Ann. Anim. Sci., Vol. 19, No. 1 (2019) 85–96 *DOI: 10.2478/aoas-2018-0031*

sciendo

INFLUENCE OF TYPE OF USE, AGE AND GENDER ON HAEMATOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS OF MAŁOPOLSKI HORSES*

Anna Czech[1♦], Martyna Kiesz[1], Adrian Kiesz[1], Tomasz Próchniak[2], Paweł Różański[3], Kamila Klimiuk[1]

[1]Department of Biochemistry and Toxicology,
[2]Institute of Biological Basis of Animal Production,
[3]Department of Animal Hygiene and Environment,
Faculty of Biology, Animal Sciences and Bioeconomy, University of Life Sciences in Lublin,
20-950 Lublin, Poland
♦Corresponding author: anna.czech@up.lublin.pl

Abstract

The purpose of the study was to determine whether the type of use, age and gender of Małopolski horses affect the level of selected haematological and biochemical parameters of their blood and whether there is an interaction between these factors. The research was carried out on 30 horses of the Małopolski breed, which were divided according to gender (18 mares and 12 stallions), age (2–6 years, 7–9 years and 10–16 years) and use (recreational vs. sport). To sum up, the gender of horses significantly affects erythrocyte indices, as evidenced by their significantly higher values in the stallions as compared to the mares, as well as by the interactions between gender and type of use and between the gender and age of the horse. The leukocyte and neutrophil counts increase with the age of horses, and the interaction between age and type of use indicates that the leukocyte and neutrophil counts in training horses is closely linked to their age. Systematic physical exercise in sport horses increases leukocyte and lymphocyte counts and contributes to osteogenesis (increase in ALP activity and plasma content of calcium and phosphorus), which has a beneficial effect on their health. The correlations obtained may improve breeders' awareness of the effect of various factors, such as age, gender or type of use, on blood indices in horses and can be helpful in evaluating the health of Małopolski horses.

Key words: horses, blood parameters, type of use, gender, age

In recent years the growth of various forms of horse riding has been observed around urban agglomerations and in tourist areas. Interest in equestrian sport has also increased, leading to the emergence of many new equestrian disciplines. These factors have stimulated the development of equine exercise physiology (Neuberg and Geringer de Oedenberg, 2007). One of the most native and popular Polish breeds of

*Work financed from statutory activity.

horse is the Małopolski horse. This breed is distinguished by its endurance and excellent condition (Tomczyk-Wrona, 2014). Nevertheless, during a horse's (including Małopolski horses) adaptation to physical exertion of varying intensity, specific changes in metabolic reactions occur, causing numerous changes in the body, especially in the circulatory, respiratory, endocrine, and neuromuscular systems (Lacerda et al., 2006). Changes taking place in these systems simultaneously and in an integrated manner are aimed at maintaining homeostasis in the body (Tateo et al., 2008; Trigo et al., 2010). However, long-term physical exertion may result in disturbances of homeostasis, such as energy depletion and changes in fluids, electrolytes and acid-base balance, with negative consequences for the horse's performance and health which may lead to significant changes in various haematological and biochemical blood parameters (Fielding et al., 2009). Hence blood indices not only provide information on the animal's health, but can also serve as indicators of the physical condition of sport horses, enabling individual tailoring of training regimes (Lacerda et al., 2006; Padalino et al., 2007; Brzóska et al., 2015). Horses whose blood indices are within reference ranges are likely to achieve better results, and their bodies will regenerate more quickly following intense physical exertion (Burlikowska et al., 2015; Vergara and Tadich, 2015).

The available literature provides a wealth of information about alterations in blood indices of horses during and after training (Czech et al., 2016; Bis-Wencel et al., 2012). It should be noted, however, that blood parameters may also be affected by the relatively high variability arising from differences in breed, physiological state, health status, housing conditions, gender and age (Padalino et al., 2007; Górecka-Bruzda et al., 2015; Vergara and Tadich, 2015).

Therefore, the purpose of the study was to investigate the effect of the type of use, age and gender of Małopolski horses on selected haematological and biochemical blood parameters and to test the interactions between these factors.

Material and methods

The study was conducted for two consecutive years at the turn of March and April on a farm in south-eastern Poland. Thirty riding horses of the Małopolski breed were selected for the study. The study group consisted of 18 mares and 12 stallions. The horses were divided into three age groups:

– 10 horses aged 2 to 6 years (6 mares and 4 stallions),
– 10 horses aged 7 to 9 years (6 mares and 4 stallions),
– 10 horses aged 10 to 16 years (6 mares and 4 stallions).

Horses were also divided according to use, i.e. 15 horses used for recreation (9 mares – 3 horses aged 2–6 years + 3 horses 7–9 years and 3 horses 10–16 years and 6 stallions – 2 horses aged 2–6 years + 2 horses 7–9 years and 2 horses 10–16 years) and 15 used for sport (9 mares – 3 horses aged 2–6 years + 3 horses 7–9 years and 3 horses 10–16 years and 6 stallions – 2 horses aged 2–6 years + 2 horses 7–9 years and 2 horses 10–16 years).

