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INFLUENCE OF THE BREED AND AGE ON HEMATOLOGICAL AND BIOCHEMICAL INDICATORS OF MARES FROM PUREBRED ARABIAN AND EASTBULGARIAN BREEDS

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Abstract

In the mares of purebred Arabian horse (n-8) and East Bulgarian horse (n-12) breeds, raised in the State stud farm `Kabiuk` near Shumen, Bulgaria, were studied basic hematological and biochemical blood parameters in the beginning of spring (March). It was established that the average levels for the mares from both breeds are as follows: alanine aminotransferase ALT U/L - 73.00±14.47, lactate dehydrogenase LDH U/L - 1523.88±93.11, creatinine Creat. U/L - 284.56±53.08, calcium Ca U/L - 2.06±0.4, total bilirubin TBL U/L - 78.40±12.45, phosphorus P U/L - 6.63±0.51, white blood cells WBC - 8.87±0.71, lymphocyte count Lym - 32.94±2.48, monocytes Mon-3.98±0.30, granulocytes Gra - 61.45±2.74, red blood cells RBC - 8.01±0.14, erythrocyte volume MCV - 67.07±28.42, hematocrit Hct - 35.12±1.54, hemoglobin MCH - 12.34±0.16, erythrocyte distribution width RDW-16.90±1.42, hemoglobin Hb-11.16±0.67, platelets THR -255.17±40.66, platelet volume MPV -7.81±0.19, platelet distribution width PDW-9.22±0.30, hemoglobin in erythrocytes MCHC - 27.29±0.29. The breed is a reliable ($p<0.01$) source of variation of LDH, with higher values found in the East Bulgarian breed (EBB). The mares of different ages can differ significantly ($p<0.05$) in the content of Mon in the blood. The age group in the breed had a significant effect ($p<0.05$) on Ca and PDW, with the oldest mares from EBB (10-12 years old) had decreasing amount of Ca and increasing amount of POW.

Key words*: Arabian mares, biochemistry, blood indicators, hematology, sport horses.*

INTRODUCTION

The East Bulgarian Horse is a half-blooded breed created by complex reproductive crossing of native, Arab, Anglo-Arab and half-blooded English mares with half-blooded and Thoroughbred stallions (Sabeva et al., 2010).
It is suitable for the classic disciplines of equestrian sports - dressage, show jumping, eventing and amateur riding (Sabeva et al., 2018; Sabeva and Popova, 2019). The breed was recognized in 1951.
The diverse use of this breed implies different intensities of exercise and it is important to have an effective health-program. This requires a detailed study of its biological parameters and their dynamics over time.
Hematological and biochemical parameters in horses are mainly used in clinical diagnostics and therapeutic monitoring, but also as an indicator of nutritional value, assessment of animal metabolic states, etc. (Al-Bulushi et al., 2017; Shawaf, 2017; Shawaf et al., 2018). The composition of the blood is sufficiently labile and reflects the mechanisms of adaptation of the body to changing environmental conditions (Meyer and Harvey, 2004; Hristev and Zapryanova, 2017).
Of particular interest are the correlation between haematological indices and horse performance. Thus, lightweight breeds have higher erythrocytes (RBC), hemoglobin (Hb), and hematocrit (Hct) than heavy horses (Kramer, 2000; Grondin and Dewitt, 2010).
Blood factors are influenced by many factors related to the physiological status of horses as season, diet, reproductive status, geographical location, and more. (Burlikowska et al., 2015; Aiello and Moses, 2016; Aros et al., 2017; Padiha et al., 2017).
Many authors have examined the effect of age on biochemical and hematological parameters (Cebulj-Kadunc et al. 2002; Gurgoze and Icen,

2010; Shawaf et al., 2018; Nidl et al., 2017; Ono et al., 2019, etc.).

Hematologic and biochemical parameters are breed specific (Burlikowska et al., 2015; Cruz et al., 2017; Shawaf et al., 2018) and the use of inappropriate reference values increases the risk of incorrect conclusions and inappropriate actions (Tsanq et al., 1998).

The purpose of this study is to determine the parameters of major haematological and blood biochemical parameters in mares of the East Bulgarian breed and Purebred Arabian horse, under the same breeding conditions, as well as the influence of the breed and age on them.

