

## Entansif Şartlarda Yetiştirilen Kıvırcık Kuzularda Kan Plazması ve Yapağıdaki Çinko ve Bakır Profili

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### ÖZET

Bu çalışmanın amacı süt emme dönemi sonundaki  $62 \pm 4$  günlük yaş ve  $23.80 \pm 3.19$  kg canlı ağırlığındaki (Ort.  $\pm$  S.S.) Kıvırcık kuzularında, kan plazması ve yapağıdaki çinko (Zn) ve bakır (Cu) profilini belirlemektir. Bu amaçla, kastre edilmemiş, sağlıklı erkek 48 baş Kıvırcık kuzudan (32 tekiz, 16 ikiz) kan ve yapağı örneği alınmıştır. Kan plazması, yapağı ve yem örneklerindeki Zn, Cu, demir (Fe), sodyum (Na) ve potasyum (K) konsantrasyonları alev başlıklı atomik absorpsiyon cihazı kullanılarak gerçekleştirilmiştir. Bu çalışmadan elde edilen sonuçlar doğum tipinin plazma ve yapağıdaki mineral konsantrasyonları üzerine etkisinin olmadığını göstermiştir ( $P > 0.05$ ). Ancak Zn ve Cu'nun ikiz doğan kuzularda yapağıda daha fazla birikme eğiliminde olduğu anlaşıldı. Ayrıca, plazmadaki Cu - Fe konsantrasyonları arasında negatif bir korelasyon gözlemlenirken ( $P < 0.05$ ); bunun aksine, kan plazmasındaki K - Na ile yapağıdaki Cu - Na ve Na - K arasında pozitif bir korelasyon gözlemlenmiştir ( $P < 0.05$  ve  $P < 0.01$  aralığında, değişen oranlarda). Çalışma sonunda entansif şartlarda yetiştirilen Kıvırcık kuzularda, süt emme sonunda plazma ve yapağıdaki Zn ve Cu konsantrasyonlarının normal aralıkta olduğu sonucuna varılmıştır. Diğer taraftan hayvanların fizyolojik durumu ve meradan yararlanma olanağı göz önünde bulundurulduğunda ileriki dönemlerde bu minerallere olan ihtiyaçlarının organik ve/veya inorganik kaynaklardan sağlanması gerekebileceği göz önünde bulundurulmalıdır.

## Zinc and Copper Profile of Blood Plasma and Wool in Kıvırcık Lambs Reared under Intensive Management Conditions

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### ABSTRACT

The aim of this study was to determine Zinc (Zn) and Copper (Cu) levels of plasma and wool in Kıvırcık lambs,  $62 \pm 4$  days weaning age, and  $23.80 \pm 3.19$  kg (Mean  $\pm$  SD) live weight, at the end of the suckling period. For this purpose, blood and wool samples were taken from 48 non-castrated healthy Kıvırcık male lambs, 32 single-born, and 16 twin-born. The concentration of Zn and Cu together with iron (Fe), sodium (Na), and potassium (K) in plasma, wool, and feedstuffs samples were performed by using a flame atomic absorption spectrophotometer. The result of this study showed that the birth type did not affect the wool mineral level ( $P > 0.05$ ). However, it was understood that Zn and Cu tended to accumulate more in wool in twin-born lambs. Also, there was a significant negative correlation observed in plasma Cu - Fe concentrations ( $P < 0.05$ ). In contrast, K - Na in plasma, and Cu - Na and Na - K concentrations in wool were positively correlated

(varied between  $P < 0.05$  and  $P < 0.01$ ). It was concluded that Zn and Cu concentration in plasma and wool of Kivircik lambs within the normal range in the suckling period under intensive management conditions. On the other hand, considering the physiological stage of animals and pasture allowance, supplementation of these minerals from organic and/or inorganic sources may be necessary for further times.

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## 1. Introduction

Zinc (Zn) and copper (Cu) have been demonstrated to be essential micronutrients for ruminants. Supplementation of an adequate level of Zn and Cu in the diet of animals improves their growth, reproduction, immunity, health, and productivity [1]. The inorganic form of these two minerals is generally used in vitamin-mineral premix rather than organic form. However, interaction and antagonistic effects with poor absorption and bioavailability of these minerals in diets limit their actual benefits to the animal metabolism [2].

In many cases, mineral deficiencies occur without any clinical symptom, which results in unsatisfactory growth, production, and infertility in small ruminants. Signs of Zn and Cu disorders are generally characterized by depressed and reduced growth, reduced reproductive rate, and wool growth with depigmentation of wool and hair in small ruminants [3]. Symptoms like bone fragility, anemia, dry, and cracked skin can be seen in severe deficiency [3].

