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Investigation of Heavy Metal Levels in Blood of Anatolian Water Buffalo (*Bubalus bubalis*) Raised in Bitlis Province

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In this study, which aims to determine the levels of heavy metals, in the blood of 20 Anatolian buffaloes (Bubalus bubalis) aged 12-18 months, raised in Bitlis province, Güroymak District and fed with ready-made feed ad-libitum in pasture + pen conditions. 3 macro minerals and 14 micro minerals were found in the blood. The levels of a total of 17 minerals were measured by inductively coupled plasma optical emission spectrometry (ICP-OES). Among the macro minerals levels were Ca 43.896±1.216 ppm, Mg 11.381±0.325 ppm, K 133.175±13.959 ppm and micro minerals levels were Al 0.710±0.090 ppm, As 0.027±0.006 ppm, Ba 0.038±0.006 ppm, Co 0.006±0.002 ppm, Cr 0.055±0.010 ppm, Cu 0.218±0.033 ppm, Fe 0.83 2±0.051 ppm, Mn 0.024±0.003 ppm, Mo 0.009±0.002 ppm, Se 0.084±0.009 ppm, Sr 0.050±0.004 ppm, Ti 0.046±0.011 ppm, V 0.058 ±0.014 ppm and Zn 0.168±0.025 ppm. Relationships between minerals are revealed by Pearson Correlation Coefficient ($p<0.05$). In addition, in this study, the ranking of macro and micro minerals and the relative values of some of these minerals (As/Se, Ca/Mg, Ca/Mn, Ca/Zn, Cu/Fe, Cu/Zn, K/Mg, Mn/Fe, Mn/ Mg, Zn/Fe) were presented.

Bitlis İli'nde Yetiştirilen Anadolu Mandalarında (*Bubalus bubalis*) Kandaki Ağır Metal Düzeylerinin Araştırılması

MAKALE BİLGİSİ	ÖZ

Araştırma Makalesi

Geliş: 09.11.2023
Kabul: 19.12.2023

Ağır metal düzeylerinin belirlenmesinin amaçlandığı bu çalışmada Bitlis ili, Güroymak, İlçesi'nde yetiştirilen ve mera+ağıl koşularında ad-libitum olarak hazır yemle beslenen Anadolu mandalarından (Bubalus bubalis) 12-18 aylık yaştaki 20 malağının kanlarında, 3'ü makro mineral ve 14'ü mikro mineral olmak üzere toplam 17 mineralin düzeyi indüktif eşleşmiş plazma optik emisyon spektrometresi (ICP-OES) ile ölçülmüştür Makro minerallerden Ca 43.896±1.216 ppm, Mg

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Anahtar Kelimeler

Ad-libitum besleme
Anadolu mandası
Kan mineralleri
Ağır metaller
ICP-OES

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11.381±0.325 ppm ve K 133.175±13.959 ppm iken; mikro minerallerden Al 0.710±0.090 ppm, As 0.027±0.006 ppm, Ba 0.038±0.006 ppm, Co 0.006±0.002 ppm, Cr 0.055±0.010 ppm, Cu 0.218±0.033 ppm, Fe 0.832±0.051 ppm, Mn 0.024±0.003 ppm, Mo 0.009±0.002 ppm, Se 0.084±0.009 ppm, Sr 0.050±0.004 ppm, Ti 0.046±0.011 ppm, V 0.058±0.014 ppm ve Zn 0.168±0.025 ppm düzeyinde olduğu belirlenmiştir. Mineraller arasındaki ilişkiler Pearson Korelasyon Katsayısı ($p<0.05$) ile ortaya konulmuştur. Ayrıca bu çalışmada makro ve mikro minerallerin sıralaması ve bu minerallerden bazılarının oransal değerleri de (As/Se, Ca/Mg, Ca/Mn, Ca/Zn, Cu/Fe, Cu/Zn, K/Mg, Mn/Fe, Mn/ Mg, Zn/Fe) sunulmuştur.

