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Investigation of Serum Trace Element Levels in Sheep in Diyarbakır Province and Districts

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ABSTRACT

Background: In this study, the serum of sheep raised on pasture in the province and districts of Diyarbakır was tested for concentrations of the essential trace elements Cr, Cu, Se, Zn, Co, Mn, ultra-trace elements like Ni, V and Ag and non-essential toxic trace elements like Al, Pb, As, Cd and Ti.

Methods: The animals used in the study consisted of 313 healthy sheep at the end of lactation obtained from different districts of Diyarbakır. The closed system method of wet decomposition was used to transfer serum trace elements to the soluble phase in blood samples. Serum trace elements were analyzed using an ICP-MS device.

Result: This study contributed to obtaining normal levels of Al, Ag, Ni, Ti and V trace elements in ewes at the end of lactation. It was observed that there was no difference between all districts in terms of serum Al, Ni, Cu and Zn trace element levels. In the study, differences were found in the serum Cr, Se, As and Cd trace element levels of sheep between the districts; however, it was determined that no significant deficiency or excess occurred and these levels were within normal limits. The Co and Mn levels we obtained in Diyarbakır are much lower than the average levels reported by the researchers. We can say that the addition of Co and Mn trace elements in appropriate proportions to the rations of animals can positively affect animal health and productivity.

Key words: Pasture, Serum, Sheep, Trace elements.

INTRODUCTION

The utilization of pastures with low quantity and quality depends on sheep breeding. However, a lack or surplus of trace elements in the soil may have unfavorable effects on the interactions between the soil, plants and animals. Although trace elements, which have structural, physiological, catalytic and regulatory functions in the organism (Kaneko, 1997), are necessary for body functions at low levels, their high levels may cause toxic effects (Pandey and Madhuri, 2014). Trace element concentrations in pasture and feed can vary. Sheep raised on pasture are typically susceptible to trace element deficiency. In contrast, in pastures with high concentrations of trace elements, animals raised for a long period of time accumulate these elements in the liver and may negatively impact body physiology. A diet with excessive amounts of trace elements may harm systems like immunity, reproduction, growth and yield (Erdoğan *et al*., 2002; McDowell, 1992; Poppenga *et al*., 2012). Some of the trace elements in the sheep blood serum that we investigated in our study are: essential trace elements such as chromium (Cr), copper (Cu), selenium (Se), zinc (Zn), cobalt (Co) and manganese (Mn), ultra-trace elements such as nickel (Ni), vanadium (V) and silver (Ag), non-essential toxic trace elements such as aluminum (Al), lead (Pb), arsenic (As), cadmium (Cd) and titanium (Ti).

Specific reference intervals are needed to determine and interpret serum trace element values of each species for various diagnostic parameters and to evaluate population health. Many studies have attempted to determine the mineral reference values of sheep in Turkey (Erdoğan *et al*., 2002; Kulcu and Yur, 2003; Or *et al*., 2005; Puls,1990; Vıcıl *et al*., 2012). However, a comprehensive mineral substance level study was not found in sheep raised in Diyarbakır province and its districts. Therefore, our first priority is identifying the reference values unique to the area where the animals are raised. However, in the average sheep trace element reference values in Turkey, in the literature reviews, normal levels of elements such as serum Cr, Cu, Pb, Se, Zn, As and Cd could be reached, while normal levels of elements such as Al, Ag, Ni, Ti and V could not be reached. It will be simpler to reveal the serum trace, ultra-trace and toxic trace element levels of sheep fed on pasture, their relationship with nature, the diagnosis of diseases, the monitoring of their treatment, increase the efficiency of the breeder and provide literature data for future studies because laboratory determinations of these elements are now possible, thanks to developing technology.

