, Tears, Saliva, and Hair Samples in Kilis Goats with ICP-MS

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Abstract: This study is focused on the evaluation of macro and trace elements concentration including sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), selenium (Se), nickel (Ni), titanium (Ti), manganese (Mn), cobalt (Co), and chromium (Cr) of the serum, saliva, tears, and hair samples in Kilis goats. The study involved 33 goats without clinical signs of disease. Individual serum, saliva, tears, and hair samples from each goat were collected for element quantifications through the inductively coupled plasma mass spectrometry (ICP-MS) method after acid digestion in a microwave system. Element concentrations varied and depended on the sample type. The most predominant element in serum and saliva samples was Na (3265±53.6 and 3559±55.2 mg/L, respectively), followed by K>Ca>Mg. Potassium was the most abundant element analyzed in tears and hair samples (3506±305 and 4664±100.5 mg/L, respectively). The major trace element was Fe in all sample types except hair samples. Nickel was detected only in serum samples (83.9±6.35 μg/L) and Mn was detected only in hair samples (12903±3142 μg/L). In all samples, Co was below the detection limit. There was a significant correlation of some elements between serum and hair (Na, K, Mg, Cu), tears and hair (K, Mg, Fe), serum and tears (K) samples. Trace elements concentration did not pose a risk for deficiency or excess. It was concluded that tears and saliva are potential alternative materials for element analysis and simultaneous tears, saliva, and hair along with serum could be useful in predicting the inorganic metabolic status of goats.

Keywords: *Element, Goat, Hair, ICP-MS, Saliva, Tears*

Kilis Keçilerinde Serum, Gözyaşı, Tükürük ve Kıl Örneklerinin Makro ve Eser Element Düzeylerinin ICP-MS ile Belirlenmesi

Öz: Bu çalışma, Kilis keçilerinde serum, tükürük, gözyaşı ve kıl örneklerinde makro ve eser elementlerden sodyum (Na), potasyum (K), kalsiyum (Ca), magnezyum (Mg), demir (Fe), bakır (Cu), çinko (Zn), selenyum (Se), nikel (Ni), titanyum (Ti), manganez (Mn), kobalt (Co) ve krom (Cr) konsantrasyonlarını belirlemeye odaklanmıştır. Klinik hastalık belirtisi göstermeyen 33 Kilis keçisinin herbirinden, mikrodalga sisteminde asitle parçalamanın ardından indüktif eşleşmiş plazma-kütle spektrometresi (ICP-MS) yöntemiyle element miktar tayini için serum, tükürük, gözyaşı ve kıl örnekleri toplandı. Element konsantrasyonları değişkendi ve örnek tipine bağlıydı. Serum ve tükürük örneklerinde en çok bulunan element Na (sırasıyla 3265±53.6 ve 3559±55.2 mg/L) ve ardından K>Ca>Mg'du. Potasyum, gözyaşı ve kıl örneklerinde analiz edilen en bol elementti (sırasıyla 3506±305 ve 4664±100.5 mg/L). Kıl örnekleri hariç tüm örnek tiplerinde ana eser element Fe idi. Nikel sadece serum örneklerinde (83.9±6.35 μg/L) ve Mn sadece kıl örneklerinde (12903±3142 μg/L) tespit edildi. Tüm örneklerde Co, tespit limitinin altındaydı. Serum-kıl (Na, K, Mg, Cu), gözyaşı-kıl (K, Mg, Fe) ve serum-gözyaşı (K) örnekleri arasında bazı elementlerde önemli korelasyonlar vardı. Eser element konsantrasyonları eksiklik veya fazlalık için bir risk oluşturmamaktaydı. Gözyaşı ve tükürüğün element analizi için potansiyel alternatif materyaller olduğu ve eş zamanlı gözyaşı, tükürük ve kılın serum ile birlikte keçilerin inorganik metabolik durumunu tahmin etmede faydalı olabileceği sonucuna varıldı.