Introduction

The buffalo is a farm animal that is bred mainly on the Asian continent. On this continent, India (56.4%) is the country with the highest presence of buffalo wealth in the world. While Pakistan (18.8%) and China (11.7%) took their places in the ranking, they actually undertook a large part of buffalo breeding in the Asian continent. When we look at the European continent, although Italy is the most common buffalo breeding country, it has only 0.2% of the global buffalo wealth. On the other hand, the presence of buffalo in Turkey (0.08%) ranked 19th in the world (Sarıözkan, 2011). In 2016, India ranked 1st with the number of 105 million buffaloes, while Turkey ranked 11th in the world with 134 thousand buffaloes (Anonymous, 2016).

Turkey is a model country known for its intensive buffalo breeding. It has a significant potential with the buffalo population scattered throughout its territory in 7 geographical regions. According to TUIK (2021) data, Samsun (22015 heads), Diyarbakır (2026 heads), Istanbul (15864 heads) and Bitlis (11250 heads) were the leading provinces in terms of buffalo breeding. According to 2020 data, Güroymak district of Bitlis province, which ranks 4th, had the highest buffalo presence (92.8%) in the province with 9809 head of livestock (TUIK, 2020). The advantage of Bitlis in terms of buffalo farming is that it has rich water resources. Even in the macro temperature of Eastern Anatolia, which reaches -30 degrees in winter, the hot spring waters of Güroymak, which reach 40 degrees, were expressed as a perfect fit for buffaloes (Anonymous, 2018). Nemrut Crater Lake and Warm Lake, which are located in the caldera of Nemrut volcano and whose waters are sweet and cold, are among the habitats where buffaloes love to spend time (Anonymous, 2023a). Güroymak plain, where buffalo breeding is intensive, is the continuation of Rahva plain and muş plain. The largest plateau of Bitlis is the Duap plateau, which is also located within the borders of Güroymak district (Anonymous, 2023a). It is also very important to know the healthy blood reference values of these animals, which are so widely breeded.

Like most animal species (McCaughan, 1992, Mc Dowell, 1992), buffaloes require a variety of macro and micro minerals (elements) for health and yield. Some of these minerals are: Calcium (Ca), Potassium (K), Magnesium (Mg), Aluminum (Al), Arsenic (As), Barium (Ba), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Selenium (Se), Strontium (Sr), Titanium (Ti), Vanadium (V) and Zinc (Zn). Although minerals are present in low concentrations in the organism, they participate in the structure of many tissues and act as cofactors of various enzymes (Spears, 1996, Johnson and Socha, 1998).

In the absence or excess of minerals, significant dysfunctions are formed in the organism. Particular emphasis is placed on 15 minerals that are essential for buffaloes in the organism (Fiest, 1999). These minerals are Calcium (Ca), Chlorine (Cl), Cobalt (Co), Copper (Cu), Iron (Fe), Iodine (I), Potassium (K), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Sodium (Na), Phosphorus (P), Sulfur (S), Selenium (Se) and Zinc (Zn) (Bülbül, 2010). Deficiency or excess of these minerals may adversely affect reproduction, fertility and immune systems (Mc Dowell, 1992), physiological and/or metabolic problems may occur. Since the early 1980s, some trace elements, which are considered as a subgroup within the microelement's family, have started to be called "ultra-trace elements" (Çelik and Okuyucu, 2005). At the same time, these minerals, which are from the group of minerals and are harmful to the organism, are called "heavy metals". The most common heavy metals, the amount of which is measured through blood tests; Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb). Less commonly tested heavy metals are Aluminum (Al), Copper (Cu), Thallium (Ta) and Zinc (Zn) (Anonymous, 2023b). Evaluation of the biochemical parameters of animals is important for clinical approaches (Groff and Zinkl, 1999) and it is also important to know the reference values of the region where the animals are raised. This study aimed to reveal the reference values of some minerals in the blood of healthy buffaloes and the relationship between them.

Materials and Methods

In the decision of Van Yuzuncu Yil University Animal Research Local Ethics Committee, in the Approval Certificate dated 31.01.2023 and numbered 2023/03-01; It was stated that "Animal Research Ethics Committee Approval is not required for the relevant research project."

Ecosystem

The alive material of the study consisted of buffaloes grown in the Güroymak district of Bitlis. Güroymak is at 38° 34' 35.1660" North and 42° 1' 14.2248" East gps coordinates.