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MATERIALS AND METHODS

This study was supported by the Dicle University Scientific Research Projects Coordinatorship in Diyarbakır in 2014-2017 (Project number 14-VF-115). The animals for the study consisted of 313 healthy ewes, which were obtained from a total of 14 breeders in Diyarbakır districts (Bismil (n=64), Yenişehir (n=36), Kocaköy (n=55), Eğil (n=51), Silvan (n=44) and Karacadağ (n=63), who were healthy at the end of lactation, non-pregnant, not given lick blocks and not given any mineral substance application, with an average age of 2-5 years, 45±5 kg and raised on pasture under similar conditions, without gender discrimination. Without compromising the cold chain, blood samples collected from the sheep's vena jugularis in July using vacuum tubes without any anticoagulant. The blood samples were transported to the laboratory and centrifuged at 3000 rpm for 15 minutes to separate the serum. The serums obtained were transferred into 1.5 ml eppendorf tubes and stored in a deep freezer at -26°C until analysis. The analysis of serum Al, Cr, Cu, Pb, Se, Zn, Ag, Ag, As, Cd, Cd, Co, Mn, Ni, Ti and V in the soluble phase was carried out using a closed system method of wet decomposition. The samples obtained by the method of wet decomposition were analyzed by ICP-MS [Agilent 7700x, inductively coupled plasma mass spectrometry (ICP-MS), Multi-Element Aqueous CRM, Calibration Standard for 6020, 2% HNO_3, Tartaric Acid, VHG-LCAL6020-100, USA]. In the study, statistical comparisons of serum trace element levels of sheep in Bismil, Yenişehir, Kocaköy, Eğil, Silvan districts and Karacadağ region were made between districts. Independent-Samples t-test was used in the evaluation of serum trace element levels. All values were expressed as mean±standard deviation ($\bar{X}$±SD) with P<0.05. Statistical analyses of the data were evaluated in SPSS 18.0 package program.

RESULTS AND DISCUSSION

The animals-used in the study was selected from healthy sheeps that were pasture-fed, at the end of lactation and not pregnant, owned by 14 breeders in six districts of Diyarbakır. The mean serum trace element results of all sheeps used in the study by districts are shown in Table 1. There was no statistical difference between the districts in the comparison of serum Ni, Al, Cu and Zn trace element levels (p>0.05). It was observed that Cu and Zn trace element levels were within the physiological limits (Altındaş, 1993; Poppenga *et al*., 2012; Puls, 1990) in sheep and were in parallel with our study. In our study, As and Ti in Karacadağ, As in Kocaköy, Ti in Eğil, Pb, Cd and V element levels were below the detectable limit (UDL) in Silvan (p<0.05). The other elements, except for trace element V, are toxic non-essential elements. Thus, it can be said that the lack of the UDL mentioned above components in sheep raised in these areas does not result in any nutritional problems or adverse side effects. Additionally, it could be argued that these districts' soil and vegetation are deficient in the UDL mentioned above components. For this situation to be determined, more research is required.

Ni, one of the essential trace elements for animals, plays a role in urease, hydrogenase and carbon monoxide dehydrogenase enzymes. Although it is an essential element at low concentrations, it can be toxic at high concentrations. Ni acts as a cofactor in the building blocks of membranes and metalloenzymes (Bradl, 2005). Animal health and trace amounts of Al and Ag are not fully understood to be related. However, it has been reported that the trace element Al accumulates in humans when the kidneys cannot perform their functions (Klein, 2003) and on the toxicity of Ag, blue skin disease occurs when it is used in high amounts in the treatment of open wounds (Özcan, 2014). The average serum Ag concentrations in our study were determined as 2.8 ppb.

Table 1: Serum trace element levels of sheep in Diyarbakır districts ($\bar{X}$±SD).

		Bismil	Yenişehir	Kocaköy	Karacadağ	Eğil	Silvan	Avarage	Ref. ranges	Sig.
Al		2.5±.39	2.7±.33	2.2±.62	3.2±.94	2.8±.07	2.8±.05	2.7±.69	-	-
Cr		0.10±.07^{abc}	0.28±.03^{ad}	.22±.03^{e}	0.07±.02^{b}	0.13±.02^{f}	0.06±.01^{cdef}	0.12±.08	0.03-0.68	***
Cu	(ppm)	0.49±.07	0.57±.07	.55±.08	0.40±.1	0.62±.07	0.48±.04	0.49±.01	0.5-2.5	-
Pb		0.35±.04	0.41±.02	.14±.01^{a}	0.60±.09^{a}	0.33±.04	UDL	0.39±.16	0.02-0.25	***
Se		0.79±.08^{a}	0.31±.06	.13±.01^{a}	0.52±.06	0.54±.07	0.52±.04	0.48±.21	0.08-0.5	*
Zn		0.50±.08	0.93±.1	.47±.08	0.53±.1	0.96±.09	0.88±.05	0.64±.22	0.22-1.84	×××
Ag		1.9±.4	4.0±.6	1.2±.1^{a}	4.3±.9^{a}	1.5±.3	1.5±.1	2.8±.0	-	*
As		9.5±2^{ab}	15±1.6^{cd}	UDL	UDL	49±8^{ac}	27±6^{bd}	40±.06	10-80	***
Cd		16±3^{a}	29±6	26±5	32±9^{ab}	13±4^{b}	UDL	24±.01	4-200	*
Co	(ppb)	1.9±.3^{abc}	3.9±1^{ad}	2.9±.8	4.6±.9^{be}	3.8±.7^{cf}	1.1±.1^{def}	3.1±.0	1.3-700	acdef*** b**
Mn		50±6.9^{ab}	51±6.3^{cd}	26±5.2	31±5.4	15±2.9^{ac}	14±2.3^{bd}	33±.01	60-5100	**
Ni		6.3±.7	5.0±.5	6.9±1	6.5±1.1	5.3±.8	3.9±.7	5.9±.0	-	-
Ti		4.3±3.4^{abc}	1.1±.03^{ad}	0.26±.06^{bd}	UDL	UDL	0.28±.05^{c}	2.3±.0	-	abc*** d*
V		68±8^{a}	73±7.2^{b}	25±5.2^{c}	14±2.3^{abd}	85±14^{cd}	UDL	45±.03	-	ab** cd***

