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Research Article

ELEMENT CONCENTRATIONS IN HORSE BLOOD AND RELATION BETWEEN AGE, GENDER, BREED, HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

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ABSTRACT. The aim of this study was to determine the concentrations of macro and essential and non-essential trace elements and correlation between biochemical and hematological parameters, age, gender, and breed in horses. The whole blood samples of 20 horses were collected from İstanbul (n=11) and Tekirdağ (n=9) cities. Macro (Ca, K, Mg, and P), trace essential (Cu, Fe, Se, and Zn) and non-essential (Al, Cd, Hg, and Pb) element concentrations were determined by ICP-OES. In addition, hematological and biochemical parameters levels were determined. Ca, K and Mg concentrations were within the normal reference range, P concentrations were higher than the reference in horses. Blood concentrations were sorted as K>Ca>P>Mg for macro (mg L^{-1}) elements, were Fe>Cu> Zn>Se for essential trace (µg L^{-1}) elements and as Al>Pb>Cd>Hg for non-essential trace (µg L^{-1}) elements. The negative and positive correlation between the hematological and biochemistry parameter levels and element levels were significant ($p < 0.05$, $p < 0.01$) between Mg-TRIG, Mg-CHOL, Zn-TB, Al-LDL, and Hg-CREA.

Keywords: *Horse, essential elements, non-essential elements, biochemistry, hematology.*

INTRODUCTION

Elements are classified as; macro (calcium [Ca], potassium [K], magnesium [Mg], and phosphorus [P] etc.) and micro/trace (aluminum [Al], arsenic [As], cadmium [Cd], copper [Cu], iron [Fe], mercury [Hg], lead [Pb], selenium [Se], and zinc [Zn], etc.) according to body (in human and animals) concentrations, essential (Cu, Fe, Zn, etc.) and non-essential (As, Cd, and Pb, etc.) according to their biological requirements, or metal (Al, Cd, Cu, Fe, Hg, Pb, and Zn, etc.), non-metal (astatine [At], bromine [Br], carbon [C], chlorine [Cl], [F], iodine [I], nitrogen [N], oxygen [O], sulfur [S], and P etc.), and metalloid (As, boron [B], germanium [Ge], silicon [Si], and tellurium [Te]) according to their chemical properties [1, 2, 3]. Deficiencies of essential macro and/or micro/trace negatively affect optimal health status [4]. On the other hand, some of the trace elements (Al, As, Cd, chromium [Cr], Cu, Fe, Hg, manganese [Mn], Ni, Pb, tin [Sn], and Zn) are potentially toxic (PTEs) (in majority by as a contaminant in food/feeds) above tolerable levels even though some are necessary for biological processes in animals and humans and animals

[4, 5, 6]. Due to their toxicity, increasing use and resistance to biological degradation, PTEs are among the most frequently monitored toxicants in both environmental, food/feeds and biological samples [1, 6, 7]