Animal material

The alive material of the study consisted of 20 male buffalo calves, which were the offspring of Anatolian buffaloes (*Bubalus bubalis*) in the Mediterranean buffalo group aged 12-18 months, which were raised by the public. Internal-external parasite control was carried out on the animals and routine vaccinations were applied. The animals were fed ad-libitum with ready-made feed consisting of 70% concentrate feed and 30% roughage containing an average of 13-15% protein and 2600 Kcal ME in a semi-intensive system (pasture + pen runs) fattening enterprise.

Blood collection

Before the procedure, the animals were fasted for one night (12 hours). The next morning, after the necessary containment measures were applied to the buffaloes, blood samples were taken from V. jugularis (Kelly, 1984) with non-ionized sterile 10 cc syringes and transferred to gel tubes. The samples brought to the laboratory by cold chain were centrifuged at room temperature (68 to 72°F / 20 to 22°C) at 3000 rpm for 10 minutes to separate the serum portions. Serums were transferred to 1.5 cc polyethylene tubes using a clean pipette tip for each sample. These tubes were stored in a -20°C freezer until blood analysis was performed (Delves and Camphell, 1988).

Laboratory analysis

The tubes containing the frozen serums were left alone at room temperature to thaw completely. Before the serums were sent to the laboratory, they were transferred to dry tubes and diluted in certain proportions. For this purpose, Triton X-100 (Merck Triton X-100 1.08643) solution was diluted to 1% with deionized water, and serum samples were diluted 10 times with this solution and made ready for heavy metal reading (Dündar et al., 2018). Triton X-100 reagent is frequently used in micromineral analysis of biological fluids in living organisms. This reagent increases the fluidity of fluids such as blood and reduces the heterogeneity that can result from the dilution process.

Today, ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometry), which is used especially in micro mineral analysis, is among the most popular techniques. In this study, the analyses of macro minerals (Ca, K, Mg) and micro minerals (Al, As, Ba, Co, Cr, Cu, Fe, Mn, Mo, Se, Sr, Ti, V, Zn) were carried out at Van Yuzuncu Yil University, Science Application and Research Center. For this, Thermo Scientific brand, CAP6300DOU model device was used. The determination limit of the minerals is 1 μg/L and the confidence interval of the analysis results is 99%. The results of the analysis were evaluated as the average of 2 measurements.

Statistical Analysis

SAS 9.4 (2014) package program was used for the statistical evaluation of the data obtained as a result of the research, Proc means were used for introductory statistics and Porc t test procedures were used for the comparison of groups. The relationships between minerals were revealed by Pearson Correlation Coefficient ($p<0.05$). In addition, the ranking of macro and micro minerals (Patkowska-Sokola et al., 2009) and the proportional values of some of these minerals (As/Se, Ca/Mn, Ca/Mg, Ca/Zn, Cu/Fe, Cu/Zn, Mn/Fe, Mn/Mg, K/Mg, Zn/Fe) are also presented in this study (Jacobson et al., 1971; Mortimer et al.1999). In the interpretation of the literature in the discussion section, the following unit translations were applied.

1 ppm= 1 mg/L = 1000 μg/L= 0.1 mg/dL= 1 mg/kg
1 mmol/L= 1000 μmol/L
1 μg/100 ml = 0.001 ppm = 1 ng/g

Fe:	1 mg/L = 0.0250 mmol/L	(Anonymous, 2023c)
	1 μmol/L = 5.5865 μg/dL	(Anonymous, 2023d)
K:	1 mg/L = 0.0256 mmol/L	(Demir et al., 2020)
Cu:	1 mg/dl = 160 μmol/L = 0.16 mmol/L	(Anonymous, 2023d, 2023e)
Zn:	1 μmol/L = 6.538 μg/dl	(Anonymous, 2023f)
Mn:	1 *mEq/L* = 0.0364 *mg/L*	*(*Anonymous, 2023g)

Results

Each mineral and the selected wavelengths (ƛ) for that mineral were presented in Table 1. The concentration values used to plot the six-point calibration curve at these wavelengths were 0.000, 0.500, 1.000, 3.000, 5.000, 7.000 µg/L. An example Ca graph was presented in Figure 1.

