



## Comparing Trace Element Concentrations in Serum and Claw Tissue Samples of Buffaloes and Brown Swiss Cattle\*

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**Abstract:** The aim of this study was to compare some trace elements in serum and hoof tissues of healthy Brown Swiss and Anatolian buffaloes raised in Turkey. Thus, the data generated in this study may have some potentials to contribute to future studies in this field. A total of 20 animals, 10 Anatolian buffalo cows and 10 Brown Swiss dairy cattle, were included in the study. The health status of all animals was examined and those without any systemic disease and lameness were enrolled in the study. Hoof tissue samples were taken from the dorsal hooves (*paries unguiae*) and solear hooves (*solea unguiae*) of the lateral of the right hind feet of all animals for trace element analysis. Cr, Mn, Fe, Cu, and Zn levels were determined by ICP-MS in all claw samples. *Paries unguiae* Cu and Zn levels were significantly higher than in the *solea unguiae* in both groups ( $P<0.05$ ). In solear hoof samples, a positive correlation between Cr and Fe, Cr and Cu, and Cr and Zn levels in buffalo group was observed but not statistically significant. In conclusion, Cu and Zn levels in *paries unguiae* are higher than *solea unguiae* in both species; however, more comprehensive studies should be performed to determine trace element levels in hooves of buffaloes and cattle with claw lesions.

**Keywords:** Buffalo, Cattle, Claw, Copper, Trace element.

## Manda ve Montofon İrki Sığırlarda Serum ve Tırnak Dokusu Bazı İz Element Düzeylerinin Karşılaştırılması

**Öz:** Bu çalışmanın amacı, Türkiye'de yetiştirilen sağlıklı Montofon ve Anadolu mandalarında serum ve tırnak dokusu bazı iz element düzeylerini karşılaştırmak, sağlıklı ayak ve tırnak yapısına sahip bu iki gruptan elde edilecek sonuçların ilerideki bu alanda yapılacak çalışmalara literatür katkısında bulunmaktır. 10 Anadolu manda ineği ve 10 Montofon süt sığırları olmak üzere toplam 20 hayvan çalışmaya dahil edildi. Tüm hayvanların sağlık durumları incelendi, sistemik hastalığı ve topallığı bulunmayan hayvanlar çalışmaya alındı. İz element analizleri için tüm hayvanların sağ arka ayak lateral tırnağının dorsal duvarından (*paries unguiae*) ve taban bölgesinden (*solea unguiae*) tırnak dokusu örneği alındı. Tüm tırnak örneklerinde Cr, Mn, Fe, Cu ve Zn seviyeleri ICP-MS ile belirlendi. Her iki grupta *paries unguiae* Cu ve Zn düzeyleri, *solea unguiae*'dan istatistiksel olarak anlamlı düzeyde yüksekti ( $P<0.05$ ). Manda grubunda, taban tırnak örneklerinde, Cr ve Fe, Cr ve Cu ve Cr ve Zn düzeyleri arasında istatistiksel olarak anlamlı olmayan pozitif korelasyon gözlemlendi. Sonuç olarak; manda ve sığırlarda *paries unguiae* Cu ve Zn düzeylerinin *solea unguiae*ya göre oldukça yüksek olduğu, özellikle çeşitli tırnak lezyonu bulunan mandalar ve sığırlarda tırnak dokusu iz element düzeylerinin belirlenmesi için ileride daha kapsamlı çalışmaların yapılması gerektiği kanısına varılmıştır.

**Anahtar Kelimeler:** Bakır, İz element, Manda, Sığır, Tırnak.

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## INTRODUCTION

Trace elements (TE) such as zinc (Zn), copper (Cu) and manganese (Mn) are a highly important components in dairy cattle feeding practice because TE play crucial roles in formation of connective tissue, vitamin metabolism, protein synthesis and immune system functions (1-3). Supplementation of TE in cattle ration may also positively affect claw structure, fertility, lactation, and immune responses (2,4,5).