Al is a non-essential trace element, and although the information on the toxic effects and dose in horses is insufficient, generally has adverse effects on the growth and essential element used in animals [4]. The nervous system is the major target of Al while also accumulates in the brain, bone, kidneys, liver, and hematopoietic tissues. Al can cross the blood-brain barrier and leads to neurologic effects (ataxia, paralysis, and behavioral changes such as memory loss) and cognitive impairment; anemia, bone abnormalities, osteoarthritis, and myocardial infarction [8]. Ca is an essential macromineral of skeletal, nervous tissues, skeletal and cardiac muscle contraction, blood clotting, and is a component of milk and exposure in high single doses results in hypercalcemia. Long-term overexposure generally does not cause toxicity in animals with normal calcium hemostasis [8]. Cd is a non-essential trace element, absorbed within a rate of 1-5% of intake and the target organs of accumulation are those primarily containing metallothioneins, like the kidney and liver [4, 8, 9]. The biological half-life varies from months to years depending on the species. Leads to renal lesions, liver, testis, myocardial, and neurological damage, anemia, infertility, osteoporosis, osteomalacia, hypertension, and death [8, 9, 10]. In addition, Cd is defined as a mutagen, carcinogen, teratogen and endocrine disruptor [8, 9]. Cu is an essential trace element as a component of enzymes, yet excessive exposure can cause gastroenteritis, hemolysis, kidney and liver damage, and death in horses [4]. Fe is an essential trace element and mostly found as bound to plasma proteins in Fe^{2+} form while the rest is involved in the structure of enzymes (peroxidase, catalase, and cytochrome C) [11]. Above tolerable levels, as a result of the insufficient binding capacity of the tissues, free Fe levels increase and leads to peroxidative damage in tissues (especially the liver) [4]. While the most affected system is cardiovascular, Fe can damage the digestive system, liver, and brain [11]. Pb is a non-essential trace element and has been known to be toxic since B.C. [4]. Pb absorption can increase with Ca, Fe, Zn or vitamin D deficiency. Oral acute and chronic toxic dose (mg kg^{-1}) ranges for horse are indicated as 500-750 and 2.4-7 respectively. Clinical symptoms in horses are seizures and death in acute cases, depression, weight loss, dysphagia, dysphonia, laryngeal paralysis, facial nerve deficits, aspiration pneumonia, seizures, and death in chronic cases [11]. Mg is an essential macromineral that is a cofactor for enzymatic reactions. The concentrations above tolerable limits name hypermagnesemia lead to sedation, diarrhea, inappetence, and so reduced performance [4]. Hg is a non-essential trace element and toxicity is probably result from binding to proteins or sulfhydryl containing enzymes. Kidney damage, anemia, abnormal gait, stomatitis, salivation, pharyngitis, gastric disorders, vomiting, diarrhea, dehydration, tremors, and shock are general signs of acute and chronic intoxications [4, 11]. P is an essential macromineral and play a part in structure of bone and soft tissues. In horses, diseases called fibrous osteodystrophy and bran disease (or big head disease) occur due to high P secretion from the bones as a result of low serum calcium level or high P intake in the diet compared to Ca. K is an essential macromineral that plays role in the regulation of biological processes and as an activator or cofactor in many enzymatic reactions. The big part of K (98%) is founded in cells. The concentrations above tolerable limits name hyperkalemia lead to arrhythmias that can result in death. Also, exposure to high levels in the diet can cause muscle tremors and excessive salivation (a hereditary genetic defect, hyperkalemic periodic paralysis disease) in horses. Se is an essential trace element as a

component of enzymes and proteins [4]. The hoof lesions (lameness, hoof overgrowth, hoof deformation, and rings below the coronary band of the hoof wall), hair loss of mane and tail (causing the bobtail view) without neurological disorders are the main signs of chronic intoxication in horses named alkali disease [4, 11]. Zn is essential for many metalloenzyme structures. The absorbed Zn rapidly disturbs and accumulates in target tissues like liver, muscle, and bone. The Zn metabolism in monogastric animals can alter due to its electron configuration and various factors. Chronic Zn exposure limits the absorption of Fe, Cu, Ca and leads to osteochondrosis in horses. Vomiting, diarrhea, depression, icterus, pale mucous membranes, tachycardia, hemoglobinemia, hemoglobinuria, pathologically intravascular hemolysis, anemia, leukocytosis, and neutrophilia also can be clinically observed. Due to the complex metabolism of Zn by virtue of interactions with other elements, the toxic dose for most species has not been established [11].

The use (for determining the mineral concentration and as an indicator of metal contamination) of biological samples (whole blood, serum, plasma, hair, hoof, urine, milk, liver, kidney, etc.) has been a long-standing method for determining and monitoring the health status of humans, animals, and environment. [2, 12, 13, 14, 15, 16, 17, 18] Whole blood metal levels reflect acute or chronic exposure [18]. Among the target species, horses are exposed to metals for a long time due to their lifespan and diet [12, 19]. Therefore, the determination of metal levels in indicator tissue and organ samples from animals (horses, dog, fish, bird etc.) provides data on regional ecosystem health as well as predicting possible effects on humans and animals living in the region [12, 19, 20, 21, 22].