'Recreational' horses were used 3–4 times a week for riding lessons at a riding school (mainly walk and trot) and for short treks. The 'sport' horses were trained to compete in hurdling competitions at the regional level (up to 120 cm). Their jumping training took place three times a week, and on other days they took part in supplementary exercises (lunging, gymnastic jumps, etc.).

Before blood samples were collected, horses were in training preparing for the start in competition for about 60 days. In this period the horses trained intensively 6 times a week for about one hour a day. All horses were clinically healthy.

The horses received the following daily: meadow hay (8 kg), crushed oats (4 kg – recreational horses; 6 kg – sports horses), wheat straw as litter (8 kg) and a commercial vitamin and mineral mix (20 g per 100 kg body weight). One kilogram of the vitamin and mineral mix consisted of calcium – 16%; phosphorus – 4%; sodium – 2%; magnesium – 4%; vitamin A – 500,000 IU; vitamin D_3 – 50,000 IU; vitamin E – 10,000 mg; vitamin C – 5,000 mg; vitamin B_1 – 400 mg; vitamin B_2 – 400 mg; vitamin B_6 – 250 mg; vitamin B_{12} – 1,000 mg; niacin – 5,000 mg; biotin – 20,000 mcg; folic acid – 200 mg; Ca-D pantothenate – 200 mg; choline chloride – 10,000 mg; iron – 2,000 mg; zinc – 1,000 mg; zinc chelate – 1,000 mg; manganese – 500 mg; manganese chelate – 500 mg; copper – 250 mg; copper chelate – 250 mg; iodine – 30 mg; selenium – 10 mg; live yeast cultures (*Saccharomyces cerevisiae* SC 47) – 5×10^{10} CFU; and soybean oil – 1%.

The composition of the diets satisfied the daily requirements for energy, protein, minerals and vitamins, based on the average food ration for the species according to Brzóska et al. (2015).

During the experiment, horses were kept in individual stalls with free access to fresh water. In each year of the study, blood was collected for analysis twice (at two-week intervals) from the same horses, from the external jugular vein.

Blood was collected in the morning before watering and the first feeding. The blood was taken by a veterinary surgeon for routine testing, and therefore the ethics committee was not required.

The following haematological parameters were determined in whole blood: haematocrit (Ht), haemoglobin content (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), as well as the white blood cell count (WBC) and the percentage composition of white blood cells (leukogram), i.e. the percentage of neutrophils (NEU), lymphocytes (LYM) and the sum of monocytes, eosinophils and basophils (MID). The determinations were made in an ABACUS-Vet analyser.

Biochemical indices, i.e. total cholesterol (TCH), HDL cholesterol and triacylglycerols (TG), were determined in the plasma by spectrophotometry using Cormay monotests. The content of the low-density lipoprotein cholesterol fraction (LDL) was calculated from the Friedewald et al. formula (1972):

$$LDL\ (mmol\ l^{-1}) = total\ cholesterol - HDL - triglycerides/2.2$$

Activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), as well as the content of phosphorus, calcium, magnesium, were determined by spectrophotometry.

Statistical analysis

The numerical data were analysed by analysis of variance (ANOVA) according to the following model:

$$y_{ijklm} = \mu + a_i + g_j + u_k + y_l (a*g)_{ij} + (a*u)_{ik} + (g*u)_{jk} + e_{ijklm}$$

where:

μ – constant value,

a_i – effect of i[th] age on the value of the variable for i = I (2–6 years), II (7–9 years), III (10–16 years),

g_j – effect of j[th] gender on the value of the variable for j = F (mares), M (stallions),

u_k – effect of k[th] type of use on the value of the variable for k = R (recreation), S (sport),

y_l – effect of year in which the blood was collected (Y),

e_{ijklm} – random factor, assumed to have distribution N(0,1).

The model included the interactions between the factors age × gender (A×G); age × type of use (A×U); and type of use × gender (U×G), i.e. the sum of the effects of the independent variables on the phenomenon tested.

Significance of differences between means was determined by Tukey's test at the level of significance $P \leq 0.05$; $P \leq 0.01$.

Due to the fact that blood was collected in two subsequent years, the authors included this in statistical calculations. However, there were no significant differences between the analysed blood indicators, therefore this was not described in the paper and was removed from the table.

Results

The blood parameters of the horses were within reference values provided by Winnicka (2015). Horses from the group in the range of 10 to 16 years of age had significantly higher levels of haemoglobin, while younger horses from 2 to 6 years of age had significantly lower MCV and MCH than horses aged 7 to 9 years ($P<0.05$) (Table 1). The type of use had no significant effect on erythrocyte indices, but they were significantly influenced by gender. Stallions had significantly higher Hb (by about 26.6%), Ht (by about 39.4%) and RBC (by about 38.6%) than mares, and lower MCV and MCH (Table 2). An interaction was noted between age and gender and between type of use and gender for basic red blood cell indices, i.e. Hb, Ht and RBC (Table 3).