Table 1. Mineral wavelengths (ƛ)
Tablo 1.Mineral dalga boyu (ƛ)

Mineral	Mineral subgroup	Mineral name	Mineral symbol	Mineral wavelength (λ): nm
Macro		Calcium	Ca	393.366
		Potassium	K	766.490
		Magnesium	Mg	279.553
Micro	Micro	Cobalt	Co	228.616
		Chromium	Cr	283.563
		Copper	Cu	324.754
		Iron	Fe	259.940
		Manganese	Mn	257.610
		Molybdenum	Mo	202.030
		Selenium	Se	196.090
		Zinc	Zn	213.856
	Ultra-trace	Aluminium	Al	167.079
		Arsenic	As	167.079
		Barium	Ba	455.403
		Strontium	Sr	407.771
		Titanium	Ti	334.941
		Vanadium	V	309.311

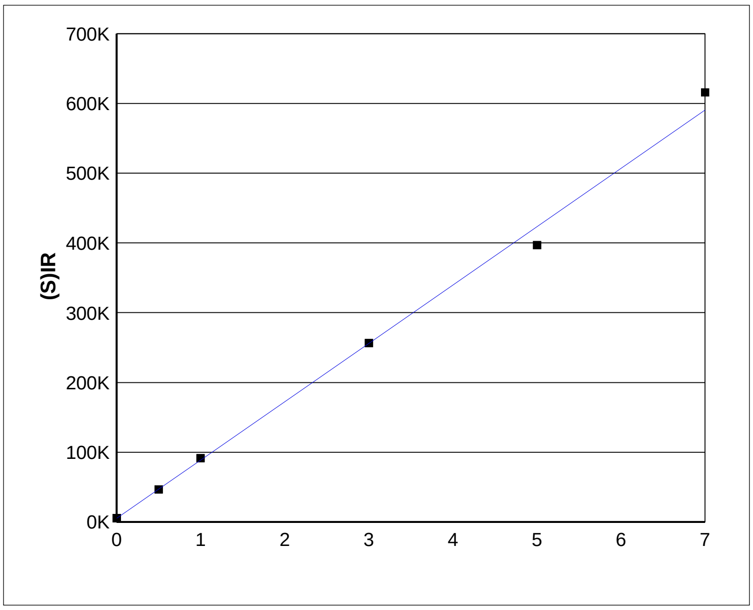

Figure 1. Calibration graph of the element Ca measured at a wavelength of 393.366 nm
Figure 1. 393.366 nm dalga boyunda ölçülen Ca elementinin kalibrasyon grafiği

Mean concentration±standard error (Mean±SE) and minimum-maximum values of serum macro and micro mineral values of Anatolian malaks were presented in Table 2.

Table 2. Serum mineral values of Anatolian buffalo calves (ppm)
Tablo 2. Anadolu malaklarının serum mineral değerleri (ppm)

Mineral	Mineral subgroup	Mineral symbol	Mean ± SE	Minimum-Maximum	Median
Macro		Ca	43.896±1.216	30.970-51.824	44.353
		K	133.175±13.959	61.834-315.904	111.335
		Mg	11.381±0.325	8.448-14.105	11.677
Micro	Micro	Co	0.006±0.002	0.001-0.030	0.005
		Cr	0.055±0.010	0.004-0.094	0.055
		Cu	0.218±0.033	0.016-0.624	0.188
		Fe	0.832±0.051	0.448-1.427	0.811
		Mn	0.024±0.003	0.005-0.041	0.023
		Mo	0.009±0.002	0.001-0.029	0.009
		Se	0.084±0.009	0.017-0.158	0.084
		Zn	0.168±0.025	0.026-0.403	0.138
	Ultra-trace	Al	0.710±0.090	0.118-1.416	0.621
		As	0.027±0.006	0.006-0.105	0.017
		Ba	0.038±0.006	0.002-0.081	0.036
		Sr	0.050±0.004	0.028-0.091	0.048
		Ti	0.046±0.011	0.001-0.118	0.032
		V	0.058±0.014	0.000-0.194	0.044

The ranking of minerals according to the literature (Patkowska-Sokola et al., 2009) was presented in Table 3.

Table 3. Ranking of the mean (x) of serum mineral values of Anatolian buffalo calves
Tablo 3. Anadolu manda buzağılarının serum mineral değerlerinin ortalama (x) sıralaması

Makro minerals			Mikro minerals													
K>	Ca>	Mg>	Fe>	Al>	Cu>	Zn>	Se>	V>	Cr>	Sr>	Ti>	Ba>	As>	Mn>	Mo>	Co

The values of serum macro and micro minerals of Anatolian buffalo calves regarding Pearson correlation coefficients were presented in Table 4, Table 5 and Table 6, respectively.