The aim of this study is to i) determine the macro/micro, essential/non-essential element levels in horse blood and reveal the relations between ii) biochemical and hematological parameters, iii) age, gender, and breed.

MATERIALS AND METHODS

Animal Material

A total of twenty blood samples were collected from horse in Istanbul and Tekirdağ provinces of Turkey. The samples (10 ml each) were taken for hemogram analysis and metal analysis (tubes with EDTA), for biochemical (tubes without anticoagulant). The serum (obtain by 10 min centrifuge at 3000 rpm) samples for use biochemical analysis and whole blood samples for use metal analysis were stored at -80°C. The study was conducted under permission (Tekirdağ Namık Kemal University Animal Experiments Local Ethics Committee 31.01.2020/T2020-403). To investigate relations of elements, horses were divided into age (≤5 n=7, 6-12 n=7, ≥13 n=6), gender (male n=7, female n=13), and breed groups (Arabian n=12, English n=8).

Hematological and Biochemical analysis

The levels of hematological parameter (corpuscular volume [MCV], mean corpuscular hemoglobin [MCH] amount, mean corpuscular hemoglobin concentration [MCHC], and levels of erythrocyte [RBC], hematocrit [HCT], leukocyte levels [WBC], hemoglobin [HGB], and thrombocyte [PLT]) were determined by the blood analyzer (Exigo-Eos Vet, Sweden). The levels of biochemical parameters (glucose [Gluco], Urea [URE], Creatine [CREA], total protein [TP], albumin [ALB], globulin [Glob], total bilirubin [TB],

triglyceride [Trig], cholesterol [Chol], [BUN], [AST], [ALT], [ALP], [LDL], and [GGT]) in blood were determined by the blood analyzer (Olympus, USA).

Element Analysis

1 ml (wet weight) of whole blood samples digested by microwave (CEM; Mars X press, USA) system (1200 W 100% power, 10 min ramp time, 150°C, 10 min hold time). Whole blood concentrations of Ca, K, Mg, P, Cu, Fe, Se, Zn, Al, Cd, Hg and Pb were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Spectro, Germany). Limit of quantitation (LOQ), recovery, correlation coefficients (R^2), and relative standard deviations (RSD) parameters were performed.

Statistical analysis

Parametric (one-way analysis of variance), non-parametric (Mann–Whitney U test, Kruskal-Wallis test), and Spearman correlation tests were performed with SPSS software (IBM, USA) after normality and homogeneity tests. Statistically, significance was considered at the p-values of <0.05, and <0.01.

RESULTS AND DISCUSSION

The concentrations of macro (mg L^{-1}) essential (Ca, K, Mg, and P), trace (μg L^{-1}) essential (Cu, Fe, Se, and Zn), and non-essential (Al, Cd, Hg, and Pb) elements in whole blood samples collected from horses within the scope of the study were presented in Table 1 as arithmetic and geometric mean, median, standard error, and minimum and maximum concentrations. The lowest and highest concentrations were as follows: Ca (82.81-150.80), K (180.90-159.09), Mg (22.64-28.56), P (88.26-160.30), Cu (631.60-1620.80), Fe (685.15-6372.60), Se (72.75-332.20), Zn (545.75-1823.75) for macro and trace essential elements and Al (12.50-40.39), Cd (*ND*-1.21), Hg (*ND*-1.03), and Pb (0.08-6.63) for non-essential elements. The mean concentrations sorted for macro essentials as K>Ca>P>Mg, for trace essential as Fe>Cu>Zn>Se, and for non-essentials as Al>Pb>Cd>Hg. The concentration of macro and trace essential and non-essential elements in age (≤5, 6-12, ≥13), gender (male and female), and breed (Arabian, English) groups were presented in table 2 as mean ± standard error (mean ± SD) and statistical differences indicated with different letters ($p<0.05$). Even though the differences were not statistically significant, the concentrations are higher in male horses except the Mg, Se, Zn, and Cd ($p<0.05$). Al levels in ≥13 years old horses were found to be significantly higher than other age groups ($p<0.05$). Even though the differences were not statistically significant, the concentrations are higher in English horses except the Ca, K, P, Se, Hg, and Pb ($p<0.05$).