Anahtar sözcükler: *Element, Gözyaşı, ICP-MS, Keçi, Kıl, Tükürük*

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Introduction

Goat breeding is a global activity and Turkey makes a significant contribution to this activity. Kilis goats, consist of nearly half a million of Turkey's goat population and are mostly spread in the South-eastern region of Anatolia, draw attention to their high milk capacity and breeding performance under comprehensive farming systems [1].

Elements are vital for the metabolism of all mammals as they are essential in biological processes [2]. Essential elements are classified as macro and trace elements, depending on the concentration needed in diet and animal tissues. The macro elements exist in all tissues and fluids, and they act a crucial part in nerve transmission, muscle contraction, a healthy immune system, and acid-base homeostasis [3]. Trace elements are vital components of wide biological functions such as oxygen transport, vitamin and hormone synthesis, cell metabolism, collagen synthesis, energy production, and enzyme activity [4]. An increase or decrease in trace element concentrations is generally associated with abnormalities in metabolic, reproductive, immunological, and hormonal functions. This imbalance could be of varying severity and occasionally causes characteristic clinical signs [5,6]. Element deficiencies that cause reproductive disorders, reduced growth and milk production, and high mortality in small ruminants may not always be accompanied by clinical symptoms. Therefore, reliable biological material research involving different tissue, body fluids and hair comes into prominence in the diagnosis of inorganic metabolism imbalances in veterinary medicine [7].

The tear is essential for the health and homeostasis of the ocular surface [8]. Information on the elemental concentration of the tear fluid is scarce. The balance of the components of the tear fluid is disturbed in many systemic diseases. Therefore, it has the potential to be a reliable source of information on many disease states. There are current studies on the use of tears in the diagnosis of many diseases, including systemic sclerosis, cancer, thyroid disorders, diabetes mellitus, diabetic peripheral neuropathy, diabetic retinopathy, Alzheimer's disease, Parkinson's disease, multiple sclerosis (MS), migraine, and cystic fibrosis in humans [9].

Saliva reflects serum biomarkers, as serum components could reach the entire saliva by passive diffusion or gingival crevicular fluid [10]. In recent years, various studies on different animal species have reported that the analysis of health status markers in saliva samples is more sensitive than those performed in serum samples. At the same time, more economical, easy, fast, non-invasive sampling with minimal equipment makes saliva a good choice as an alternative diagnostic fluid. Saliva is more than a reflection of animal serum biomarkers [11,12].

Hair has a very active metabolism during its growth and is affected by health and nutrition status. Due to its structure, elements that cannot be detected in the blood at the same time can be found in the hair at a significant concentration, which has led to the evaluation of the hair as an important biological material in element analysis [13]. The analysis of animal hair is considered one of the fast and most effective ways to obtain sufficient information to assess inorganic metabolism and health status [14,15].

The below-average production and reproductive performance of ruminants require assessment of metabolic status. In the evaluation of the inorganic metabolic state, which is an important part of the general metabolic state, studies that include the determination of reliable biological materials and methods are valuable. The studies on the element concentrations in the various body fluids and tissue of goats are quite limited. The purposes of this study were to (i) determine inorganic metabolism status by simultaneously detecting the macro and trace element concentrations of serum, saliva, tears, and hair samples in Kilis goats, (ii) state the suitability of different biological materials for the ICP-MS method, and (iii) indication the correlation between the element concentrations of serum, tears, saliva, and hair samples.

Material and Methods

Ethical Statement

The study was conducted with the permission of Harran University Animal Experiments Local Ethics Committee (HRUHADYEK) with the number 2021/009/02.

Animals and Sampling

One-three years old 33 without clinical signs of disease Kilis goats lived in mid-lactation period were included in the present study. All animals were fed on pasture in Şanlıurfa (37°10'N: 38°47'E) at an altitude of 518 m above sea level. Fresh water was available *ad libitum*. The serum, tears, saliva, and hair samples were collected once from each goat in April-May.