Table 4. Pearson Correlation Coefficients of serum mineral values of Anatolian buffalo calves-I
Tablo 4. Anadolu malaklarının serum mineral değerlerinin Pearson Korelasyon Katsayıları-I

	Al	As	Ba	Ca	Co	Cr	Cu	Fe	K
Al	1.000	-0.131	0.242	-0.401	-0.352	-0.622	-0.324	-0.230	-0.184
As		1.000	0.159	-0.114	0.010	0.365	0.722*	0.247	0.720**
Ba			1.000	0.215	-0.276	0.101	0.227	-0.540	0.044
Ca				1.000	0.524*	0.060	0.173	0.240	0.097
Co					1.000	0.147	0.120	0.698*	-0.122
Cr						1.000	0.039	-0.025	0.641
Cu							1.000	0.345	0.421
Fe								1.000	0.228
K									1.000

* $p < 0.05$, ** $p < 0.01$, statistically significant coefficient of correlation

Table 5. Pearson Correlation Coefficients of serum mineral values of Anatolian buffalo calves-II
Tablo 5. Anadolu malaklarının serum mineral değerlerinin Pearson Korelasyon Katsayıları-II

	Mg	Mn	Mo	Se	Sr	Ti	V	Zn
Mg	1.000	-0.019	0.302	0.235	-0.070	-0.053	0.043	-0.381
Mn		1.000	-0.495	-0.477	-0.174	-0.686*	-0.287	-0.356
Mo			1.000	0.607*	0.270	-0.294	0.194	0.472
Se				1.000	0.033	0.168	0.083	0.499*
Sr					1.000	0.090	-0.302	0.031
Ti						1.000	0.305	-0.027
V							1.000	0.118
Zn								1.000

* $p < 0.05$, statistically significant coefficient of correlation

Table 6.Pearson Correlation Coefficients of serum mineral values of Anatolian buffalo calves-III
Tablo 6. Anadolu malaklarının serum mineral değerlerinin Pearson Korelasyon Katsayıları-III

	Mg	Mn	Mo	Se	Sr	Ti	V	Zn
Al	-0.233	0.161	0.007	-0.031	-0.017	-0.074	-0.321	0.232
As	-0.203	-0.066	0.007	0.269	0.055	-0.311	-0.013	0.327
Ba	0.081	0.617*	-0.671*	-0.404	-0.409	0.007	-0.626*	-0.636*
Ca	0.800	0.044	0.082	-0.025	-0.290	0.057	0.103	-0.497*
Co	0.637**	-0.304	0.807**	0.438	0.040	-0.323	0.228	0.203
Cr	-0.036	0.122	0.147	0.020	0.484	0.436	-0.046	-0.406
Cu	-0.114	0.373	-0.216	0.019	-0.387	-0.504	0.269	0.071
Fe	0.183	-0.206	0.622*	0.097	0.124	-0.457	0.269	0.347
K	-0.015	-0.083	-0.010	0.083	0.035	0.002	-0.026	0.189

* $p < 0.05$, ** $p < 0.01$, statistically significant coefficient of correlation

Table 7. Proportional relationships of serum mineral values of Anatolian buffalo calves
Tablo 7. Anadolu malaklarının serum mineral değerleri arasındaki orantısal ilişkiler