***Table 1**. Macro essential (mg L^{-1}) and trace (µg L^{-1}) element levels in horse blood samples (n=20)*

	Macro Essential Elements				Trace Essential Elements				Trace Non-Essential Elements			
	Ca	**K**	**Mg**	**P**	**Cu**	**Fe**	**Se**	**Zn**	**Al**	**Cd**	**Hg**	**Pb**
Arithmetic mean	116.76	132.82	25.90	116.28	1037.40	2374.88	173.18	935.79	19.17	0.23	0.20	0.87
Standard error	4.17	3.37	0.40	4.33	45.46	247.16	12.92	66.13	1.41	0.07	0.07	0.32
Median	117.76	132.82	25.92	113.34	1004.40	2166.39	163.55	848.25	17.97	0.12	0.00	0.42
Geometric Mean	115.35	132.01	25.84	114.85	1019.58	2185.24	164.75	899.97	18.41	0.00	0.00	0.49
Minimum	82.81	108.09	22.64	88.26	631.60	685.15	72.75	545.75	12.50	0.00	0.00	0.08
Maximum	150.81	159.09	28.56	160.30	1620.80	6372.60	332.20	1823.75	40.39	1.21	1.03	6.63
Referans [28, 29,48]	100-160	96-193	22-28	17-65								

***Table 2.** Essential/Non-essential macro (mg L^{-1}) and trace (µg L^{-1}) element levels by gender (male n=7, female n=13), age groups (≤5 n=7, 6-12 n=7, ≥13 n=6), and breed origin (Arabian n=12, English n=8) (mean±SE)*

	Macro Essential Elements				Trace Essential Elements				Trace Non-Essential Elements			
	Ca	**K**	**Mg**	**P**	**Cu**	**Fe**	**Se**	**Zn**	**Al**	**Cd**	**Hg**	**Pb**
Male	119.02±4.80	135.75±4.38	25.86±0.54	120.29±4.25	1041.12±55.41	2455.96±346.10	171.64±15.77	927.25±83.56	19.96±2.08	0.20±0.08	0.25±0.09	0.92±0.48
Female	112.55±8.21	127.36±4.90	25.97±0.61	108.85±9.39	1030.48±85.33	2224.30±319.36	176.04±24.20	951.64±116.47	17.69±1.12	0.28±0.12	0.10±0.10	0.76±0.26
P	*0.65*	*0.44*	*0.42*	*0.08*	*0.70*	*0.82*	*0.44*	*0.76*	*0.94*	*0.31*	*0.35*	*0.44*
≤5	115.28±5.95	129.06±6.29	25.51±0.85	122.63±8.64	936.00±65.67	1876.81±246.99	143.14±17.32	80.218±20.4.13	16.91[a]±1.73	0.13±0.05	0.30±0.09	0.40±0.14
6-12	118.42±8.38	130.04±6.06	26.49±0.50	144.38±5.63	1139.43±91.78	2944.42±602.36	202.78±25.95	1075.75±145.93	16.65[a]±0.75	0.35±0.16	0.10±0.03	1.51±0.87
≥13	116.53±8.44	140.44±4.51	25.66±0.74	111.09±8.53	1036.67±59.32	2291.50±213.27	173.68±18.40	928.37±229.69	24.74[b]±3.34	0.21±0.09	0.19±0.06	0.66±0.16
P	*0.96*	*0.35*	*0.57*	*0.87*	*0.18*	*0.22*	*0.16*	*0.07*	*0.02*	*0.06*	*0.09*	*0.09*
Arabian	118.33±4.74	135.68±4.34	25.78±0.55	119.08±5.89	1023.88±54.71	2223.42±203.41	170.80±15.91	932.77±74.79	18.99±2.08	0.21±0.07	0.24±0.10	0.66±0.16
English	114.38±7.97	128.53±5.31	26.07±0.61	112.10±6.40	1057.67±82.87	2602.07±552.02	176.75±23.09	940.31±128.38	19.43±1.78	0.25±0.08	0.14±0.05	1.17±0.38
P	*0.65*	*0.31*	*0.74*	*0.44*	*0.91*	*0.79*	*1.00*	*0.73*	*0.57*	*0.79*	*0.57*	*1.00*