The strength and quality of claw horns are under the influences of metabolic, genetic, hormonal, environmental, and nutritional factors in cattle (6-8). Nutritional factors such as fatty acids, TE, minerals, vitamins, and amino acids are particularly important components for a high-quality hoof formation and sustainability (6,9). The TE, particularly Cu, Zn, calcium (Ca), iron (Fe), iodine (I), selenium (Se), molybdenum (Mo), and chrome (Cr) are closely associated with hoof development and claw diseases (10,11).

Zn has been shown to play a crucial role in keratinization (12-14) and is an important mineral effective in the production of proper hoof tissue. Due to its catalytic, structural, and regulatory functions, Zn affects all processes that necessary for the healthy hoof growth (7,15,16). Cu is involved in the activation of many enzymes similar to zinc. The most important enzyme in hoof cell keratinization is thiol-oxidase which is activated by Cu (7, 17,18).

Mn, an essential element for hoof production, is important for the pyruvate carboxylase enzyme activation. It influences gluconeogenesis and cell energy production (9,16,19). Mn indirectly plays a role in the keratinization mechanisms and minimizes the claw disorders by contributing to hoof tissue formation (18,20).

Fe fundamentally affects the formation and structure of hemoglobin and myoglobin molecules (1,21). This may explain why Fe is a highly crucial entity for the transportation of oxygen from one location to terminal oxidases within the body. Moreover, in the keratinization process, the optimum Fe supply is essential for oxygen delivery. In this process, energy is obtained from the Calvin cycle in the form of ATP, in which oxygen and glucose are the main substrates (21). Cr potentiates the activity of insulin exclusively in insulin-sensitive tissues. It was shown that cattle rations supplemented with Cr improved immunity, glucose clearance, and milk yield (22).

This study aimed to compare some trace elements in serum and hoof tissues of healthy Brown Swiss and Anatolian buffaloes raised in Turkey. Thus, the data generated in this study may have some potentials to contribute to future studies in this field.

## MATERIALS and METHODS

The study was conducted after the approval of Afyon Kocatepe University Animal Experiments and Local Ethics Committee (No: 49533702/172-29.11.2017). A total of 20 animals, 10 Anatolian buffaloes (mean age:  $5.3 \pm 1.05$ ) and 10 Brown Swiss cattle (mean age:  $5.3 \pm 1.33$ ), were included in the study. The health status of all animals was examined and those without any systemic disease and lameness were enrolled in the study. The criteria given in Table 1 were used for the evaluation of lameness. Animals with a score of 1 according to the lameness assessment were selected and fed *ad libitum* with a complete ration prepared according to the formulations. Each 1 ton of concentrated feed mix contained 15.000.000 IU Vitamin A, 2.100.000 IU Vitamin D, 28.000 mg Vitamin E, 45.000 mg Mn,

26.000 mg Fe, 140.000 mg Zn, 25.000 mg Cu, 250 mg Co, 1400 mg I, and 1200 mg of Se. All cows were fed the same ration composition (Table 2).

**Table 1.** Lameness scoring system used in this study (2).

**Tablo 1.** Çalışmada kullanılan topallık skorlama sistemi (2).

Score	Description	Gait characteristics
1	Sound	Flatback, smooth head wobble frequency, joint activities with ease, uniform stride
2	Imperfect gait	Slightly arched back, smooth head wobble frequency, mild joint stiffness, slightly uneven gait, no visible lameness
3	Slight lameness	Arched back, uneven head bobs, joint stiffness, uneven gait, slightly lameness
4	Noticeable lameness	Obvious arched back, obvious head bobs, joint stiffness, hesitant gait, obvious lameness
5	Obvious lameness	Severely arched back, very obvious head bobs, joint stiffness, difficulty walking, severely lameness

