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ORIGINAL PAPER

EFFECT OF SHEEP`S DIFFERENT NUTRITIONAL CONDITIONS ON MİCROELEMENTS (Cu, Zn, Co) IN THEIR BLOOD SERUM

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Abstract

This study aimed to compare the levels of copper, zinc, and cobalt minerals in the blood serum of sheep under different nutritional conditions. The study was conducted on 1-2-year-old, healthy Norduz sheep raised at the Agricultural Research and Implementation Center of Van Yuzuncu Yil University. A total of 30 sheep made up three equal groups: a control group (group C; *n*=10) and two experimental groups (groups 1 and 2; *n*=10 in each group). Randomly selected animals from all the groups were grazed on low-quality pastures during a 4-week experiment. Each animal in group C was supplied with a total of 900 g day^{-1} of feed containing 850 g day^{-1} of mixed grass-clover and 50 g day^{-1} of barley. Each animal in groups 1 and 2 was supplied with 850 g day^{-1} of mixed grass-clover and weekly increasing quantities of barley during the experiment as follows: 915 g day^{-1} in the first week, and 945 g day^{-1}, 960 g day^{-1}, 980 g day^{-1}, in the second, third and fourth weeks, respectively. Animals in the second group were additionally fed with 500 g day^{-1} of feed concentrate per head throughout the 4-week experimental period, unlike the other groups. This study determined that – in addition to pasture grazing – feeding with mixed grass-clover hay, extra barley and concentrates should be provided to ensure adequate Cu levels. Moreover, it was also found that pasture grazing could be sufficient for Zn intake, in contrast to Co levels, which fall below normal limits even if the quality of a diet is improved.

Keywords: sheep, diets, blood serum, microelement parameters.

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INTRODUCTION

Mineral deficiency in animal diets may contribute to more fatalities than other nutritional deficiencies (Alçiçek, Yurtman 2009). The nutritional needs of sheep, which are mostly supplied from pastures, cannot be achieved with roughage only, particularly during such physiological periods as pregnancy and lactation (Alçiçek, Yurtman 2009), when the intake of microelements becomes insufficient (McDowell 1992, Erdoğan et al. 2002, Küçükkaya 2010). In addition, variations in soil and vegetation may lead to low levels of trace mineral concentrations and bioavailability (Erdoğan et al. 2003, Genther, Hansen 2014). It has also been reported that the use of high amounts of phosphorus and nitrogen as soil fertilizers reduces the mineral content of barley (Hill et al. 1978, Loneragan et al. 1979, Andersson, Siman 1991, Sönmez, Yılmaz 2000). For this reason, concentrated feeds may be required to supply the energy, protein, mineral substance and vitamin requirements of sheep (Alçiçek, Yurtman 2009).

Animal health depends on adequate and well-balanced trace element concentrations. The serum copper (Cu), zinc (Zn) and cobalt (Co) levels are directly related to the Cu, Zn and Co concentrations of the diets in ruminants (Oldfield 1987, Van Ryssen, Bradfield 1992, Tuncer et al. 2020).

Copper plays an important role in the activation of many critical enzymes (Küçükkaya 2010). It has functions in cellular respiration, immune system, lipid metabolism, growth, wool quality, hemoglobin synthesis, bone formation, connective tissue development, carbohydrate, and protein synthesis. Cu is also important for the synthesis of melanin, a pigment of leather and wool (Erdoğan et al. 2002, 2003, İpek, Keskin 2007, Küçükkaya 2010). It has been reported that Cu absorption in animals fed with concentrated feed given in addition to roughage is twice as high as in animals that graze only on pasture (Küçükkaya 2010). As reported by Tiftik, Doğanay (1997), while there is information indicating that Zn in the diet reduces Cu absorption, there are also opinions that it has no effect (Turner et al. 1987).

Zinc is essential for vital activities, such as growth, cell health, immune system, nervous system, reproduction and protein synthesis (Kozat et al. 2007, Bengü et al. 2018). Zn is found in all animal tissues, particularly in muscles, bones, blood, glands, genital organs, skin, hair, wool and nails (Gabor 1991, Tuncer et al. 2020). It has been reported that consumption of high-fiber diets can reduce Zn levels (Bengü et al. 2018).