Different letters on the same line are statistically significant ($p<0.05$).

Table 3. *Hematological parameter levels (n=20)*

Parameter	WBC*	RBC□	HGB**	HCT△	MCV#	MCH***	MCHC	RDW△	PLT*	MPV#	PCT	LYM△	MON△	NEU△	EOS△
Arithmetic mean	6.59	8.33	13.83	38.22	45.85	16.67	36.43	21.57	101.20	8.11	0.08	63.15	2.99	29.99	2.86
Standard error	0.30	0.18	0.31	0.72	0.44	0.15	0.21	0.14	7.91	0.29	0.01	2.13	0.20	2.11	0.34
Median	6.76	8.11	13.75	38.75	45.00	16.70	36.45	21.60	110.50	8.25	0.08	63.55	2.85	29.55	2.35
Geometric Mean	6.47	8.29	13.76	38.09	45.81	16.66	36.42	21.56	91.01	8.01	0.07	62.48	2.87	28.16	2.40
Minimum	4.65	7.19	11.90	32.60	43.00	15.40	35.10	20.30	15.00	6.30	0.02	43.70	1.80	7.90	0.30
Maximum	10.69	10.09	17.40	45.90	49.00	17.70	38.20	22.60	151.00	10.50	0.12	88.20	5.20	51.20	6.40
Reference [45, 46]	**5.4 - 14.2**	**6.8 - 12.9**	**11 - 19**	**32 - 53**	**37 - 59**	**12.3 - 19.7**	**31.0 - 38.6**	-	**100 - 350**	-	-	**19.8 - 58.9**	**1.4 - 10.5**	**0 - 100**	**0 - 8.7**

□10^{12}/l, #fl, △%, *10^{9}/l, **g dL^{-1}, ***pg

Table 4. *Biochemical parameter levels (n=20)*

Parameter	ALB△	ALT	TP	TRIG	UREA	LDL	HDL	I	BUN□	AST	BILT□	CHOL□	CREA□	GLUC
Arithmetic mean	3.28	8.58	7.00	28.27	31.06	7.25	56.75	3.70	14.65	294.16	2.27	91.19	1.39	85.09
Standard error	0.05	0.73	0.16	2.34	1.40	0.79	8.15	0.25	0.64	36.93	0.16	1.90	0.03	4.41
Median	3.32	8.20	6.92	26.75	30.40	7.00	53.50	3.50	14.50	228.60	2.12	90.55	1.41	88.80
Geometric Mean	3.27	8.05	6.97	25.40	30.47	0.00	45.38	3.54	14.39	269.28	2.18	90.82	1.38	79.09
Minimum	2.90	4.30	5.91	3.00	22.10	0.00	10.00	2.00	10.00	196.30	1.27	78.30	1.15	8.90
Maximum	3.59	16.50	8.47	47.40	44.20	14.00	151.00	6.00	21.00	910.10	3.57	108.50	1.63	105.80
Reference [46, 47]	**2.9 - 3.8**	**3 - 23**	**6.0 - 7.7**	**4 - 44**	-	-	-	-	**10 - 24**	**220 - 600**	**1 - 2**	**46 - 180**	**0.9 - 1.9**	**75 - 115**

□mg dl^{-1}. # U L^{-1}. △g dl^{-1}

Table 5. *Correlation between hematological parameter levels and macro and trace essential and non-essential elements levels*