**Table 2.** Ingredient composition of the basal diet (% of DM).

**Tablo 2.** Günlük rasyon içeriği (KM %).

Physical composition	Water buffalo diet (% DM)	Cow diet (% DM)
Corn silage	26.45	22.21
Barley straw	26.58	13.39
Straw	8.51	24.52
Barley (Rolled)	10.33	16.79
Premix*	27.29	22.24
Sodium bicarbonate	0.84	0.85
<u>Chemical composition</u>		
Crude protein	15.01	12.15
NEI Mcal/kg	1.46	1.42
Ether extract	3.13	3.01
NDF	37.16	41.24
ADF	23.41	25.89
NFC	35.65	33.41
Ca	0.82	0.76
P	0.46	0.41

\*Each 1 ton of concentrated feed mix contained; 15.000.000 IU Vitamin A, 2.100.000 IU Vitamin D, 28000 mg Vitamin E, 45.000 mg Mn, 26.000 mg Fe, 140.000 mg Zn, 25.000 mg Cu, 250 mg Co, 1400 mg I, 1200 mg Se. DM: dry matter, NDF: neutral detergent fiber, ADF: acid detergent fiber, NFC: nonfiber carbohydrate

## Hoof Tissue Sampling

Before the hoof sampling, the animals were placed in a specially prepared hoof trimming chute. Then, the hooves of Anatolian buffalo and cattle were washed with a brush to clean thoroughly and remove all foreign objects, faeces etc. Routine hoof trimming of all animals was accomplished during the study, and the excess parts of the hoof were cut and removed. Approximately 300-500 mg hoof tissue samples were taken from the dorsal hoof (*paries unguulae*) and solear hoof (*solea unguulae*) of the lateral of the right hind foot of all animals. The surfaces of the removed hoof biopsies were completely cleaned and rinsed through 99.5% acetone, 96% ethanol, and distilled water and then stored in polyethylene bottles until analyzed. Blood samples (8 ml) were collected via jugular vein, then centrifuged at 3000 rpm for 10 minutes and the serums were separated and stored in a freezer at -20 °C until TE analysis.

## Measurement of Trace Elements

Analyses were carried out by a laboratory (Bayburt University, Central research Laboratory, Turkey). Briefly, a closed system microwave burning method was carried out for the analysis of hoof tissue samples. For this purpose, Ethos Easy microwave closed system with teflon containers was used. Each 0.2 g of hoof sample was loaded for separate teflon and 8 ml of 65% HNO<sub>3</sub> was added. After the addition of 1 ml of 30% H<sub>2</sub>O<sub>2</sub>, it was left for 15-20 minutes at room temperature. Lids of teflon containers were tightly squeezed and burned for 10 minutes by applying 200 °C heat treatment. After treatment, the solutions were cooled and diluted by 50 ml with ultra-pure water. Then, the solution taken into plastic

tubes was filtered through 25 µm down to 0.45 µm filters and stored at 4°C until reading. Cr, Mn, Fe, Cu, and Zn levels were determined by ICP-MS (Agilent 7800) according to the method of Zhao et al. (3). The minimum detection limit was 0.0001 ppm. Results were expressed in ppb.

### Statistical Analysis

Mann Whitney U test was used to determine the differences between Cr, Mn, Fe, Cu, and Zn concentrations in serum and hoof tissues. For correlations between findings, Spearman's correlation test was used. Data were expressed as mean ± standard deviation, and P<0.05 was considered statistically significant.

### RESULTS

Cr levels in *paries unguulae* and *solea unguulae* were not significantly higher in the hoof of cattle than in buffaloes (P>0.05) (Table 3). A similar trend was also observed in serum Cr level that was higher in cattle than buffalo groups (P>0.05). Hoof tissue Fe levels were higher in the cattle group than the buffalo group; however, serum Fe levels were lower in the cattle group compared to the buffalo group but not statistically significant (P>0.05). *Paries unguulae* Cu and Zn levels were higher than in the *solea unguulae* in both groups, and the difference was significant (P<0.05). Cu and Zn levels in cattle hoof tissue were significantly higher in *paries unguulae* than in *solea unguulae*. It was noted that serum Cu and Zn levels were similar in buffalo and cattle groups (P>0.05).