MATERIALS AND METHODS

The study was conducted in the Kabiyuk National Park, Shumen, with mares from East Bulgarian (EBB) and Purebred Arabian (PAB) breeds. The animals were fed a ration including haymaking and rolled concentrated fodder (a mixture of triticale, corn and black oats). The haymaking is with dry matter - 33.23%, which includes - crude protein - 32.17%, crude cellulose - 27.57%, crude fat - 4.58%, BEV - 28.3% and crude ash - 7.38%. One kilogram of hay contains Ca - 5.45 g, Mg - 1.37 g, Cu - 6.84 mg, Fe - 0.36 g, Zn - 23.19 mg, Mn - 52.91 mg. The dry matter in the concentrated feed is 91.66%, in which the crude protein is 12.27%, the crude cellulose - 11.16%, the crude fat - 4.66%, BEV - 69.35%, crude ash - 2.26%. The content of Ca in one kilogram of concentrated feed is 61.85 mg, of Mg - <1.00 mg, Cu - 2.89 mg, Fe - 2.24 mg, Zn - 1.40 mg, Mn - 0.20 mg.

In March, at the beginning of the spring season, blood samples were taken from 12 mares from EBB and 8 mares from PAB. The blood was taken from the jugular vein between 6-8 in the morning before a meal, with the animal having constant access to water. Hematological parameters were examined with a MS4 + apparatus (Switzerland), biochemical analyses spectrophotometrically on a Mindray BA88 A using the following parameters and reagents: ALT-Tris buffer, 100 mM; pH 7.5, L Alanine 500 mM, 2 Oxoglutarate 15 mM, NADH 0.18 mM; LDH ≥ 1700 U/L and a wavelength of 340 nm; Ca-CPC 0.14 mM, 8-quinolinol 25 mM, HCl pH 1.20 and a wavelength of 575 nm; Creatinine-picnic acid 0.14 mM, NaOH 0.18M, Na tetraborate 10 mM and a wavelength of 510 nm; LDH-phosphate buffer 50 mM, pH 7.5, sodium pyrivate 0.60 mM, NADH 0.18 mM, wavelength 340 nm; P-ammonium molybdate 0.4 mmol/L, sulfuric acid 0.21 mol/L, wavelength 340 nm; total bilirubin-hydrochloric acid 0.1M, 3,5-dichlorphenyldiazonium salt 2 mM, wavelength 510 nm.

Data processing was done with the software product SPSS 21. A mixed linear model of the following statistical type was used:

$Yijk = \mu + BRi + AGj + BAij + eijk,$

where: Yijk - observation vector; μ - total mean constant; BR and AG have fixed effects of the breed (i = 2) and age group, respectively (k = 3: A - from 4-6 years; B - from 7-9 years; C - from 10-12 g .in.); BAij is a random effect of the age group in the breed; eijk … - residual variant.

RESULTS AND DISCUSSIONS

The studies show that, despite identical feeding and rearing conditions, the blood indices of the breeds studied differ to a lesser or greater extent (Table 1). In PAB, alanine aminotransferase (ALT) values, lymphocyte count (Lym), hematocrit (Hct), erythrocyte distribution width (RDW), hemoglobin (Hb), platelets (THR), relative thrombocyte ratio are higher. volume (PDW) and the concentration of hemoglobin in a number of red blood cells (MCHC). All other studied indicators are higher in mares from EBB. In both breeds, ALT, lactate dehydrogenase (LDH), creatinine (Creat), phosphorus (P), total bilirubin (TBL) indicators were elevated, while mean hemoglobin (MCH), mean hemoglobin in erythrocytes (MCHC) and calcium (Ca) are below normal.

The most significant and reliable difference is the activity of lactate dehydrogenase (LDH) (Table 2). The enzyme is responsible for the anaerobic conversion of pyruvate to lactate. Studying the haematological and biochemical parameters in 20 adult sport horses Burlikowska et al. (2015) report significantly lower than the values obtained by us 335.60 ± 24.80 U * L^{-1}. Similar to the authors' cited levels, LDH levels have been reported by

Gurgoze and Icen (2010). They examined 90 purebred Arab mares, ages 6-12 and 14-20, in Turkey, and obtained enzyme values of 236.66 ± 14.18 U/L and 416.77 ± 122.58 U/L for the respective age group.