The same superscripts (* , **and***) in the same row denote significant difference at (p<0.05, P<0.01, P<0.001) respectively.
UDL= Under definable limit.

In the literature review, reference values of Al, Ag, Ni, Ti and V trace elements in sheep were not found. However, literature values of Ni in goats (2.7-4.4 ppb) and cattle (1.2-5.6 ppb) and V only in cattle (1.2-1.4 ppb) were found (Puls, 1990). While our study was similar to the Ni levels reported by Puls (1990) for goats and cattle, it was higher than the values reported for V. Additional studies are needed to evaluate these data more accurately. In statistical comparisons between districts, the average levels of Al (2.7 ppm), Ag (2.8 ppb), Ni (5.5 ppb), Ti (2.3 ppb) and V (45 ppb) were determined in our study (Table 1). However, it was impossible to tell from the animals' health whether their deficiencies or excesses were within physiological bounds. However, the fact that the animals appear healthy leads us to believe that the above trace elements are within acceptable ranges and can serve as a source of benchmark values for sheep.

Insufficient Cr intake is closely associated with carbohydrate, lipid and nucleic acid metabolism disorders. Significant differences were found in the evaluation of serum Cr trace element levels between districts ($p<0.001$). The highest level was observed in Yenişehir and Kocaköy, while the lowest level was observed in Karacadağ and Silvan districts ($p<0.001$). The average serum Cr level was determined to be 0.12 ppm. This level was much lower than the serum Cr poisoning limit of 4 ppm reported by Kaya and Akar (1998). In general, serum Cr levels are reported to be 0.03-0.06 ppm in sheep (Puls, 1990; Vıcıl *et al*., 2012) and 0.68 ppm in goats (Donia *et al*., 2014). The serum Cr levels in our study were similar to the average serum Cr values reported by these researchers for sheep.

Exposure to low levels but prolonged exposure to Pb causes various physiological, biochemical and behavioral disorders in ruminants. Anemia occurs due to the suppression of enzymes that synthesize blood proteins. Red blood cell membrane durability decreases (Erdoğan *et al*., 2005; McDowell, 1992). In the study, serum concentrations of Pb ($p<0.001$) and Ag ($p<0.05$) in Karacadağ were significantly higher than in the Kocaköy district, while no difference was found between the other districts. This statistical difference suggests that - Karacadağ being a volcanic mountain, the soil structure may cause regional differences. In the present study, serum Ag concentration was determined as 2.8 ppb and Pb as 0.39 ppm. Serum Pb levels were found to be much higher than the average levels reported by some investigators (Or *et al*., 2005; Puls, 1990; Vıcıl *et al*., 2012). This overall situation demonstrates that regional and pasture differences might be the reason. The high serum Pb concentrations suggest that further Pb trace element research on animals is necessary.

Se is an important trace element that causes *muscular dystrophy* in calves and sheep when deficient in vitamin E (Küçükaslan, 2011). When the serum Se levels in different districts were compared the serum Se levels in the Bismil district were significantly higher than in the Kocaköy district ($p<0.05$). Øvernes *et al*., (1985) reported that serum Se levels in sheep should be between 0.15-0.35 ppm and *white muscle disease* occurs when the serum Se level falls below 0.10 ppm. In our study, the mean serum Se levels were 0.48 ppm in all sheep of the region. Mean serum Se concentrations did not fall below this value in any district. These levels were similar to the serum Se levels reported by Poppenga *et al*. (2012) and Puls (1990). However, they were generally higher than the levels reported in some other studies (Erdoğan *et al*., 2002; Kock *et al*., 1987).