Cobalt is necessary for the synthesis of vitamin B12 in the rumen. If there is insufficient Co in the body, rumen microorganisms cannot produce enough vitamin B12 (Kiliçkap, Kozat 2017, Bengü et al. 2018, Tuncer et al. 2020). Co is essential for optimal development and function of the rumen microflora, and ruminants are particularly susceptible to Co deficiency (Sharman et al. 2008, Radwińska, Żarczyńska 2014). It has been seen that consumption of Co-enriched feed has positive effects on the microbial population, rumen fermentation and digestion of low-quality feeds in sheep (Stangl et al. 1999, Bengü et al. 2018, Tuncer et al. 2020).

Sheep are grazing animals, obtaining most of their nutrients from pasture and local roughage. Therefore, due to local differences between the levels of essential elements in grass and pasture plants, they are vulnerable to nutrient insufficiencies, and Cu, Zn, and Co deficiencies are considered clinical problems in many countries (Ademi et al. 2017).

The aim of this study was to evaluate the effect of sheep`s different nutritional conditions on microelements (Cu, Zn, CO) in their blood serum.

MATERIAL AND METHODS

Study area

The study was carried out at the Van Yuzuncu Yil University Agricultural Research and Implementation Center in Tusba (38°34′03.5″N 43°17′24.5″E). Tusba district in Van province, where the study was carried out, had moderate dry climatic conditions and an average of 46.3% relative humidity during the study period. Van is a city in the Eastern Anatolia region of Turkey, with an average temp. of 9.4°C according to long-term meteorological data (1939-2020) (Anonymous 2021). The soils of this study area have slightly basic Ph (7.94-8.16), poor organic matter content (1.69-1.87%) and high lime content (21.06-21.41%) – Çimrin, Boysan (2006).

Animals and diets

The study was carried out on 1-2-year-old sheep, with normal eating habits, healthy hair coat or fleece, known be healthy. They were randomly selected Norduz sheep (n=30) reared at the Agricultural Research and Implementation Center of Van Yuzuncu Yil University. A total of 30 sheep made up three equal groups: a control group (group C; n=10) and two experimental groups (groups 1 and 2; n=10 in each group). The study design complied with the ethical standards of the Provincial Directorate of Agriculture and Forestry (2020/44762815-1191429).

For the practice of ration formulation, this study followed feeding standards developed by the US National Research Council nutrient requirements of sheep (NRC 1985). Dry matter, crude protein, crude fiber and ash contents were determined according to standard AOAC procedures. Crude protein was calculated by multiplying the nitrogen content by a factor of 6.25. Phosphorus was determined colorimetrically using potassium dihydrogen phosphate as the standard. Calcium was analysed with an atomic absorption spectrophotometer according to the method described by Chapman & Pratt (Wanasundera, Ravindran 1994).

Randomly selected animals from all the groups were grazed on low-quality pastures with poor organic matter content during a 4-week experiment. Each animal in group C was supplied with a total of 900 g day^{-1} of feed containing 850 g day^{-1} of mixed grass-clover and 50 g day^{-1} of barley. Each animal in groups 1 and 2 was supplied with 850 g day^{-1} of mixed grass-clover and weekly increasing quantities of barley during the experiment as follows: 915 g day^{-1} in the first week; and 945 g day^{-1}, 960 g day^{-1}, 980 g day^{-1}, in the second, third, and fourth weeks respectively. Animals in group 2 were additionally fed with 500 g day^{-1} of feed concentrate per head throughout the 4-week experimental period, unlike group 1. The composition and nutrient contents of the concentrate feed are given in Table 1.