Table 1. Hematological and biochemical parameters in both breed mares (Mean ± SE)

Parameters	Mean ± SE	Breed	
		PAB	EBB
LDH, U/L	1,524±93.11	1,090±130.8	1,813±128.4
ALT, U/L	73.00±14.47	75.63±20.33	71.25±19.95
Creat., µmol/L	284.6±53.08	262.1±74.56	299.5±73.17
Ca, mmol/L	2.06±0.41	1.27±0.58	2.59±0.57
TBL, µmol/L	78.40±12.45	61.05±17.49	89.97±17.17
P, mmol/L	6.63±0.51	6.15±0.72	6.95±0.71
WBC, 1000/ µL	8.87±0.71	8.21±1.00	9.31±0.98
Lym, %	32.94±2.48	34.69±3.48	31.78±3.41
Mon, %	3.98±0.30	3.81±0.42	4.09±0.42
Gra, %	61.45±2.74	60.83±3.85	61.87±3.78
RBC, m/mm^3	8.01±0.14	8.34±0.20	7.79±0.20
MCV, fl	67.07±28.42	44.43±39.92	82.17±39.18
Hct, %	35.12±1.54	36.85±2.16	33.96±2.12
MCH, pg	12.34±0.16	12.23±0.22	12.42±0.22
RDW, %	16.90±1.42	19.30±1.99	15.30±1.96
Hb, g/L	11.16±0.67	12.24±0.94	10.45±0.93
THR, m/mm^3	255.2±40.66	318.4±57.12	213.0±56.05
MPV, fl	7.81±0.19	7.89±0.27	7.76±0.26
PDW, %	9.22±0.30	9.84±0.42	8.81±0.41
MCHC, g/dL	27.29±0.29	27.68±0.41	27.03±0.40

In 39 Noma horses, Ono et al. (2019) have established values for LDH 488 ± 270 U/L (from 217-758 U/L), indicating that values are higher than those of the Kisso breed (431 ± 161 U/L) and lower than Japanese race horses - 550 ± 134 U/L. According to the authors, the differences are due to the different diet. Our LDH values are similar to those reported by Aros et al. (2017) - 807 ± 515 IU/L (353 to 1,746 IU/L) and from Winnicka (2011) - 520-1,480 U * L^{-1}.

Alanine aminotransferase (ALT) is an enzyme released by the cytoplasm from hepatocytes when they are destroyed and it is considered to be very specific for the liver. According to Mircheva (2006), this indicator is rarely an indicator of clinical certainty, unless it exceeds at least twice the upper limit of the reference values, which in horses are 3-25 U/L. The values obtained from the mares of both breeds are twice above the reference value specified by the author. Probably the reason is the significant breeding differences or temporary discomfort of the animals at the end of the winter. The latter is probably more likely, given the values of the indicator reported by other authors: Ju et al. (1993) - for 46 mares in Taiwan - 6.49 ± 3.14 µ/L; Burlikowska et al (2015) - 8.80 ± 0.61 IU * L^{-1} for competition horses. Gurgoze et al. (2010) examined the effect of age on blood counts of Purebred Arabian horses and reported slightly elevated ALT values in horses between 6-13 months of age - 31.66 ± 10.44 U/L. With age, the values of the indicator decreased, with mares at 6-12 years of age and 14-20 years of age ALT being 7.66 ± 2.96 U/L and 4.22 ± 0.82 U/L, respectively.

Table 2. Influence of the breed, age group, group in the breed on the hematological and biochemical parameters of EBB and PAB mares, F-criterion and degree of reliability

Parameters/factors	Breed	Age group	Age group in the breed
df	1	2	1
LDH, U/L	13.446**	0.192	1.911
ALT, U/L	0.211	0.975	0.569
Creat., µmol/L	0.050	0.040	2.427
Ca, mmol/L	1.462	2.606	4.042*
TBL, µmol/L	2.651	1.020	0.012
P, mmol/L	1.799	2.814	2.714
WBC, 1000/µL	2.369	1.949	0.040
Lym, %	1.185	0.895	0.226
Mon, %	0.218	3.120*	0.059
Gra, %	0.345	0.645	0.027
RBC, m/mm^3	1.683	1.170	1.724
MCV, fl	0.803	0.677	0.710
Hct, %	0.563	1.682	2.110
MCH, pg	1.669	1.513	0.005
RDW, %	3.087	0.726	0.349
Hb, g/L	2.075	0.549	0.001
THR, m/mm^3	1.130	0.287	0.518
MPV, fl	0.237	0.320	1.645
PDW, %	0.565	1.825	7.947*
MCHC, g/dL	0.924	1.228	0.213