	Macro Essential Elements				Trace Essential Elements				Trace Non-Essential Elements			
	Ca	**K**	**Mg**	**P**	**Cu**	**Fe**	**Se**	**Zn**	**Al**	**Cd**	**Hg**	**Pb**
RBC	.325	-.161	-.074	-.180	.060	.206	.078	.083	.048	-.088	.194	.120
MCV	-.073	-.008	.029	-.079	.205	.165	.165	.171	-.145	.201	-.304	.090
RDW	-.040	-.232	.049	.442	-.667**	-.520*	-.687**	-.657**	.011	-.669**	.713**	-.643**
HCT	.317	-.214	-.026	-.324	.292	.435	.311	.291	-.019	.105	-.012	.323
PLT	-.083	-.007	-.524*	-.320	.054	.070	.056	.008	.449*	.042	-.121	.079
MPV	.011	.178	.137	-.217	.111	.087	.152	.146	-.100	.272	.035	.215
WBC	.077	-.212	.189	.480*	-.397	-.350	-.465*	-.418	.230	-.512*	.543*	-.492*
HGB	.417	-.019	-.033	-.276	.205	.353	.200	.201	.078	.033	.073	.230
MCH	.163	-.034	-.017	-.137	.152	.209	.131	.113	-.017	.135	-.189	.085
MCHC	.157	.116	.026	-.265	.045	.107	.075	.040	.234	.149	-.054	.127
LYM	.289	-.031	.202	.236	.084	-.005	.046	.139	-.020	.028	.041	.087
NEUT	-.168	-.010	-.252	-.077	-.099	.002	-.077	-.153	.059	-.110	.018	-.113
MONO	-.429	.376	-.311	-.189	-.160	-.252	-.152	-.171	.573**	-.006	.165	-.100
EO	-.041	.009	.361	.032	-.008	-.047	-.021	.020	-.534*	-.036	-.205	-.045

* (p<0.05), ** (p<0.01)

Table 6. *Correlation levels between biochemical parameter levels and macro and trace essential and non-essential elements levels*

	Macro Essential Elements				Trace Essential Elements				Trace Non-Essential Elements			
	Ca	**K**	**Mg**	**P**	**Cu**	**Fe**	**Se**	**Zn**	**Al**	**Cd**	**Hg**	**Pb**
GLUCO	-.242	.074	.083	-.038	-.358	-.334	-.331	-.372	-.155	-.166	.099	-.346
URE	.080	-.323	.248	.340	.045	.038	-.017	.021	-.208	-.190	-.087	-.131
KREA	-.180	-.205	-.361	-.270	.284	.275	.324	.283	.336	.278	-.475*	.277
ALT	.040	.035	.009	.225	.050	-.005	.025	.047	.109	.003	-.201	.051
AST	.147	-.072	.088	.251	.140	.108	.051	.157	.111	-.059	-.119	.086
TRIG	.114	-.129	-.523*	-.202	.036	.128	.083	.065	.326	.043	.237	.078
CHOL	-.121	-.150	-.816**	-.168	-.274	-.234	-.219	-.229	.369	-.147	.311	-.108
TP	-.278	.209	.230	.385	.071	-.080	-.018	.096	-.050	.026	.020	-.023
ALB	-.041	-.406	.097	.029	.105	.102	.141	.129	-.400	.089	-.232	.090
TB	.247	.081	.174	-.065	.487	.415	.448	.475*	-.077	.262	-.296	.346
BUN	.021	-.242	.311	.234	.147	.119	.081	.119	-.230	-.062	-.197	-.028
LDL	.057	.162	-.327	-.232	.067	.128	.087	.082	.515*	.052	.144	.210
HDL	-.168	.032	-.173	-.247	-.171	-.182	-.083	-.143	-.092	.026	-.153	.028

* (p<0.05), ** (p<0.01)

A total of fourteen species of macroalgae were collected from the Mae Ram The levels of hematological and biochemical parameters in whole blood samples collected from horses within the scope of the study were presented in Table 3 and 4 as arithmetic and geometric mean, median, standard error, and minimum and maximum concentrations. The correlation between levels of hematological and biochemical parameters with macro and trace essential and non-essential elements in whole blood samples collected from horses within the scope of the study were presented in Table 5, 6 and statistical differences indicated with sings; * ($p<0.05$) and ** ($p<0.01$).