**Table 3.** The concentrations of Cr, Mn, Fe, Cu, and Zn in hoof tissue and serum samples in buffaloes and cattle (mean±SD).

**Tablo 3.** Manda ve sığırlarda tırnak dokusu ve serum örneklerinde Cr, Mn, Fe, Cu ve Zn düzeyleri (Ort±SD).

		Cattle	Buffalo	P
Paries unguulae	Chrome (ppb)	2240.98±3447.86 <sup>a</sup>	540.20±249.86 <sup>ab</sup>	0.07
	Manganese (ppb)	4277.39±3527.12	3094.33±1289.15	0.35
	Iron (ppb)	82703.30±54309.19 <sup>a</sup>	45573.35±55087.41 <sup>ab</sup>	0.13
	Cooper (ppb)	1952.21±1533.64 <sup>ab</sup>	5341.36±9203.36 <sup>a</sup>	0.09
	Zinc (ppb)	136548.78±33767.77 <sup>a*</sup>	120957.91±14682.68 <sup>a</sup>	0.02
Solea unguulae	Chrome (ppb)	1597.30±1861.19 <sup>ab</sup>	666.82±305.05 <sup>ab</sup>	0.65
	Manganese (ppb)	4101.61±4550.78	-	-
	Iron (ppb)	67126.01±52711.51 <sup>a</sup>	42059.98±27186.46 <sup>ab</sup>	0.36
	Cooper (ppb)	567.22±500.99 <sup>b</sup>	605.51±213.85 <sup>b</sup>	0.17
	Zinc (ppb)	81255.92±32390.58 <sup>b</sup>	71199.93±9213.31 <sup>b</sup>	0.29
Blood Serum	Chrome (ppb)	8.79±4.18 <sup>b</sup>	6.70±4.05 <sup>b</sup>	0.27
	Manganese (ppb)	3.79±4.46 <sup>*</sup>	5.74±1.97	0.02
	Iron (ppb)	2395.73±1656.58 <sup>b</sup>	3039.98±2484.15 <sup>b</sup>	0.29
	Cooper (ppb)	627.80±153.79 <sup>b</sup>	628.67±112.78 <sup>b</sup>	0.70
	Zinc (ppb)	676.92±151.03 <sup>c</sup>	959.19±506.65 <sup>c</sup>	0.17

<sup>abc</sup> The difference between values bearing different letters in the same column is statistically significant (P<0.05)

<sup>\*</sup>Indicate statistically significant differences between buffalo and cattle (P<0.05)

<sup>abc</sup> Aynı sütunda farklı harf taşıyan değerler arasındaki fark istatistiksel olarak önemlidir (P<0.05)

<sup>\*</sup>Sığır ve mandalar arasındaki istatistiksel olarak önemli farklılıkları göstermektedir (P<0.05).

In the buffalo group, there was a positive correlation between Cr and Fe levels in the dorsal surface of the hoof, but this correlation was not

statistically significant (P>0.05). On the other hand, there was a negative correlation between Cr and Cu, and Cr and Zn concentrations in the dorsal surface of

the hoof in the buffalo group ( $P>0.05$ ) however a positive correlation between Zn and Cu levels was noted.

In solear hoof samples, a positive correlation between Cr and Fe, Cr and Cu, and Cr and Zn levels in

buffalo group was observed but not statistically significant ( $P>0.05$ ). The correlation between serum Fe and Cu was negative, whereas it was positive between serum Fe and Zn in the buffalo group (Table 4).