Mineral Ratios	Proportional Values	Interaction Direction*	Interaction Mode**	Literature
Al/ Co	118.333	Belirtilmemiş	-	Mortimer et al. 1999
Al/ Mg	0.062	Belirtilmemiş	-	Mortimer et al. 1999
As/ Se	0.321	→	-	Jacobson et al., 1971; Mortimer et al. 1999
Ca/ Mg	3.857	→	Antagonism	Jacobson et al., 1971
Ca/ Mn	1.829	→ ←	Antagonism	Jacobson et al., 1971; Mortimer et al. 1999
Ca/ Zn	261.286	→	Antagonism	Jacobson et al., 1971; Mortimer et al. 1999
Co/ Fe	0.667	Belirtilmemiş	-	Mortimer et al. 1999
Cu/ Fe	0.262	→	Antagonism	Jacobson et al., 1971; Mortimer et al. 1999
Cu/ Mo	24.222	Belirtilmemiş	Stimulation	Mortimer et al. 1999
Cu/ Zn	1.298	→	-	Jacobson et al., 1971; Mortimer et al. 1999
K/ Mg	11.702	→	Antagonism	Jacobson et al., 1971
Mn/ Fe	0.029	→	Antagonism	Jacobson et al., 1971; Mortimer et al. 1999
Mn/ Mg	0.002	→	-	Jacobson et al., 1971; Mortimer et al. 1999
Zn/ Fe	0.202	→	Antagonism	Jacobson et al., 1971; Mortimer et al. 1999

* "Direction of interaction of minerals" reported by Jacobson et al. (1971)
** "Mode of interaction of minerals" reported by Georgievskii (1982)

There are also close relationships between minerals in terms of the use of minerals by animals (Okuyan, 1997). Based on this, according to the literature (Jacobson et al., 1971; Mortimer et al. 1999) the proportional relationships of minerals to each other were presented in Table 7. The direction of interaction (Jacobson et al., 1971) is indicated by arrows, and the mode of interaction (Georgievskii, 1982) was added to the same table.

Discussion and Conclusion

Macro minerals

Ca: According to the results of a study conducted by Hagawane et al. (2009), the mean Ca values of lactated healthy buffaloes was found to be as 11.21±0.19 mg/dl. In a study conducted by Chhabra et al. (2015) on buffaloes, the Ca values of blood plasma in summer and winter months were measured as 8.92±0.26 mg/dl and 9.74±0.30 mg/dl, respectively. In a study investigating the effect of season on blood minerals in Iraqi buffaloes (Kadhim and Al-Dulaimi, 2015), the Ca values were recorded in autumn, winter, spring and summer were reported as 2.39, 2.41, 2.22, 2.51 mmol/L, respectively. According to the results of a study conducted by Runa et al. (2022) on Murrah buffaloes, the average blood Ca values of male and female young calves was 8.78±0.57 mg/dl; The Ca level of male animals was determined as 8.30±0.30 mg/dl. In this study, it was determined that the mean blood Ca value of the buffalo calves used lower than the values reported in the aforementioned literatures.

K: Khadjeh et al. (2005) found the serum K level of Iranian Khuzestan male buffaloes to be as 5.35±0.98 mmol/L. Souza et al. (2019) determined the blood K level as 4.89±0.46 mmol/L on the 30th day after birth in buffaloes with multiple births. According to the results of a study conducted by Runa et al. (2022) on Murrah buffaloes, the mean blood K values of male and female young buffalo calves was 5.23±0.08 mmol/L; the K level of male animals was determined as 5.25±0.12 mmol/L. In this study, it was determined that the mean blood K value of the buffalo calves used lower than the values reported in the aforementioned literatures.

Mg: Hagawane et al. (2009) determined the blood Mg level of lactating buffaloes raised in the city of Parbhani as 3.50±0.17 mg/dl. According to Shoushtari et al. (2014), blood Mg levels of female water buffaloes was 4.27±0.21 mg/dl before puberty and 5.71±0.48 mg/dl at estrus. Dhamsaniya et al. (2016) reported the serum Mg level of the control group as 3.57±0.12 mg/dl in Surti buffaloes. In this study, it was determined that the mean blood Mg value of the buffalo calves used lower than the values reported in all the literatures.

Micro minerals

Al: As a result of the study conducted by Gaafar (2008) in Egypt, it was stated that the plasma Al average of buffaloes was 25.30 μg/100 ml, while the plasma Al average of cattle was 20.61 μg/100 ml. It was determined that the mean blood Al value of the buffalo calves used in this study considerably higher than the values reported by Gaafar (2008) about both buffaloes and cattles.

As: According to Khan et al. (2020), average As levels in Niliravi buffaloes were between 0.023-0.069 mg/L in winter; it ranged between 0.020-0.064 mg/L in summer. Puis (1981) reported that the toxic limit for As in buffaloes was 1 ppm. Mean blood As value of buffalo calves used in this study were determined by Khan et al. (2020) was slightly higher than the value reported. However, it was found to be well below the toxic limit reported by Puis (1981).