Table 1

Composition and nutrient content of the concentrated feed provided to sheep in group 2

Ingredients	(g kg^{-1})
Barley	646.9
Soyabean meal	131.0
Wheat bran	187.6
Mineral-vitamin premix	2.5
Ground limestone	24.5
Common salt	7.5
Chemical composition (DM basis)	(%)
Dry matter	89.0
Crude protein	17.9
Crude fiber	7.8
Ether extract	2.4
Crude ash	3.9
Calcium	1.1
Phosphorus	0.6
Metabolizable energy (Mcal kg^{-1})	3.0

Blood samples and analysis

Blood taken from the jugular vein of the sheep was transferred to tubes without anticoagulants and delivered to the laboratory in cold chain conditions. The blood samples were centrifuged at 4°C, 3000 rpm for 15 min, and serum was separated. The resulting serum samples were placed in Eppendorf tubes and frozen at -20°C until analyses, which began two weeks later. Then, the mineral parameters were determined in the serum using the ICP-OES (inductively-coupled plasma-optic emission spectroscopy) device. ICP-OES is one of the most popular techniques used today for the analysis of trace elements in many sample types. It is based on the measurement of the emission of atoms excited by passing into the gas phase, spraying the sample into the plasma at a temp. of approximately 6000-10000°C.

Statistical analysis

Data relating to the study results were presented as mean and standard error (mean ± SE). A one-way analysis of variance was used for micromineral parameters. The significance of differences between the sub-factors was determined by the Duncan's test according to the result of the analysis of variance. Then, the relationships between minerals were analyzed and the associations were revealed with the Pearson correlation coefficient. SAS statistical software (SAS 2014) was used for the statistical analyses.

RESULTS

The effects of different feeding methods on the micromineral parameters of Norduz sheep are presented in Table 2, and the distribution of these parameters in separate groups is shown as box plots in Figures 1, 2, and 3.

Table 2

Effects of different diets on the micromineral parameters of Norduz sheep

Parameters ($\mu g\ dl^{-1}$)	Groups			*P*
	C (*n*=10) (mean ± SE)	1 (*n*=10) (mean ± SE)	2 (*n*=10) (mean ± SE)	
Cu	31.77±2.50*a*	46.45±1.03*b*	66.83±2.27*c*	<0.01
Zn	153.52±1.74*a*	166.90±1.07*b*	180.94±2.52*c*	<0.01
Co	0.51±0.03*a*	0.60±0.03*b*	0.74±0.01*c*	<0.01

Group C – control group, *a*, *b*, *c* – different lower case letters in the same line represent statistically significant differences.

As can be seen in the table and the figures, significant differences were found in the Cu, Zn, and Co levels of all groups ($P<0.01$). In Table 3, the results of the correlation of the data obtained from this study are presented. The table shows that highly significant correlations were found among Cu, Zn, and Co ($P<0.01$).

DISCUSSION

This study shows (Table 2) that Cu, Zn, and Co levels in the blood serum increase significantly as the nutrient content increases in sheep feeding programs ($P<0.01$). Microminerals are vital for the health and growth of livestock; however, low dietary concentrations or variability in soil and plants lead to low micromineral concentrations and bioavailability (Genther,