**Table 4.** Correlation coefficients between Cr, Fe, Cu, and Zn in hoof tissues of buffaloes.

**Tablo 4.** Mandalarda tırnak dokusu Cr, Fe, Cu ve Zn arasındaki korelasyon katsayıları.

		CrBPU	CrBSU	FeBPU	FeBSU	CuBPU	CuBSU	ZnBPU	ZnBSU
CrBPU	<i>p</i>	1							
	<i>r</i>	.							
CrBSU	<i>p</i>	.103	1						
	<i>r</i>	.777	.						
FeBPU	<i>p</i>	.539	-.200	1					
	<i>r</i>	.108	.580	.					
FeBSU	<i>p</i>	.,345	.067	.067	1				
	<i>r</i>	.328	.855	.855	.				
CuBPU	<i>p</i>	-.455	.273	-.188	.273	1			
	<i>r</i>	.187	.446	.603	.446	.			
CuBSU	<i>p</i>	.406	.406	.236	-.612	.200	1		
	<i>r</i>	.244	.244	.511	.060	.580	.		
ZnBPU	<i>p</i>	-.018	.115	-.103	-.091	.382	.539	1	
	<i>r</i>	.960	.751	.777	.803	.276	.108	.	
ZnBSU	<i>p</i>	.030	.006	.188	.067	.200	.018	.479	1
	<i>r</i>	.934	.987	.603	.855	.580	.960	.162	.

\* $P<0.05$ , BPU: *Buffalo paries unguiae*, BSU: *Buffalo solea unguiae*, BPU: *Manda paries unguiae*, MSU: *Manda solea unguiae*

An insignificant positive correlation between Fe and Cr, Fe and Cu, and Fe and Zn levels in the dorsal wall tissue were observed in cattle. On the other hand, a statistically significant positive correlation was found between Fe and Cr levels in cattle solear tissue ( $P<0.01$   $r=0.879$ ). Furthermore, the difference between Mn and Fe levels in the dorsal wall was also significant with a positive correlation ( $P<0.01$

$r=0.860$ ) (Table 5). The correlation between serum Cu and Fe and Zn and Fe levels in the cattle group was positive, whereas the correlation between serum Zn and Cu concentrations were negative. In serum, Mn and Cr levels in the cattle group were significantly different and correlated positively ( $P<0.05$   $r=0.648$ ) (Table 6).

**Table 5.** Correlation coefficients between Cr, Fe, Cu, Zn, and Mn in hoof tissue in cattle.

**Tablo 5.** Sığırlarda tırnak dokusu Cr, Fe, Cu, Zn ve Mn arasındaki korelasyon katsayıları.

		CrCSU	FeCPU	FeCSU	CuCPU	CuCSU	ZnCPU	ZnCSU	MnCPU	MnCSU
CrCPU	<i>p</i>	1,000								
	<i>r</i>	.								
CrCSU	<i>p</i>	.382	1,000							
	<i>r</i>	.276	.							
FeCPU	<i>p</i>	.503	.527	1,000						
	<i>r</i>	.138	.117	.						
FeCSU	<i>p</i>	.055	.879**	.430	1,000					
	<i>r</i>	.881	.001	.214	.					
CuCPU	<i>p</i>	.297	.406	.321	.139	1,000				
	<i>r</i>	.405	.244	.365	.701	.				
CuCSU	<i>p</i>	.248	-.212	.103	-.152	-.297	1,000			
	<i>r</i>	.489	.556	.777	.676	.405	.			
ZnCPU	<i>p</i>	.139	.333	.333	.224	.370	-.164	1,000		
	<i>r</i>									

**Table 5.** Correlation coefficients between Cr, Fe, Cu, Zn, and Mn in hoof tissue in cattle (Continue).**Tablo 5.** Sığırlarda tırnak dokusu Cr, Fe, Cu, Zn ve Mn arasındaki korelasyon katsayıları (Devamı).

	CrCSU	FeCPU	FeCSU	CuCPU	CuCSU	ZnCPU	ZnCSU	MnCPU	MnCPU
ZnCSU	<i>p</i> .701	.347	.347	.533	.293	.651	.		
	<i>r</i> .612	.236	.212	.103	.588	.188	-.055	1.000	
MnCPU	<i>p</i> .060	.511	.556	.777	.074	.603	.881	.	
	<i>r</i> .505	.649*	.860**	.560	.294	.000	.451	.287	1.000
MnCSU	<i>p</i> .136	.042	.001	.092	.410	1.000	.191	.422	.
	<i>r</i> .276	.500	.455	.619	-.321	.231	.112	-.067	.529
	<i>r</i> .440	.141	.187	.056	.366	.521	.758	.854	.116

\*: P<0.01, \*\*: P<0.05 CPU: Cattle paries unguiae CSU: Cattle solea unguiae, CPU: Sığır paries unguiae, CSU: Sığır solea unguiae.