Blood samples were collected from V. jugularis into vacuum serum tubes, 10 mL tubes without anticoagulant with a negative pressure system for serum. A capillary tube was used to collect tears samples. The standing animals had their heads tilted slightly and their eyelids opened gently. Tears accumulated in the lateral canthus were transferred to eppendorf tubes by capillary tubes [16]. For saliva samples, the sponge was placed on the cheek of the goat for an average of 1 minute with the help of forceps. Sponges were placed in tubes and transported to the laboratory [12]. All blood, tears, and saliva samples were separated by centrifugation (3000 rpm for 10 min) and stored at -20°C until analysis.

The hair samples were collected from the shoulder area using ethanol-precleaned stainless steel scissors and before being analyzed were washed in acetone in 10-15 minutes and then rinsed thrice in ultrapure water [17].

Chemicals and Standard Solutions

The chemicals, 37% (v/v) HCl, 65% (v/v) HNO_3, 30% (v/v) H_2O_2 were purchased from Merck (Germany). Stock standard solutions of elements were provided by Agilent Japan: Lot Number: 10-160YPY2. Standart certified reference material (NIST SRM 8435) was obtained from Nova Chimica (Milano, Italy). Argon gas (99.9990%) was provided by Linde Gases (Linde Group, Turkey).

Sample Preparation and Microwave Acid Digestion

For the purpose of eliminating possible contamination of elements, the whole equipment was previously kept for one night in 10% HNO_3 and then washed with ultrapure water. A closed microwave-assisted digestion procedure was used for all samples mineralization. Each serum (1.0 mL), saliva (100 µL) and tears (100 µL) samples were taken in tetrafluoroethylene vessels was mixed with 0.5 mL HNO_3, 1.5 mL HCl, and 0.25 mL H_2O_2. Hair samples (100 mg) taken into tubes were mixed with 4 mL HNO_3 and 2 mL H_2O_2. After digestion was complete tetrafluoroethylene vessels were cooled to room temperature. To optimize the pH for analysis by ICP-MS, the samples were transferred into a 50 mL polyethylene volumetric flask and diluted to 50 mL with ultrapure water (MES MP Mini pure, Türkiye). They were stored at +4°C until analyzed. The microwave digestion procedure of blanks was the same as the samples.

ICP-MS Procedure and Quality Assurance

Element analyses were determined on an Agilent 7500ce with an octopole reaction system inductively coupled plasma-mass spectrometer with an autosampler (Cetac ASX-520) and a nebulizer (Agilent, Japan). All samples were analyzed in three times. These isotopes were preferred to minimize interferences and maximize sensitivity. Recovery of 13 elements in all samples was between 96.8-102.0%.

Statistical Analyses

Statistical calculations were carried out using SPSS 22.0 software (SPSS Inc., Chicago, USA). Element concentrations of serum, tears, saliva and hair were compared by analysis of variance with repeated measures. A paired sample t-test was used for multiple comparisons. Relationships among numeric variables were developed using the Spearman rank correlation coefficient. The P-value lower than 0.05 was considered statistically significant. The graphics were created using the R "Hmisc" and "corrplot" packages [18,19]. The correlations between the elements of serum, tears, saliva, and hair were calculated while the significance value was evaluated.

Results

The concentration and comparison of macro and trace elements in serum, tears, saliva, and hair samples are presented in *Table 1*. There were statistical differences between the Na, K, Ca, Mg, Fe, Cu, Zn, Se, Ti, and Cr concentrations of different biological material samples ($P<0.05$). Nickel was detected only in serum samples and Mn was detected only in hair samples. In all samples, Co was below the detection limit.

Sodium was the most abundant macro element in serum and saliva samples (3265±53.6 and 3559±55.2 mg/L, respectively), followed by K>Ca>Mg. The major trace element found in serum samples was Fe (3.33 mg/L), followed by Zn>Ti>Cu>Cr>Se. Manganese and Co levels were below the detection limit in serum samples. In saliva samples, the most abundant trace element was Fe (1.79 mg/L), followed by Cr>Zn>Cu>Se>Ti. In saliva samples, Ni, Mn, and Co levels were below the limit of detection.