Also, Zn and Cu are an important constituent of several enzymes that have vital functions in metabolism [4]. While Zn is an essential micronutrient for immune function, Cu is required for normal red blood cell formation [5, 6]. Therefore, knowing the concentration of these elements in plasma and wool is very important to manipulate their concentration in the diets. On the other hand, there had been some research concerning effects of season and pasture on blood serum and wool samples of native sheep breeds in Turkey; but no study was found to determine Zn and Cu levels of blood and wool of lamb to manipulate their diets on fattening period [7, 8]. Therefore, this study aimed to determine Zn and Cu levels of plasma and wool of lambs at the end of the suckling period.

## 2. Material and Methods

All animal care and handling procedures were reviewed and approved by the Ethical Committee of the Sheep Breeding Research Institute, Bandırma, Balıkesir, Turkey (Approval number: 3760010). All efforts were made to minimize any discomfort during the sampling of blood and wool.

### 2.1. Animal care and feeding conditions

The data in this study originated from a research trial conducted to evaluate the effects of activated clinoptilolite and inactive brewer's yeast mixture on fattening Kivircik male lambs. All lambs, used in this study, were with their dam for the first 15 days and free to suckle. Up to the weaning period, they suckled their dam twice a day and had free to access commercial starter feed and alfalfa hay *ad libitum*. At the weaning period, a total of 48 healthy (16 twin-born, 32 single-born) non-castrated male Kivircik lambs were selected with an average  $62 \pm 4$  days weaning age and  $23.80 \pm 3.19$  kg live weight to conduct the trial. When lambs were allocated randomly into the trial groups, blood and wool samples were collected within each lamb to determine Zn and Cu levels of plasma and wool. Also, concentrate feed and roughage samples, used in the suckling period, were taken for further chemical analysis.

### 2.2. Blood and wool sampling

Blood samples of lambs were obtained from the jugular vein using vacutainer tubes containing sodium heparin and stored at  $-20^{\circ}\text{C}$  for Zn, Cu, and other selected mineral analyses. Plasma samples were separated by using laboratory centrifuge (Elektro-mag, M4812P) at 4000 rpm for 10 minutes and store in 2 ml polypropylene microcentrifuge tubes.

Wool samples were taken from *fossa paralumbalis* by stainless steel sheep clippers. Feed and other foreign particles were removed from the wool and washed by warm water, then

they are soaked into ethanol for 5 minutes to remove organic dust and oven-dried at 60 °C for at least 24 h.

### 2.3. Microwave digestion of plasma and wool samples

For selected mineral analysis, 1 ml of plasma sample was pipetted into a vessel, resistant to temperature and pressure, with 8 ml nitric acid and 2 ml of hydrogen peroxide for microwave digestion. The digested sample was then allowed to cool before being transferred quantitatively into clean falcon tubes and completed to 25 ml final volume with deionized water and analyzed in the flame atomic absorption spectrophotometer [9]. The same procedure was applied to wool and feed samples, which weighed into vessels about 0.3 g and 0.5 g.

### 2.4. Analysis of mineral contents

The concentration of Zn, Cu, iron (Fe), sodium (Na), and potassium (K) in plasma and wool samples were measured using Shimadzu AA-7000 flame atomic absorption spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

### 2.5. Chemical analysis of feedstuffs:

Dry matter (DM), crude protein (CP), ash, ether extract (EE) and, crude fiber (CF) compositions of concentrate feed and alfalfa hay, used in the suckling period, were analyzed according to AOAC methods [10]. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents of samples were analyzed according to the methods reported by Van Soest et al. [11]. All chemical analyses of feedstuffs were carried out in triplicate.

### 2.6. Statistical analyses

Mineral contents of blood and wool samples were analyzed using SAS (JMP, version 13.2). The data of plasma and wool minerals were subjected to one way ANOVA using the birth type as a variable. The differences between means statistically compared with Student's t-test. Pearson correlation analyzes performed to determine the relationship between minerals.

## 3. Results and Discussion

The analyzed chemical and mineral compositions of concentrate and alfalfa hay, which used in the suckling period, are presented in Table 1. Plasma and wool mineral concentrations of lambs according to birth type are given in Table 2 and Table 3.

Most of the studies about minerals have been carried out to determine the effect of pasture allowance, season, and breed type on serum/plasma and wool of small ruminants [7, 8, 12, 13]. The considerable variation observed in these studies about the mineral concentration of serum/plasma and wool. In this study, there were no significant differences observed in both plasma and wool mineral concentrations according to birth types ( $P > 0.05$ ). Although Zn and Cu concentrations of plasma were higher in single-born lambs compared to twin-born, on the other hand, Zn and Cu concentrations of wool in twin-born lambs were higher than single-born.