Hematological and biochemical parameter level analyzes are the most common clinical methods used to evaluate the physiological and pathological conditions of animals and the early diagnosis of diseases [13, 23]. Limited information is available on essential and non-essential element levels in horses and their correlation with hematological and biochemical parameters. According to this; the study was aimed to determine the levels of essential and non-essential metals and their effects on biochemical and hematological parameters in horses. Especially in the recent years, the interest in the role of these elements in biological systems has increased. As the knowledge about the biochemical and metabolic interactions of these elements or minerals is gained, their role in diagnosis and treatment is revealed [24]. Blood consists of plasma and cells (red blood cells, white blood cells, and platelets), and the term whole blood is the sample that includes all of them [25]. The concentrations of macro (mg L^{-1}) essential (Ca, K, Mg, and P), trace (μg L^{-1}) essential (Cu, Fe, Se, and Zn), and non-essential (Al, Cd, Hg, and Pb) elements were determined in whole blood samples. In the current study, the element concentrations in the whole blood of horses were investigated, since there is limited information about their relationship with hematological and biochemical parameters. Element concentrations were measured with ICP-MS, which is a fast, effective and sensitive analysis method that is widely preferred in clinical toxicology today [26, 27]. ICP-MS is a suitable method for the rapid and sensitive measurement of many elements, especially trace elements, and is gaining more and more importance in diagnosis in human medicine. Faster and more accurate detection of these substances will contribute to early intervention and a positive prognosis. Determining the only serum concentrations of some elements such as Cu, Mg, K, Se, and Zn, is not sufficient for accurate evaluation as they act in cells. Element analysis in the cell allows for optimum evaluation of trace element balance [24].

In this study, Ca, K and Mg concentrations were in normal ranges, while P concentrations were higher than reference concentrations [28, 29, 30]. Also, K and Ca concentrations were found to be lower, P concentration was higher and Mg concentration was similar in the study conducted by Massai et al [13] compared to the current study. Potassium (K) is the most important cation of the intracellular environment K concentrations were within the normal reference range in the current study but should be attended to artificially high K concentrations. The most important cause of hyperkalemia is renal dysfunction, but to prove the existence of a true hyperkalemia, conditions that may cause hyperkalemia (use of tourniquet while taking blood sample, emptying the blood sample from the needle tip or quickly into the tube, hemolysis, drug intake, acute and chronic renal failure) should be questioned or as a sample tube containing EDTA, it is commonly used in laboratory analysis, resulting in artificially high K concentrations. K is mostly found inside the cell; therefore small deviations can cause large changes in the determined concentrations [31]. The high K concentrations in the current study can probably be explained by the high K content of the erythrocytes, using EDTA-containing

tubes or hemolysis [32]. Supporting this, Hawkins et al. [33] showed that more than 33% of hypokalemic cases were missed when whole blood was used to determine K concentrations. Therefore, efforts should be made to minimize these factors that will contribute to the spurious elevation of K. And when laboratory concentrations are not compatible with clinical concentrations clinicians should be suspicious of this situation and consult the laboratory by sending an appropriate sample [31].

In the present study, negative (Mg-PLT, Cu, Fe, Zn, Cd and Pb-RDW, Se, Cd, Pb-WBC, Al-EO) and positive (Hg, P-WBC, AL-PLT, MONO, Hg-RDW) correlations were determined between hematological parameters and elements.