Creatinine is a product of the breakdown of creatine phosphate in muscle tissue. It is exclusively excreted by glomerular filtration through the kidney. The values of this indicator reported in the literature vary widely. In the studies of Meyer and Harvey (2004), Boediker (1991) and Gurgoze and Cetin (2004), Creat is in the range of 88.4-167.96 µmol/L. Gurgoze and Icen (2010), in Purebred Arabian mares, found an increase in values of the indicator with increasing of the age - 66.30 ± 9.72 µmol/L in mares 14-20 years, 86.63 ± 5.30 µmol/L at 6-12 years and 97.24 ± 17.68 µmol/L for mares over 20 g. In the mares we studied, creatinine was elevated in both breeds - 262.1 ± 74.56 µmol/L at PAB and 299.5 ± 73.17 µmol/L at EBB, and according to Kaneko et al, (1997), creatinine was strongly influenced by diet and the muscle mass of an animal

The levels of Hb and Hct found by us are similar to those of other authors: - Ono et al. (2019) for the Noma breed - 12.8 ± 2.6 g/L and 35.5 ± 7.1%, respectively for Hb and Hct, for the Kisso breed - 11.6 ± 1.5 g/L and 32.9 ± 4.0%, and for Japanese race horses - 16.1 ± 1.4 g/L and 42.2 ± 4%; Aros et al. (2017) for local working horses in Chile - 120 ± 16.2 g/L and 33.6 ± 5.1%; Qadri et al. (2018) have found Hb values of 12.8 ± 0.68 g/L in mountain horses in Kashmir.

Mircheva (2006) indicates that total serum bilirubin in horses and ponies varies between 1 and 2 mg/dL (17-34 µM/L) and is dependent on the latest food intake. In the mares we studied, bilirubin was 61.05 ± 17.49 µM/L for PAB and 89.97 ± 17.17 µM/L for EBB.

The calcium in both breeds has significantly lower than those reported by other authors - 1.27 ± 0.58 mmol/L for PAB and 2.59 ± 0.57 mmol/L for EBB. Thus, Padilha et al. (2017) reported 13.22 ± 0.59 mg/dL in the Brazilian Sport Horse breed; Aros et al. (2017) report 4.5 ± 3.4 mmol/L in local horses in Chile. Among the main causes of hypocalcemia are insufficient Ca content in feed, low levels of magnesium in the blood, which reduces the activity of parathyroid hormones, etc. (Arnbjerg, 1980; Ramiro, 2011; Raimundo et al., 2017).

In contrast to the Ca, amount of P, exceed the limit of reference values, reported by (Mircheva, 2006). This disturbs the balance between Ca and P. Blood calcium levels can be adjusted for nutritional supplements. It is advisable to intake supplements with vitamin D and magnesium.

In experiment, made by Popov et al. (1974), who tested the effect of compound feed on the feeding of 18 Thoroughbred mares during pregnancy and lactation, found that the

granulated compound feed enriched with trace elements and vitamins that fed the mares from the experimental group had a beneficial effect on them both during pregnancy and on the growth and development of foals.

Table 3. Hematological and biochemical parameters of the mares by age group in the breed (Mean ± SD)

Breed	PAB		EBB		
Age group	A (4-6 y)	C (10-12 y)	A(4-6 y)	B(7-9 y)	C(10-12 y)
LDH, U/L	81.25±28.75	70.00±28.75	71.75±28.75	33.00±33.19	109.0±40.65
ALT, U/L	1,275±185.0	904.8±185.0	1,748±185.0	1,743±213.6	1,949±261.6
Creat., µmol/L	182.9±105.5	341.4±105.5	393.0±105.5	321.4±121.8	184.1±149.1
Ca, mmol/L	1.39±0.82	1.14±0.82	4.35±0.82	3.00±0.95	0.40±1.16
TBL, µmol/L	53.48±24.74	68.63±24.74	95.48±24.74	57.73±28.57	116.7±34.99
P, mmol/L	5.92±1.02	6.38±1.02	5.57±1.02	5.48±1.18	9.80±1.45
WBC, 1000/µL	7.96±1.42	8.47±1.42	10.71±1.42	6.63±1.63	10.59±2.00
Lym, %	37.40±4.92	31.98±4.92	28.80±4.92	37.93±5.68	28.60±6.96
Mon, %	3.53±0.60	4.10±0.60	3.05±0.60	5.27±0.69	3.95±0.85
Gra, %	59.08±5.45	62.58±5.45	61.65±5.45	56.80±6.29	67.15±7.70
RBC, m/mm^3	8.34±0.28	8.33±0.28	7.52±0.28	7.51±0.33	8.33±0.40
MCV, fl	43.58±56.46	45.28±56.46	153.4±56.46	44.50±65.19	48.65±79.84
Hct, %	36.20±3.06	37.50±3.06	28.68±3.06	33.30±3.53	39.90±4.32
MCH, pg	12.13±0.31	12.33±0.31	12.60±0.31	11.90±0.36	12.75±0.44
RDW, %	18.00±2.82	20.60±2.82	14.33±2.82	18.37±3.26	13.20±3.99
Hb, g/L	11.63±1.34	12.85±1.34	9.53±1.34	11.17±1.54	10.65±1.89
THR, m/mm^3	381.3±80.78	255.5±80.78	220.3±80.78	194.3±93.27	224.5±114.2
MPV, fl	8.33±0.38	7.46±0.38	7.58±0.38	7.90±0.44	7.80±0.54
PDW, %	10.50±0.59	9.18±0.59	8.13±0.59	7.77±0.69	10.55±0.84
MCHC, g/dL	28.03±0.58	27.33±0.58	27.70±0.58	27.00±0.67	26.40±0.82