The most abundant macroelement in tears samples was K (3506 mg/L), followed by Na>Ca>Mg. The major trace element found in tears samples was Fe (5.02 mg/L), followed by Cr>Zn>Cu. Selenium, Ni, Ti, Mn, and Co levels were below the limit of detection in tears samples.

According to our study results, in hair samples the most predominant macroelement was K (4664 mg/kg), followed by Ca>Mg>Na. As for the trace elements, Zn (118.0 mg/kg) was the most notable in the hair samples, followed by Fe>Ti>Se>Mn>Cu. The levels of Ni, Co, and Cr were below the detection limit in hair samples.

The correlation of elements in serum, tears, saliva, and hair samples are presented in *Table 2*. Most of the significant correlations among elements were found in the hair samples. In all samples, the elements most correlated with other elements were Na, K, and Fe. *Fig.1* shows a correlation heatmap between serum, tears, saliva, and hair elements.

Table 3 represents correlations for pairs of elements between serum, tear, saliva, and hair samples. These correlations were verified, taking into concideration only those significant coefficient ($P<0.05$). A very strong correlation was detected between the serum and hair Na concentrations (rho: 0.703) ($P<0.01$). In terms of K values, there was a strong correlation between serum and tears (rho: -0.530), serum and hair (rho: -0.383), and tears and hair (rho: 0.492). In addition, a significant correlation was detected in Fe concentrations between the tears and hair samples (rho: -0.475) and in Cu concentrations between serum and hair samples (rho: 0.400).

Table 1. *Element concentrations and comparison in serum, tears, saliva, and hair samples*

Element	Serum		Tears		Saliva		Hair		P Value
	Min-Max	X ± Sx (n)	Min-Max	X ± Sx (n)	Min-Max	X ± Sx (n)	Min-Max	X ± Sx (n)	
Na (mg/L)	2477-4031	3265±53.6^{a} (33)	448-3111	1989±159^{b} (33)	3088-4120	3559±55.2^{c} (33)	382-2709	1534±88.6^{1,b} (33)	***
K (mg/L)	148.8-362.1	232.3±10.1^{a} (33)	1410-5011	3506±305^{b} (33)	166.1-491.1	243.6±17.6^{a} (33)	3464-5684	4664±100.5^{1,c} (33)	***
Ca (mg/L)	61.4-112.7	89.8±2.05^{a} (33)	16.5-64.7	37.2±3.0^{b} (33)	5.34-23.24	14.18±1.17^{c} (33)	454.5-4678.5	2731±215.1^{1,d} (33)	***
Mg (mg/L)	14.4-38.4	24.9±0.76^{a} (33)	9.2-48.4	20.8±1.96^{b} (33)	5.57-36.8	10.7±1.6^{c} (33)	15.9-882.7	437.3±39.6^{1,d} (33)	***
Fe (mg/L)	1.1-19.1	3.33±0.52^{a} (33)	0.84-7.87	5.02±2.35^{b} (33)	1.15-3.09	1.79±0.1^{c} (33)	44.9-593.4	114.8±19.7^{1,d} (33)	***
Cu (µg/L)	271.7-1212.5	435.4±34.7^{a} (33)	53.8-315.6	193.4±21.9^{b} (33)	177.7-395.1	285.7±14.9^{c} (33)	8440-11725	10007±192.4^{2,d} (33)	***
Zn (mg/L)	0.194-2.684	1.392±0.574^{a} (33)	0.591-3.117	1.669±0.168^{a} (33)	0.057-1.334	0.370±0.107^{b} (12)	87.56-186.6	118.0±4.56^{2,c} (33)	***
Se (µg/L)	169-537	231.2±25.2^{a} (30)	-	<LOD	133-382	136±13.8^{b} (33)	5812-20037	13802±655^{2,c} (33)	***
Ni (µg/L)	53.3-131.1	83.9±6.35 (12)	-	<LOD	-	<LOD	-	<LOD	N.A.
Ti (µg/L)	355.2-606.9	497.2±10.7^{a} (33)	-	<LOD	90.2-179.8	125.3±5.58^{b} (33)	11780-86820	18699±931^{2,c} (33)	***
Mn (µg/L)	-	<LOD	-	<LOD	-	<LOD	632-70050	12903±3142^{2} (33)	N.A.
Co (µg/L)	-	<LOD	-	<LOD	-	<LOD	-	<LOD	-
Cr (µg/L)	73-845	427.7±43.6^{a} (23)	1474-1898	1733.4±62.2^{b} (6)	93-1283	821±93.4^{c} (33)	-	<LOD	***