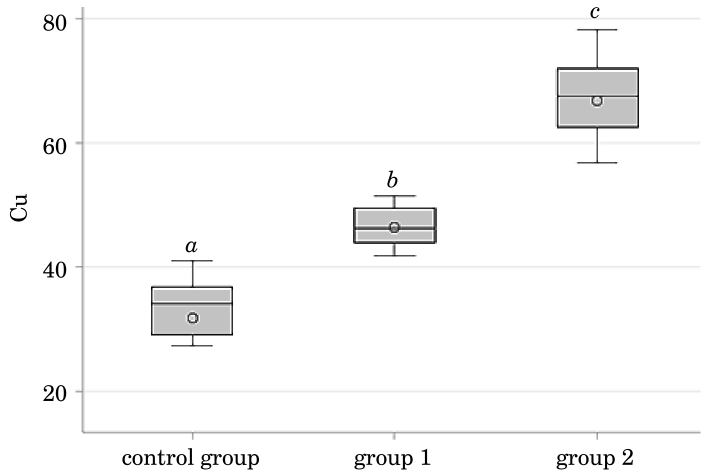

Fig. 1. Box plots for Cu levels

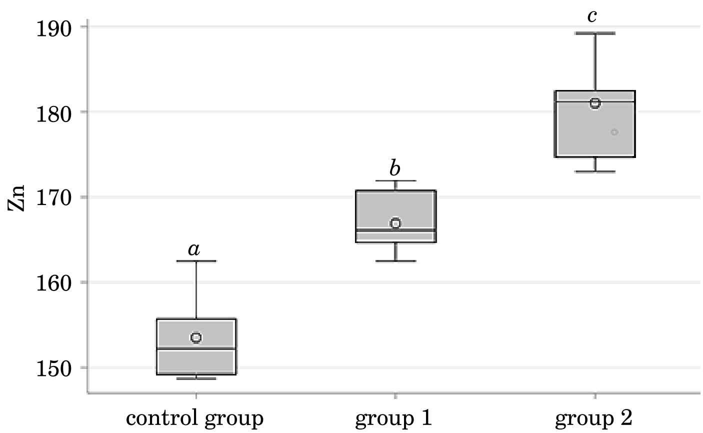

Fig. 2. Box plots for Zn levels

Hansen 2014). Therefore, nutritional programs that increase mineral substance values are beneficial for the reproductive performance and efficiency of sheep.

This study revealed that the plasma Cu levels of healthy animals in group C (31.77 μg dl^{-1}), which were fed with a limited amount of barley, pasture and grass-clover, are below the normal range of 58-160 μg dl^{-1} (Kaneko 1980, Altıntaş, Fidancı 1993, Montgomery et al. 1996). Although the Cu levels increase significantly ($P<0.01$) in the blood serum of the sheep in group 1 with supplemental barley, the normal Cu reference values for sheep are only observed in the sheep in group 2, whose diets were supplemented with concentrated feed (Table 2, Figure 1). The reason for the low level of serum Cu in the sheep in group C may be the decrease in Cu absorption as a result of the formation of unused copper sulfide in the rumen of the sheep fed on