**Table 6.** Correlation coefficients between serum Cr, Fe, Cu, Zn, and Mn in buffaloes and cattle.**Tablo 6.** Manda ve sığırlarda serum Cr, Fe, Cu, Zn ve Mn arasındaki korelasyon katsayıları.

	CrBS	CrCS	FeBS	FeCS	CuBS	CuCS	ZnBS	ZnCS	MnBS	MnCS
CrBS	<i>p</i> 1									
	<i>r</i> .									
CrCS	<i>p</i> -.164	1								
	<i>r</i> .651	.								
FeBS	<i>p</i> -.103	.127	1							
	<i>r</i> .777	.726	.							
FeCS	<i>p</i> -.479	.491	-.248	1						
	<i>r</i> .162	.150	.489	.						
CuBS	<i>p</i> -.539	.273	-.164	.297	1					
	<i>r</i> .108	.446	.651	.405	.					
CuCS	<i>p</i> .188	-.261	.164	.200	-.127	1				
	<i>r</i> .603	.467	.651	.580	.726	.				
ZnBS	<i>p</i> -.333	.345	.345	.248	-.030	.079	1			
	<i>r</i> .347	.328	.328	.489	.934	.829	.			
ZnCS	<i>p</i> -.164	.394	-.042	.115	-.018	-.479	.261	1		
	<i>r</i> .651	.260	.907	.751	.960	.162	.467	.		
MnBS	<i>p</i> .212	.830**	.127	.176	-.091	-.212	.321	.164	1	
	<i>r</i> .556	.003	.726	.627	.803	.556	.365	.651	.	
MnCS	<i>p</i> -.176	.648*	-.091	.430	.321	-.491	.370	.564	.370	1
	<i>r</i> .627	.043	.803	.214	.365	.150	.293	.090	.293	.

\*: P<0.01, \*\*: P<0.05 BS: Buffalo serum, CS: Cattle serum, BS: Manda serumu, CS: Sığır serumu

## DISCUSSION and CONCLUSION

Cu is an important mineral to maintain the strength of the hoof capsule, and it is crucial for good hoof quality at certain concentrations (7,13,23,24). It is actively involved in different physiological events such as melanin synthesis and the formation of sulfhydryl amino acids, including methionine, and cysteine. These amino acids are linked by disulfide bonds, generating a very strong three-dimensional network in digits where the greater portion of the weight of animal embarks (9,25,26). There are few studies evaluating TE in hoof tissue in Anatolian buffaloes such as Cu, Zn, Mn and Fe. Assis et al. (27) examined hoof quality and biochemical composition

of female buffaloes (n=14) and showed that the dorsal wall contained more Cu than the sole horn. Sadeghi Nasap et al. (28) reported that the hoof contains 10.34 µg/g Cu in healthy cattle. In the same study, there was a positive correlation between Cu and Zn levels in cattle (28). Elsewhere, the Cu level in healthy Holstein cattle was 0.19 ppm, with a positive correlation between Cu and Zn levels in hoof horn tissue (29). In our study, Cu levels in the dorsal wall and sole horn in the buffalo group were determined as 5341.36 and 605.51 ppb, respectively, as reported previously by Assis et al. (27), who stated that the dorsal wall contained significantly more Cu than sole horn.

In cattle group, Cu levels in dorsal wall and sole were 1952.21 and 567.22 ppb, respectively. Similar findings were also generated previously and a positive correlation was seen between Cu and Zn levels (28,29). We, in this study, observed that dorsal wall horn was found to contain approximately 2.5-3 times more Cu in the water buffalo group than the cattle group.