Erythrocyte counts, such as mean erythrocyte volume (MCV), MCH (mean hemoglobin concentration in erythrocytes), MCHC (mean hemoglobin concentration in erythrocytes), show the efficiency of hemoglobin synthesis and its oxygen transport capacity. The results we have obtained for this parameters correspond to those reported by other authors. Burlikowska et al. (2015) found that for mares used for jumping, the MCV was 44.17 ± 1.05 fl, MCH - 16.75 ± 0.43 pg, MCHC - 37.88 ± 0.20 g * dl^{-1}; Shawaf et al. (2018) cite MCV 45 ± 1.8fl, MCH 9.1 ± 1.12 pg and MCHC 22.28 ± 0.20 g/dL for Scottish ponies in Saudi Arabia etc.

A number of studies have reported reductions in some blood parameters in older animals in different animal species (Nakai et al., 1992; Atanasova et al., 2008; Valcehv et al., 2009; Markova et al., 2018). Table 2 and Table 3 clearly shows that the age group in the breed has a significant effect on Ca levels. With advancement of age in both breeds the Ca content in the blood decreases as in the PAB reaches up to 1.14 ± 0.82 mmol/L and at EBB - 0.40 ± 1.16 mmol/L. Gurgoze and Icen (2010) reported that levels of Ca (2.80 ± 0.07 mmol/L) and P (0.84 ± 0.05 mmol/L) in PAB mares with advancing age (14-20 years) dramatically decrease as the cause is probably due to a decrease in bone metabolism. Young animals have been shown to absorb calcium from the food more efficiently and have much higher values than adult animals (Braithwaite, 1975).

With advancing of the age, after 10 years, TBL levels increased (by 22.07% for PAB and 50.53% for EBB), P (by 7.21% for PAB and 43.16% for EBB) and HcT (by 3.47% for PAB) and by 28.12% for EBB). The differences, although significant, are unreliable, with probable cause being the small number of animals and the wide variation in signs. The levels of LDH (69.72%) and ALT (10.31%) were also increasing in EBB mares after 10

years of age, and Creat (50.41%) and MCV (3.69%) levels increased in PAB mares. In the animals of the highest age group, MCV values were 68.29% lower than those of the youngest. Trending decreases in LDH and ALT values have been observed in PAB mares with advancing of the age.

CONCLUSIONS

It was found that in mares from the East Bulgarian and Purebred Arabian horse breeds, at the end of the winter period, when feeding with winter ration, the mean ALT level was 73.00 ± 14.47 U/L, of LDH - 1,523.88 ± 93.11 U/L, Creat. - 284.56 ± 53.08 U/L, Ca - 2.06 ± 0.4 U/L, TBL - 78.40 ± 12.45 U/L, P - 6.63 ± 0.51 U/L. WBC is 8.87 ± 0.71 μL, Lym is 32.94 ± 2.48%, Mon - 3.98 ± 0.30%, Gra - 61.45 ± 2.74%, RBC - 8.01 ± 0.14 m/mm^3, MCV - 67.07 ± 28.42 fl, Hct - 35.12 ± 1.54%, MCH - 12.34 ± 0.16 pg, RDW - 16.90 ± 1.42%, Hb - 11.16 ± 0.67 g/L, THR - 255.17 ± 40.66 m/mm^3, MPV - 7.81 ± 0.19 fl, PDW - 9.22 ± 0.30%, MCHC - 27.29 ± 0.29 g/dL.
The breed is a reliable ($p<0.01$) source of LDH variation, with higher values reported in the Eastbulgarian breed. The mares at different ages differed significantly ($p<0.05$) in blood content of Mon. The age group in the breed had a significant effect ($p<0.05$) on Ca and PDW, with the oldest EBB mares (10-12 years old) reducing the amount of Ca and increasing that of PDW.

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