[1] Concentration is expressed as mg/kg, [2] concentration is expressed as µg/kg, limit of detection is expressed as LOD, [a-d] Different letters in the same row indicate significant differences (P<0.05)

Table 2. *The Spearman's rank significant correlation of macro and trace elements in serum, tears, saliva and hair samples*

Serum		Tears		Saliva		Hair	
Element	rho	Element	rho	Element	rho	Element	rho
Zn-Na	-0.584**	Na-K	0.627**	K-Cu	0.750**	Fe-Na	0.634**
Zn-K	-0.449**	Na-Fe	0.766**	K-Fe	0.602**	Fe-Mg	0.548**
Zn-Ca	0.647**	Na-Cu	0.775**	Ti-Mg	0.680**	Fe-Ca	0.625**
Zn-Ti	0.459**	K-Fe	0.810**	Ti-Ca	0.677**	Fe-Ti	0.754**
Zn-Fe	0.613**			Cu-Cr	0.658**	Fe-Mn	0.696**
Cu-Na	0.481**					Cu-Na	0.927**
Cu-K	0.566**					Cu-K	-0.544**
Ti- Mg	0.526**					Cu-Fe	0.600**
Ti-Ca	0.915**					Mn-Mg	0.860**
Na-K	0.688**					Mn-Ca	0.866**
Ca-Mg	0.576**					Se-Na	-0.497**
						Se-K	0.651**
						Ti-Mg	0.880**
						Ti-Ca	0.944**
						K-Na	-0.693**
						Ca-Mg	0.950**

*** P<0.01*

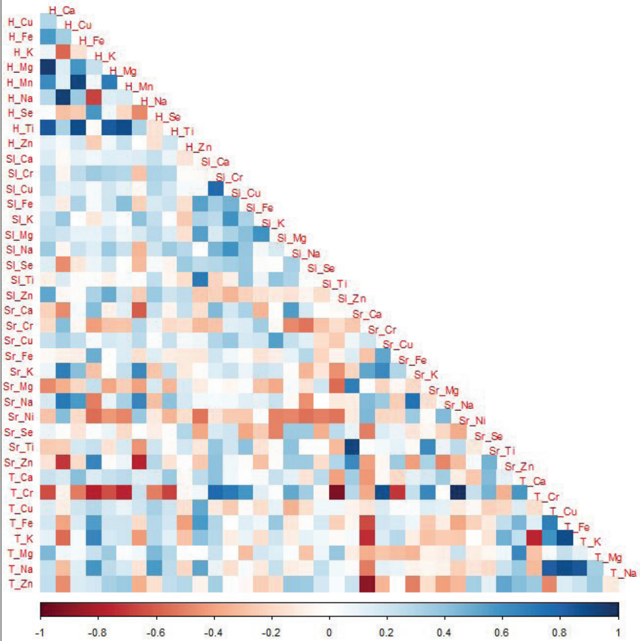

Fig 1. The correlation heatmeap of serum, tears, saliva, and hair samples. H: Hair, Sl: Saliva, Sr: Serum, T: Tear

Table 3. *Significant Spearman's rank correlations for macro and trace elements between serum, tears, and hair samples*

Element	Serum-Tears	Serum-Hair	Tears-Hair
Na-Na		0.703**	
K-K	-0.530*	-0.383*	0.492*
Mg-Mg		-0.389*	0.527*
Fe-Fe			-0.475*
Cu-Cu		0.400*	

** P<0.05, ** P<0.01*

Discussion

This study tries to determine the various macro and trace elements in the serum, saliva, tears, and hair samples of Kilis goats. The assessment of the inorganic metabolic status of livestock is a valuable tool in herd health management of animals, both individually and on a herd basis. Therefore, below-average production and reproductive performance in ruminants is a condition that should be evaluated for inorganic metabolism status.