Ba: According to Luna et al. (2019), the plasma value of the cattle whose Ba levels were investigated in the blood as 13.6±1.05 μg/L, while the serum value was 13.5±1.20 μg/L. Hussein et al. (2022) determined the lactating serum Ba level as 16 μg/L. In this study, it was determined that the mean blood Ba value of the buffalo calves used higher than the values of the cattle reported in the aforementioned literatures.

Co: Khan et al. (2018) reported the mean plasma Co of buffaloes with potentially toxic metal accumulations in the blood as 0.19-0.21 mg/L. Dhamsaniya et al. (2016) measured the serum Co level of the control group as 1.52±0.04 mg/dl in Surti buffaloes. Mc Dowell (2003) reported the critical level for Co as 0.25 mg/L. In this study, it was determined that the mean blood Co value of the buffalo calves used considerably lower than the values reported in the aforementioned literatures.

Cu: The blood Cu content of lactating buffaloes was determined as 1.21 mg/kg by Sharma and Prasad (1982). Yadav et al. (1998) reported that the average Cu content of the blood serum of buffaloes in the Rewari region as 0.47 mg/kg. According to the results of the study conducted by Mandal et al. (1996) on dairy buffaloes in Mohindergarh, the average Cu content of the blood serum was 0.67 mg/kg. Gaafar (2008) stated that the average plasma Cu as 112.30 μg/100 ml in the study conducted on buffaloes in Egypt. In a study conducted by Kadhim and Al-Dulaimi (2015), the Cu values of Iraqi buffaloes recorded in autumn, winter, spring and summer were reported as 68.63±11.35 mmol/L, 67±72.69 mmol/L, 64.15±5.22 mmol/L and 54.56±2.68 mmol/L, respectively. In the study conducted by Chhabra et al. (2015) on buffaloes, the Cu values of blood plasma in summer and winter months were measured as 12.68±0.35 μmol/L and 12.68±1.45 μmol/L, respectively. It was determined that the mean blood Cu value of the buffalo calves used in this study significantly lower than the values reported by Sharma and Prasad (1982), Mandal et al. (1996) Yadav et al. (1998) and Kadhim and Al-Dulaimi (2015), but higher than the values reported by Gaafar (2008) and Chhabra et al. (2015).

Cr: According to Luna et al. (2019), the plasma value of the cattle whose investigated Cr levels in blood was 5.72±0.49 µg/L, while the serum value as 5.06±0.44 µg/L. Hussein et al. (2022) found the serum Cr level in lactating cows to be as 83 µg/L. In this study, it was determined that the mean blood Cr value of the buffalo calves used considerably higher than the values of the cattle reported in the aforementioned literatures.

Fe: In a study conducted by Georgievskii et al. (1982), it was reported that the average serum Fe level of buffaloes fed with feed and roughage with very high Fe content varied between 1.1-2.5 ppm. Sharma et al., (1991) found that the serum Fe levels of animals fed Fe content under different mineral levels as 1.47, 1.64 and 1.69 mg/kg. In the study conducted by Chhabra et al. (2015) on buffaloes, the Fe values of blood plasma in summer and winter months were measured as 47.53±2.07 µmol/L and 51.93±5.92 µmol/L, respectively. In this study, it was determined that the mean blood Fe value of the buffalo calves used lower than the values reported in the literatures.

Mn: Sharma et al. (1991) observed that the serum Mn content of animals treated at different mineral levels as 0.59 and 0.65 mg/kg. In the study conducted by Chhabra et al. (2015) on buffaloes, the Mn values of blood plasma in summer and winter months were measured as 0.82±0.04 µmol/L and 0.80±0.09 µmol/L, respectively. According to the results of a study conducted by Runa et al. (2022) on Murrah buffaloes, the mean blood Mg values of male and female young buffalo calves was 2.04±0.04 mEq/L while the Mg level of male animals was determined as 2.03±0.06 mEq/L. The mean blood Mn value of the buffalo calves used in this study was significantly lower than the values reported by Sharma et al. (1991) while higher than the values reported by Chhabra et al. (2015) and Runa et al. (2022).