As is a toxic trace element and is non-essential for other living organisms but necessary for humans, pigs and chicks. As makes the body more vulnerable to oxidative damage by preventing the synthesis of glutathione, one of the antioxidant substances (Flora, 2011). As compounds are transmitted to the environment through the water. They tend to accumulate in animals exposed to this contamination. In the comparison of serum As levels in our study, the highest values were found in Eğil (49 ppb) and Silvan (27 ppb) districts. The mean serum As levels of the sheep in the study was 40 ppb ($p<0.001$). These mean values were similar to the serum As results reported by Puls (1990).

Cd trace element is in antagonist relationship with Cu and Zn elements. This substance, which has toxic effects on all systems in the organism, tends to accumulate, especially in the kidney and liver (Raikwar *et al*., 2008). In our study, a significant difference was found between Bismil-Karacadağ and Karacadağ-Eğil districts and Cd values in Karacadağ were higher in both districts ($p<0.05$). The average Cd values (Or *et al*., 2005; Puls, 1990) reported by the researchers for the sheep showed parallelism with the levels we obtained in our study.

Atypical findings like growth retardation, pica, weakness, delay in sexual maturation and icterus in sheep are signs of Co deficiency, also known as the White Liver Disease of lambs (Şimşek, 2016). It may be present in areas with copper and phosphorus deficiencies. In the statistical evaluation of the Co element in our study, significant differences were recorded between the districts; the highest levels were found in Karacadağ ($p<0.01$) and the lowest levels were found in the Silvan district ($p<0.001$). The results of our study are similar to the results of Puls (1990) for sheep serum Co levels. However, the mean Co levels reported by many researchers for sheep (Vıcıl *et al*., 2012; Yıldız *et al*., 1995) were significantly higher than the mean levels found in our study. This may be due to the differences in breed, region and pasture conditions of the animals.

Mn is an essential element that acts biochemically as a component of metalloenzymes and as an enzyme activator (Şimşek, 2016). The amount of Mn in feeds is affected by soil structure, pH, fertilization and plant species. Since, plants grown in acidic and sandy soils are poor in Mn, primary deficiency may be observed in animals fed on these plants. Although there were statistical differences between the districts in our study, the average Mn levels were measured as 33 ppm ($p<0.01$). Georgievskii (1982); Gupta *et al*. (2016) reported the mean serum Mn levels in sheep as 40-50 ppb, which was considered to be the closest to our

findings. Serum Mn levels were significantly lower in our study than the average values reported by numerous researchers (Kulcu and Yur, 2003; Küçükersan *et al*., 1996; Puls, 1990; Yıldız *et al*., 1995) and were, therefore, incompatible. This may indicate that pasture soils in our region are poor in Mn.

It has been suggested that Ti, whose reference values could not be reached in sheep, facilitates DNA damage and tumor formation Trouiller *et al*., (2009). When the level of Ti was compared in different districts, the highest value was observed in Bismil, while the lowest values were observed in Kocaköy, Silvan and Yenişehir, respectively. The mean serum Ti level was determined to be 2.3 ppb in general ($p<0.05$).

In the statistical evaluation of the V element whose toxicity is more easily seen, significant differences were observed between the districts; the highest value was 85 ppb in Eğil ($p<0.001$) and the lowest value was 14 ppb in Karacadağ ($p<0.01$). The average serum V level between the districts was determined as 45 ppb.

CONCLUSION

The sheep serum trace element concentrations are one of the crucial factors that should be considered for clinical diagnosis and prognosis in animal disease conditions. Normal values of Al, Ag, Ni, Ti and V trace elements in late lactation sheep fed on pasture in Diyarbakır and its districts have not been found in any previous study. In the present study, differences were found in the serum Cr, Se, As and Cd trace element levels of sheep between the districts; however, it was determined that no significant deficiency or excess occurred and these levels were within normal limits. It was noted that the Co and Mn levels found in our present study were much lower than the values reported in literature and we predict that adding Co and Mn trace elements to the animals' diets at the proper rates in all of the districts under consideration may have a positive impact on animal health and productivity. However, the fact that the sheep are healthy and the average serum Pb levels are high suggests that additional studies should be done on this subject.

Conflict of interest

It is declared that there is no conflict of interest between the authors.

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