In this study, the macro elements measured in samples were Na, K, Ca, and Mg, and their mean serum concentrations were 3265±53.6, 232.3±10.1, 89.8±2.05, and 24.9±0.76 mg/L, respectively. Overall, all the concentrations for serum macroelements were within physiological ranges in goats [20].

The serum Na concentrations observed in this study were mostly in agreement with studies with other goat breeds [21-24]. In our study serum Na levels in Kilis goats were higher than those in Guizhou black [25] and Halep goats [26], and it was lower than Kilis [27] and Angora goats [26].

The mean serum K concentration was parallel to those reported by Schweinzer et al.[2]. Previous studies have reported K concentration of different goat breeds and Kilis goat serum ranging from 119-199 mg/L [21-28].

Na is the main element responsible for maintaining the osmotic pressure and volume of the plasma, while K is responsible for maintaining the intracellular osmotic pressure. Therefore, the similarity between our results and the literature, as well as the results being within physiological ranges, suggests that these elements are less affected by external factors.

Calcium plays a significant role in maintaining the homeostasis of animals, including coagulation, mineralization of bones and teeth, hormone secretion, and neural excitability. In our study, the mean serum Ca concentration was 89.8±2.05 mg/L and it was higher than Honamlı goats [21] and Kilis goats in late gestation and parturition period [29]. It was lower than Kilis goats before and during, gestation, mid-gestation, and after the parturition period [29] and also lower than the findings of Schweinzer et al.[2], and Lima et al.[3]. Our value was similar to those previously reported in goats from China [25], South Italy [22], North-Eastern, Algeria [23], and Turkey [26,28]. Although serum concentrations of Ca vary in physiological conditions such as pregnancy and lactation, they are tried to be maintained within certain limits by homeostatic mechanisms.

Magnesium is the fourth most common cation in the body. As an intracellular cation, it functions as a catalyst or activator of hundreds of enzymes and active in all major metabolic processes. Previous studies have reported Mg concentration of goat serum ranging from 18.3-39.3 mg/L [2,21,22,25,26,28]. The mean serum Mg concentration of this study (24.9±0.76 mg/L) was agreed with those reported within the literature.

The results of our study documented that all macro elements were measured in serum, tears, saliva, and hair samples, although they differed in distribution. The macro elements exist in all tissues and fluids, and they are vital. Indeed, when compared with previous study results, although they show differences due to changes in different races, geography, physiological conditions, etc., and also the similarities of serum macro element levels and keeping them within physiological limits prove that macro element concentrations are under strict homeostatic control.

In the present study, the trace elements investigated in samples were Fe, Cu, Zn, Se, Ni, Ti, Mn, Co, and Cr. The mean Fe concentration of serum samples was 3.33±0.52 mg/L. Serum Fe concentrations in small ruminants may vary within 1.93-2.09 mg/L [5,30] thus, the results of this study demonstrate high serum concentrations of this element. Several studies have reported values between 0.779-2.93 mg/L [2,3,21,31-33]. However, the serum Fe concentration was lower than the results of the study conducted with Omani [4], Boer [34], and Guizhou black goats [25].

The physiological range of serum Cu concentrations in small ruminants was notified between 0.43-1.39 mg/L, and a concentration of less than 0.08 mg/L has been reported to cause deficiency symptoms [28,35]. The mean serum Cu concentration of this study was 435.4±34.7 μg/L, which was mostly lower than the values reported in previous studies with goats [2-4,24,25,31,33,35-38]. However, it was higher than the results of Erdogan's study (0.33 mg/L) [28].