Mo: According to Khan et al. (2020), the average Mo levels in buffaloes were between 2.61 mg/L and 2.70 mg/L in winter while it ranged between 1.82 mg/L and 2.26 mg/L in summer. Clawson et al. (1972) reported the toxic level of Mo in buffaloes as 5 ppm. In this study, it was determined that the mean blood Mo value of the buffalo calves used considerably lower than the values reported in the aforementioned literatures.

Se: Khan et al. (2018) measured the Se level of buffalo plasma as 0.02 mg/kg in their study in Jhang. According to Khan et al. (2020), the average Se levels in all buffaloes were between 0.017 and 0.028 mg/L in winter while it ranged from 0.012 to 0.024 mg/L in summer. Mc Dowell (1997) reported that the critical limit for serum Se concentration as 0.03 mg/L. In this study, it was determined that the mean blood Se value of the buffalo calves used higher than the values reported in the literatures.

Sr: According to Luna et al. (2019), the plasma value of the cattle whose Sr levels was 118±5 µg/L, while the serum value measured as 117±5 µg/L. In this study, it was determined that the mean blood Sr value of the buffalo calves used lower than the values of the cattle reported in the literature.

Ti: According to the results of a study conducted by Sarmiento-González et al. (2009), in which Ti levels in the organs and blood of rats were investigated, the blood Ti level was determined as 2.36±0.48 ng/g. It was determined that the mean blood Ti value of the buffalo calves used in this study was significantly higher than the value of rats reported in the literature.

V: Cornelis et al. (1981) stated that the normal level of V in the blood serum should be in the range of 0.016-0.939 ng/ml, and if it is above 1.0 ng/ml, it is likely that exposure to V is possible. In a study examining the effects of inorganic vanadium supplementation on antioxidant enzymes, immune status and hemato-biochemical properties of growing hybrid calves, blood V level of male Karan Fries calves (Tharparkar × Holstein Friesian) was reported as 0.97 mg/kg by Pal et al. (2018). Mean blood V value of the buffalo calves used in this study were within the physiological limits reported by Cornelis et al. (1981), while it was found to be considerably lower than the calf value reported by Pal et al. (2018).

Zn: Yadav et al. (1998) reported that the mean serum Zn content of buffaloes raised in the Rewari region was 2.76 mg/kg. As reported by Mandal et al. (1996), the average Zn content in blood serum in Milch buffaloes in Mohindergarh district was 2.80 mg/kg (Baloda and Promila, 2018). In the study conducted by Chhabra et al. (2015) on buffaloes, the Zn values of blood plasma in summer and winter months were measured as 10.90±0.45 µmol/l and 22.20±2.53 µmol/L, respectively. According to the results of a study conducted by Runa et al. (2022) on Murrah buffaloes, the mean blood Zn value of male and female young buffalo calves was 115.45±3.88 mg/dl while the Zn level of male animals was determined as 116.74±5.85 mg/dl. The blood Zn values of the buffalo calves used in this study was considerably lower than the values reported by Yadav et al. (1998) and Baloda and Promila (2018) while it was determined that it was considerably higher than the value reported by Runa et al. (2022).

Of the analyzed minerals, correlation between As-Cu, Ba-Mn, Ca-Co, Co-Fe, Mo-Se, Se-Zn were positive at $p<0.05$ level while corelation between Ba-Mo, Ba-V, Ba-Zn, Ca-Zn, Mn-Ti were negative at $p<0.05$ level. Also, a positive correlation was found between As-K, Co-Mg and Co-Mo at $p<0.01$ level. Additionally, the correlations between Ca-Zn, with a proportional value of 261.286, and Co-Fe, with a proportional value of 0.667, supported the antagonistic effect reported by Georgievskii (1982).

The most common way for heavy metals to enter metabolism is drinking water. Heavy metals taken into the body through inhalation can also cause diseases, especially in the lungs, liver and kidneys (Ayaz and Yurttagül, 2012). This situation in living things causes similar symptoms. However, it was clearly seen in the literature review that there are not many studies on heavy metal levels to be used to evaluate the situation in buffaloes. There is very little literature on blood levels of Al, Ti and V, especially in buffaloes. For this reason, there is a need for comprehensive mineral studies, especially Al, Ti and V, in which comparisons will be made with buffaloes and cattle and thus species-specific reference values will be revealed.

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