Zn is involved in the metabolism of keratin and is an important mineral for hoof horn development. Zn has three main roles in keratinization, particularly catalytic, structural, and function regulators (15,18). Given that Zn is an important element in keratin synthesis and that intense cell proliferation occurs in the corium coronarium, the hoof capsule containing the higher proportion of Zn is likely to have a faster growth rate than other parts of the hoof. Assis et al. (27) report that the dorsal wall contained less Zn than the sole horn in the water buffalo. The dorsal wall horn contains more Zn and less keratin than the sole horn, which may explain why the sole is softer than the wall. It was shown that the level of hoof tissue Zn in lame cattle was lower with respect to in non-lame cattle (9,28-30).

Kibar et al. (29) concluded that there is a positive correlation between Zn and Cu levels in the tissue of healthy cattle. We observed that the dorsal wall possessed more Zn content than sole horn in both buffalo and cattle groups. At the same time, there was a positive correlation between Zn and Cu levels of hoof tissue in both buffalo and cattle groups. All these findings are similar to the literature data given above.

In this study, the dorsal wall horn had higher concentrations of Zn content than the sole horn thus, it was speculated that it might be associated with faster growth of the dorsal wall than the sole.

Mn is indirectly effective in keratinization by acting on the activation of critical enzyme systems such as pyruvate carboxylase, which is mainly responsible for energy production (9,18). Mn is also important for the proper utilization of Cu (31). The current literature search was unrewarding since no

studies evaluating Mn levels in the hoof of Anatolian buffaloes; however, there are several investigations on Mn levels in cattle (29,32). Hoof tissue Mn levels in healthy Holstein cattle was 0.28 ppm (29), whereas it was 0,5 µg/g in crossbred cattle (32). Kibar et al. (29) reported a positive correlation between Mn and Cu and Zn levels in hoof tissue. In this study, Mn levels of buffalo hoof tissue were determined as 3094.33 ppb for dorsal wall horn. In the cattle group, Mn level was determined as 4277.39 ppb for dorsal wall and 4101.61 ppb for sole horn. Furthermore, it was observed that hoof capsule and sole horn Mn levels were quite similar in the cattle group. Mudgal et al. (33) reported that plasma Fe, Mn, Cu, and Zn levels in 18-20 months old male Anatolian buffaloes were 3.78, 0.85, 0.77, and 2.42 ppm, respectively.

In healthy female buffaloes, serum Fe, Mn, Cu, and Zn concentrations were 3.2, 0.059, 0.53, and 3.24 µmol/L, respectively (34). Sun et al. (11) evaluated the serum TE levels in cattle with different lameness scores and showed that the lowest serum Cr levels in cattle with the lowest lameness score (approximately 0.5 µg/g) and serum Cr level was the highest (approximately 4 µg/g) in cattle with the highest lameness score. They also evaluated that where the serum Fe and Cu concentration was highest in animals, the lameness score was lowest. Sadeghi-Nasab et al. (28) measured that serum Zn level in healthy cattle was 86.43 µg/dL and serum Cu level was 62.28 µg/dL. In the present study, buffaloes and cattle with the lowest lameness score without any hoof lesions were recorded.

The serum Cr level was 6.70 ppb in the buffalo group and 8.79 (0.874 µg/dL) in the cattle group. These findings are in concordance with previous studies. In this study, serum Fe, Mn, Cu, and Zn levels were 3039.98, 5.74, 628.67, and 959.19 ppb in the buffalo group and 2395.73, 3.79, 627.80, and 676.92 ppb in the cattle group, respectively. Serum Cr, Mn, Fe, Cu, and Zn levels in buffalo and cattle were close to each other.

In conclusion, Cu and Zn levels in *paries ungulae* are higher than those *solea ungulae* in both species;

however more comprehensive studies should be performed to determine trace element levels in hooves of buffaloes exclusively with claw lesions.

#### Conflict of interest

The authors declare that they have no conflict of interest.

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