In our study, the concentration of Zn in serum samples was 1.392±0.574 mg/L. It was lower than those reported by Zhou et al.[34], Yun and Mei [25], Shawaf et al.[4], and Kachuee et al.[31], while lower concentrations were found those reported in previous studies (0.210-0.967 mg/L) [2,3,28,33,36,37]. When we compare the Zn content of serum samples in our study the literature showed that the results were in agreement with those reported in literature by Nazifi et al.[32].

Selenium is an essential trace element in animal nutrition and has many actions related to animal production, fertility, and disease prevention In the present study, the mean serum Se concentration was 231.2±25.2 μg/L. Harris reported a normal serum Se concentration of 100 μg/L for goats [39]. In previous studies conducted in different geographical regions and with different methods, serum Se concentrations of goats have been reported between 11-377 μg/L [2,4,25,28,31,32,36,37,40].

Nickel has gained much attention in recent years because it is related to the activity of the urease enzyme and is also a cofactor of enzymes involved in nitrogen metabolism [41]. Although Ni imbalance causes many serious problems in animals, data on small ruminants are insufficient. In this study, Ni has been detected in only serum samples and its mean value was 83.9±6.35 μg/L. Our value was lower than reported by Bashir et al.[41], Yazar et al.[37], and the permissible limits (0.4 mg/L) determined by the National Research Council [42]. Nickel remains below the detection limit in samples other than serum samples could be explained by the serum Ni level does not exceed the threshold level for its transition to the analyzed biological materials.

Titanium plays a role in the growth of mammals and is considered a growth factor [43]. Therefore, it is an essential element in animal nutrition. However, under normal farm conditions, its insufficiency is not expected [7]. In our study the concentration of Ti in serum and saliva samples were 497.2±10.7 and 125.3±5.58 μg/L, respectively. The Ti concentration of tears samples was below the detection limit. The possible reason for this was thought to be the existence of a mechanism that prevents the transition of Ti to tears.

Cobalt concentration was below the detection limit in all samples. Studies on Co deficiency in goats are scarce, however, these results indicate that there could be a lack of Co in the ration of Kilis goats and a need for Co supplementation in the ration.

Chromium is an essential element that supports mammals using carbohydrates, lipids, and proteins as an insulin activator. The tolerable concentration of Cr was reported as 1.0 mg/L in the blood [44]. In this study, the mean Cr concentration in serum samples of Kilis goats was 427.7±43.6 μg/L and below the tolerable limit. The Cr content of goats was lower in our study than in Khan's [44] and Yazar's study [37].

The bioavailability and requirement of the trace elements is affected by many factors including species, breed, sex, and age, as well as physiological factors such as the stage of pregnancy and lactation, inflammation, antagonists, concentrations of forage (soil type, age of plant, forage species, etc.) season, and climate [2,45]. For instance, Fe requirement of the animal body is a factor that greatly affects Fe absorption. Animals with low Fe status or fed an Fe-deficient diet will have greater Fe absorption and retention for physiological needs. Similarly, Cu and Zn absorption is regulated by their dietary concentrations, related to the animal's requirements, and the physiological conditions of the organism [45]. Another factor affecting trace element bioavailability in ruminants is the interactions between the elements. Differences in the sulfur content of the feeds or the presence of cyanogenetic glycosides, which are anatagonists for Se, may cause differences in the bioavailability of Se. It is also reported that high dietary Ca may reduce Mn absorption [46]. The fact that trace element concentrations are affected by many different factors makes it difficult to determine the reference values and causes great differences between these values. For this reason, serum trace element values reported in the literature selected from studies with goats and the levels compared in this study showed variable differences.