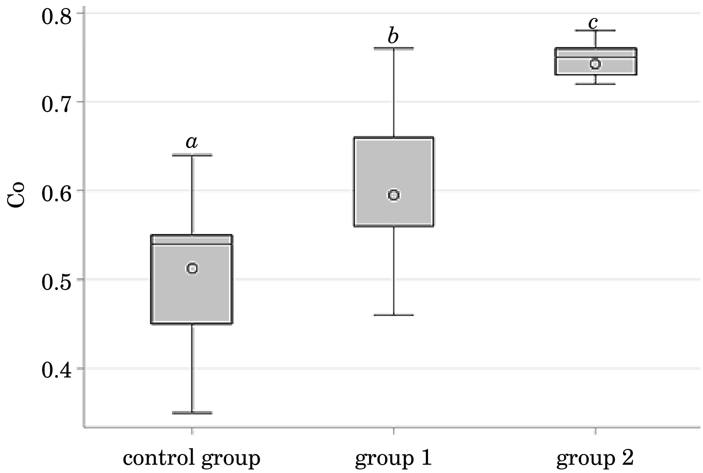

Fig. 3. Box plots for Co levels

Table 3

Correlation levels between microelements in serum samples

Microelements	Cu	Zn	Co
Cu	1	0.974**	0.780**
Zn		1	0.767**
Co			1

** P<0.01

pasture grass containing high amounts of soluble protein (Oncüer et al. 1996, Batmaz 2014). This result is consistent with available literature reports (Çimtay, Ölçücü 2000, Uyanık 2000, Batmaz 2014) suggesting that Cu deficiency occurs in animals fed mostly on pasture in different regions of the world, and nutrient compensation occurs to a certain extent in animals fed with concentrated feed. High intakes of Zn may also reduce Cu concentrations in the blood plasma (Kincaid 1999). The serum Cu level of sheep reared mostly in pastural conditions is found to be sufficient in Morkaraman (80.1 µg dl^{-1}) and Tuj (75.04 µg dl^{-1}) sheep in Kars province (Kaya et al. 1998), and just below the limit – in Dağlıç sheep in Hatay province (Erdoğan et al. 2003). However, the Cu levels of Merino (Ozan 1985) and Dağlıç (Erdoğan et al. 2002) sheep were found to be 33.72 and 32 µg dl^{-1}, respectively, thus being similar to the findings in this study's group C. These differences may be explained by various factors, such as the composition of the soil, climatic conditions, season, the pasture's concentration of Cu, genetics, and the *in vivo* antagonistic interactions of copper with some elements, such as zinc, molybdenum, lead, cadmium and sulfur (Haenlein 1980, Alonso 2000, Erdoğan et al. 2003).

In this study, it was determined that the serum Zn levels increase significantly (P<0.01) as nutritional levels increase (Table 2, Figure 2). In addition, the serum Zn levels of all groups are higher than the normal range (80-150 μg dl^{-1}) reported for sheep by some researchers (Kurt et al. 2001, Erdoğan et al. 2002, Tuncer et al. 2020). While the mean Zn level (153.52 μg dl^{-1}) of group C sheep, which were fed mostly on pasture, was just above the normal limits, significant increases (P<0.01) were detected in groups 1 and 2 with supplemental feeding. As the amount of nitrogen in a plant increase, the transport of Zn from the root zone to the green parts and grains of the plant will also increase (Barut et al. 2019), so it is normal for the Zn level to increase in the groups with additional feeding. Although it has been reported in previous studies (Eyüboğlu et al. 1997, Karaçal, Çimrin 1997, Çimrin, Boysan 2006) that significant areas of soil in Van province have depleted Zn contents, the current study shows that pasture-based feeding is sufficient for the Zn requirement of sheep. Furthermore, this study finds the serum Zn levels in Norduz sheep to be higher than in Karacabey Merino sheep (27 μg dl^{-1}; Ozan 1985), Morkaraman sheep (40.56 μg dl^{-1}; Kaya et al. 1998), Akkaraman sheep (64.81 μg dl^{-1}; Kurt et al. 2001) and Sakız-Awassi crossbred sheep (83-88 μg dl^{-1}; Yokuş et al. 2004). The Zn values of all groups in this study are lower than those in the Sakız sheep (257.19 μg dl^{-1}) but higher than those in the Kıvırcık sheep (94.9 μg dl^{-1}) in the same season in the İzmir province. However, Zn values of the group C (153.52 μg dl^{-1}) in this study are similar to those of Merino sheep (150.20 μg dl^{-1}; Tiftik, Doğanay 1997). In addition to the effect of breed, the effect of climate, pasture, feeding and age is also important in these differences.

Although the plasma Co levels in this study's sheep increased significantly as the nutritional levels increased (Table 2, Figure 3), they were below the reference values (1.7-5.1 μg dl^{-1}; Kayri, Irmak 2021) in all the groups. Low Co level in volcanic regions (Çiftçi et al. 2008, Kayri, Irmak 2021) and the volcanic characteristics of the research area (Çiftçi et al. 2008) may have caused Co deficiency in the herbage growing in this region. It is known that the level of Co in diets of ruminants is directly related to the Co level in the serum, and the low Co level in all groups can be explained by this direct relationship (Oldfield 1987, Tuncer et al. 2020). As a result, Co supplementation may be essential for high herd health outcomes.

CONCLUSION

While revealing that improved dietary quality is associated with significant increases in microelement levels in sheep, this study also determined that – in addition to pasture grazing – feeding with mixed grass-clover hay, extra barley, and concentrates should be ensured for adequate Cu levels. It is concluded that pasture grazing could be sufficient for Zn intake, in contrast to Co levels, which fall below normal limits even if the quality of a diet is improved. In order to prevent cobalt deficiency, the minimum recommended amount of cobalt should be given to sheep daily with feed and pastures. Improvement in these micromineral values of animals is very important in terms of reproductive characteristics and tolerance to disease.

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