**Table 1.** Chemical composition and mineral contents of concentrate feed and roughage.

Chemical Composition	Concentrate	Alfalfa hay
Dry Matter, % fed basis	88.97	96.18
Crude Protein, %	18.01	13.27
Ether Extract, %	2.72	1.78
Ash, %	8.22	7.82
Crude Fiber, %	12.06	40.75
Neutral Detergent Fiber, %	45.53	51.62
Acid Detergent Fiber, %	10.28	42.72
Acid Detergent Lignin, %	7.91	14.20
Zn, mg/kg	75.05	13.02
Cu, mg/kg	16.76	8.09
Fe, mg/kg	285.62	227.40
Na, g/kg	1.80	1.28
K, g/kg	8.13	6.60

**Table 2.** Plasma mineral concentrations of Kivircik lambs

	Single	Twin	Sig.
Zn, mg/kg	1.18±0.07	1.11±0.11	ns*
Cu, mg/kg	1.49±0.05	1.39±0.07	ns
Fe, mg/kg	4.20±0.14	4.43±0.20	ns
Na, g/kg	792.57±5.23	791.31±7.39	ns
K, g/kg	623.49±11.30	606.07±15.97	ns

\*ns: not significant

**Table 3.** The wool mineral concentration of Kivircik lambs

	Single	Twin	Sig.
Zn, mg/kg	120.82±1.42	123.60±1.98	*ns
Cu, mg/kg	8.31±0.27	8.38±0.37	ns
Fe, mg/kg	178.89±13.06	200.06±19.74	ns
Na, g/kg	135.17±8.88	114.65±12.79	ns
K, g/kg	617.44±38.15	547.38±53.95	ns

\*ns: not significant

The average plasma Zn and Cu level range is 0.8-1.2 and 1.0-2.0 mg/l in sheep, respectively [13, 14]. Also, White et al. [15] noted that a plasma Zn concentration above 0.5 mg/l supports normal wool growth in Merino lambs. Besides, wool fiber increases its crimp, mechanical strength, and lustrous appearance, with diets, include higher Cu concentrations [2]. Obtained results from this study indicate that there were no deficient in Zn and Cu because the plasma concentration of these two minerals was within the recommended range. Rojas et al. [16] presented evidence that supplementation of sheep diet with different zinc sources decreased serum Cu concentration. Therefore a small amount of Cu sources such as CuO must be added into diets. Correlations of plasma and wool minerals are above and below the empty diagonal cells, respectively.

**Table 4.** Pearson correlation coefficients among minerals in plasma and wool samples in Kivircik lambs

	Zn	Fe	Cu	K	Na
Zn		0.18	0.01	0.03	0.20
Fe	0.15		0.16	0.05	0.01
Cu	-0.08	-0.30*		0.13	0.30*
K	0.24	0.31*	0.07		0.45**
Na	0.15	0.05	0.04	-0.08	

\*:  $P < 0.05$ ; \*\*:  $P < 0.01$

In this study, Cu in concentrate and roughage (16.76 mg/kg and 8.09 mg/kg) was 2.82 times (together with) lower than Cu concentration in basal diets used by Rojas et al. (70 mg/kg) [16]. As shown in Table 4, there was no significant correlation observed in plasma Zn and Cu level ( $P > 0.05$ ). Although, Cu concentration tended to decrease in plasma. These results were in agreement with results obtained by Shinde et al. [2], Rojas et al. [16], and Underwood and Suttle [17]. Farrag et al. [18] suggest that it is possible to increase the Zn level in the blood plasma of lambs by mineral supplementation. On the other side of this suggestion, Beeson et al. [19] showed that supplemental zinc affects blood serum levels only used extremely high levels, such as 300 or 620 mg/kg in growing or finishing cattle. However, using more than 300 mg/kg of Zn in the diets can

cause toxic effects on lambs, due to tolerance limits [3]. In this study, the concentration of Zn and Cu in concentrate and roughage were 75.05 and 13.02 mg/kg and within the minimum requirements and tolerance limits, respectively (Table 1).

White et al. [15] noted that Zn and Cu play an essential role in protein synthesis, and their function directly affects wool growth. A normal Zn and Cu concentrations of the sheep wool ranged from 35-195 and 1.7-25 mg/kg, respectively [20,21]. Also, physiological state, supplying level in diets, and breed type affected wool Zn and Cu levels [20,22]. This study showed that the birth type did not affect the wool mineral level ( $P > 0.05$ ). On the other hand, the accumulation of Zn and Cu levels in wool was higher in twin-born lambs. This situation may be related to the metabolism rate of twin born lambs. Also, there was a significant negative correlation observed in plasma Cu and Fe concentrations ( $P < 0.05$ ). In contrast, K-Na in plasma, and Cu-Na and Na-K concentrations in wool were positively correlated (varied between  $P < 0.05$  and  $P < 0.01$ ).

It is concluded from the study that Zn and Cu concentration in plasma and wool of Kivircik lambs within the normal range in the suckling period under intensive management conditions. On the other hand, considering the physiological stage of animals and pasture allowance, supplementation of these minerals from organic and/or inorganic sources may be necessary for further times.

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