Copper is an essential element for the production of hemoglobin and a negative correlation with some hematological parameters (RBC, HGB, HCT, MCH, MCHC, MCV) was reported. Overexposure of Cu can cause anemia [13, 34, 35]. Only a statistically significant negative correlation was detected between Cu and RDW concentrations.

Excessive exposure to Cd causes anemia in horses. The increase in Cd has a devastating effect on erythrocytes and causes decreases in RBC, HCT, HGB, MCH and MCHC [13, 35]. A negative correlation between Cd and WBC was reported in some studies and is consistent with the present study [35, 36]. Contrary to these, a positive correlation between Cd and WBC were stated in some studies [13, 37].

Red blood cell distribution width (RDW), which is used as an indicator of anemia, is a risk indicator for mortality and cardiovascular disorders. Higher RDW is associated with effective in factors such as inflammation and oxidative stress that change erythrocyte homeostasis and cause anisocytosis Exposure to Cd and Pb causes hemolysis and erythropoietic deterioration, so studies show that exposure to these increases RDW concentrations. Also, Cd can cause Fe deficiency. It is thought that the positive relationship of Cd with RDW may be related to this [38].

In the present study, a significant negative correlation between Cu, Fe, Se, Zn, Cd and Pb with RDW, and a positive correlation between Hg with RDW were found.

The relationship between the RDW and the elements is not clear and studies in animals are limited. The number of samples in the present study was insufficient to clarify this relationship. More comprehensive studies in which the number of animals is increased and evaluated together with some parameters that may be associated with RDW (such as vitamin deficiencies, nutrition, age, oxidative stress) are needed.

In the present study, when we evaluated the relationship between macro, trace essential and non-essential element concentrations with biochemical parameters, negative (TRIG and CHOL-Mg, CREA-Hg) and a positive (Zn-TB, LDL-AL) correlations were determined. Similarly; Badiei et al., [39] were reported a positive relationship between CREA and Hg.

Positive (Cu-TP, Zn-TP, Pb-TB) and negative (Zn-ALP) correlations were stated in a study, different from the present study results [13].

According to a study conducted in Arabian horse serum; among the (Se), (Mn), (Cr), (Cu), (Fe) and (Zn) only Se and Cr were found to be significantly higher in females than males [40].

According to a study conducted in horse mane hair; Cd showed a significant positive, while Hg and Fe significant negative correlations with age [41]. In the present study, no significant differences were found with the elements depending on age.

Accumulation of some element such as Pb increases with age [42]. Consistent with this, in the peresnt study, Al concentration was highest in horses aged ≥13 years. However,

some studies have also reported that there is no age-related accumulation with Al [41, 43]. Whole blood Al concentration in the present study was lower than in other studies [43, 44].

CONCLUSION

Low/high concentrations of elements, which play important roles in metabolic processes, can cause many dysfunctions and susceptibility to diseases without clinical symptoms. Therefore, accurate determination of element levels, especially at trace concentrations, is clinically important. However, according to their biological functions or toxicological properties, elements are presents intra or extracellular, so performing the analysis in plasma or serum can leads to inaccurate measurement of their concentrations. In addition, generally, the concentrations of non-essential elements are not determined in routine blood biochemical parameter analyzes. Therefore, it is thought that it is a more accurate approach to perform analyzes in whole blood (for determining both intracellular and extracellular levels) using methods such as ICP (-MS and -OES) that allows detection at trace limits and determine the non-essential elements.

Interactions can occur between elements (essential or non-essential) and they can alter each other's level and biological functions. Due to the biological or toxic effects of elements on the system and tissues, it is important to monitor their levels and examine their effects. For this purpose, in order to understand the effects of some hematological and biochemical relations that were revealed in the present study, monitoring of the concentrations in horses and planning further studies to establish reference values are recommended.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: F.A.Y., M.Y., Design: F.A.Y., M.Y., N.A., Data Collection or Processing: F.A.Y., M.Y., N.A., Analysis or Interpretation: F.A.Y., M.Y., Literature Search: F.A.Y., M.Y., N.A., Writing: F.A.Y., M.Y.

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