According to the results of our study, Na, K, Ca, Mg, Fe, Cu, Zn, and Cr were detected in tear and saliva samples, although their concentrations differed. In addition, unlike tears, Se and Ti were also measured in saliva samples. The measurement of all macro elements and some trace elements in serum, tears, and saliva samples simultaneously suggests that there are different mechanisms in the transition from serum, which acts as an element transport pool, to other body fluids.

In this study, the predominant macro element in hair samples was K, and the lowest concentrations of Na were also measured in hair samples. At the same time, a significant negative correlation was detected between Na and K (rho:-0.693) in the hair samples. This result is thought to be related to the tendency of Na and K to disperse in biological systems. Sodium is an extracellular element and is closely related to water. Therefore, in water-poor tissues, K is expected to be more than Na.

The complete reference ranges of macro and trace elements of hair are not available for goats, only partial information can be found for some elements. According to the results of a study conducted by Battini et al.[47] on the hair of dairy goats, the Na, K, Ca, Mg, Fe, Cu, and Zn concentrations of normal hair were reported as 1575.99±194.22, 3310.06±229.1, 896.93±42.34, 339.65±11.42, 161.58±24.84, 4.44±0.42, 103.49±2.54 ppm, respectively. Our results for Na were similar to normal goat hair, while other element concentrations were found to be higher except Fe. The highest concentration of Fe was detected in hair samples (114.8±19.7 mg/L) in our study and it was lower than reported by Battini et al.[47] and Yun and Mai [25]. However, it was higher than Boer goats [34]. The mean hair Zn concentration was 118.0±4.56 mg/kg in this study, which was higher than the reported by Pavlata et al.[36]. According to the present study, both the mean hair Zn and Se concentrations in Kilis goat were lower than the reported for the Boer and Guizhou black goat [25,34]. Studies with various animal species have shown that hair is a good indicator of Cu [7]. In our study, there was a significant correlation between serum and hair Cu concentrations (rho: 0.400). The mean hair Cu concentration was lower than the reported value for the Boer goats [34] and it also was higher than the value reported by Yun and Mei [25].

The obtained data demonstrate that the highest values of K, Ca, Mg, Fe, Cu, Zn, Se, Ti, and Mn were measured in the hair samples. Combs et al [48] concluded that elements are accumulated in hair in higher concentrations than in blood. Our results of element concentrations of hair samples also indicate that these elements tend to accumulate in the hair.

Knowledge about Mn metabolism especially in goats is scarce. It was reported that the Mn deficient nutrition had no effect on the Mn concentrations in the serum of goats, but decreased the Mn content in the hair, so the hair was a reliable indicator of Mn in animals [49]. The fact that Mn could only be measured in hair samples in the present study, supports this knowledge. The mean Mn concentration of hair samples was measured as 12903±3142 µg/kg. This value was consistent with the reported for normal goats [7].

The conclusions of this study can be listed as follows; *(i)* Saliva, tears, and hair samples could be used as alternative biological material in macro and trace element analysis by ICP-MS method. (*ii*) In goats, it may be useful for diagnosis and follow-up through the correlation of some elements between serum-hair (Na, K, Mg, Cu), tears-hair (K, Mg, Fe), and serum-tears (K). (*iii)* Hair Mn concentrations can be considered as a reliable parameter for investigating Mn metabolism in goats. *(iv)* In particular, trace element concentrations are affected by a wide variety of factors and reference values sometimes show great differences. Thus, the determination of especially the trace element concentrations with more comprehensive studies including the factors affecting element concentrations with small ruminants in the region will be beneficial as it may prevent possible economic losses in terms of yield and reproduction.

Availability of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding author (N. Paksoy).

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Conflıct of Interest

The authors declared that there is no conflict of interest.

Ethical Statement

The study was conducted with the permission of Harran University Animal Experiments Local Ethics Committee (HRUHADYEK) with the number 2021/009/02.

Author's Contribution

NP designed and supervised the study. NP, EEÖ, ÜY, and Mİ collected the samples. NP and EEÖ performed laboratory analysis. FB analyzed the data. The first draft of the manuscript was written by NP and Mİ. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

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