Table 1. Mean of haematological and biochemical indices and macroelements in the blood of Małopolski horses according to age

Item*	Age (years)			P-value
	I 2–6 n=10	II 7–9 n=10	III 10–16 n=10	
Erythrocyte indices				
Hb (g l^{-1})	130.5 ab±3.44	120.75 b±2.88	137.83 a ±3.72	0.033
Ht (%)	33.30±3.77	31.93±4.91	30.76±3.43	0.809
RBC (10^{12} l^{-1})	8.73±1.09	8.18±1.77	7.38±1.65	0.505
MCV (fl)	36.30 b±1.47	40.75 a±1.09	41.07 a±0.89	0.021
MCH (pg)	14.81 b±0.99	16.36 a±1.00	16.96 a±0.66	0.012
MCHC (g l^{-1})	407.00±22.01	400.88±21.0	413.71±17.49	0.356
Leukocyte indices				
WBC (10^9 l^{-1})	8.15 ab±0.321	7.55 b±0.109	8.93 a±0.218	0.038
NEU (%)	33.41 b±0.89	41.69 ab±1.73	45.82 a±1.44	0.050
LYM (%)	66.41 a±1.05	58.15 ab±2.84	53.73 b±2.72	0.048
MID (%)	0.18 b±0.043	0.16 b±0.091	0.45 a±0.034	0.031
NEU/LYM	0.503 b±0.022	0.717 ab±0.068	0.853 a±0.063	0.040
Lipid parameters (mmol l^{-1})				
TCH	1.91±0.277	2.05±0.198	2.11±0.398	0.605
HDL cholesterol	1.01±0.094	1.04±0.164	1.16±0.171	0.366
TG	0.27 a±0.012	0.16 b±0.033	0.14 b±0.010	0.018
LDL cholesterol	0.78±0.066	0.94±0.072	0.88±0.043	0.741
% HDL	54.5±4.98	53.24±5.92	55.3±3.98	0.936
Enzymatic activity (U l^{-1})				
ALP	179.2 a ±6.97	128.3 b±4.87	143.9 b±8.77	0.043
AST	373.3±11.76	389.2±15.86	409.4±27.65	0.527
ALT	5.52±0.987	5.47±0.722	6.40±0.587	0.628
LDH	856.2±28.98	789.9±20.76	784.8±17.99	0.544
Macroelements (mmol l^{-1})				
Phosphorus	1.39±0.043	1.34±0.037	1.20±0.098	0.444
Calcium	2.59±0.072	2.77±0.093	2.58±0.102	0.329
Magnesium	0.74±0.033	0.75±0.049	0.73±0.037	0.968

a, b – values within a row with different letters differ at $P \le 0.05$ (where “a” is a higher value).

*Ht – haematocrit; Hb – haemoglobin content; RBC – red blood cell count, MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin, MCHC – mean corpuscular haemoglobin concentration; WBC – white blood cell count; NEU – neutrophils; LYM – lymphocytes; MID – the sum of monocytes, eosinophils and basophils; TCH – total cholesterol, HDL cholesterol – high-density lipoprotein cholesterol; LDL cholesterol – low-density lipoprotein cholesterol; TG – triacylglycerols; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; LDH – lactate dehydrogenase.

Table 2. Mean of haematological and biochemical indicators and macroelements in the blood of Małopolski horses according to gender and type of use

Item*	Type of use		P-value	Gender		P-value
	recreation n=15	sport n=15		mare n=18	stallion n = 12	
Erythrocyte indices						
Hb (g l^{-1})	124.6±7.66	136.6±9.02	0.059	123.6 b±4.28	156.5 a±5.12	0.036
Ht (%)	30.71±1.34	34.71±1.98	0.208	30.22 B±2.98	42.13 A±1.96	0.002
RBC (10^{12} l^{-1})	7.76±0.78	8.95±0.65	0.199	7.71 B±0.49	10.69 A±0.84	0.009
MCV (fl)	37.80±1.44	40.93±2.06	0.053	39.67a±1.01	35.50 b±1.11	0.041
MCH (pg)	15.52±0.98	16.44±1.59	0.169	16.20 a±0.64	14.20 b±0.87	0.016
MCHC (g l^{-1})	409.7±17.4	402.0±21.0	0.246	407.9±18.09	400.5±20.65	0.420
Leukocyte indices						
WBC (10^{9} l^{-1})	7.50 b±0.57	8.82 a±0.22	0.023	8.37±0.96	7.05±0.76	0.221
NEU (%)	44.12 a±1.77	36.36 b±1.91	0.044	39.53±2.00	37.98±1.82	0.797
LYM (%)	55.67 b±2.74	63.38 a±3.87	0.035	60.21±1.97	61.88±2.73	0.784
MID (%)	0.21±0.023	0.26±0.011	0.628	0.26±0.023	0.15±0.017	0.399
NEU/LYM	0.793 a±0.021	0.574 b±0.042	0.039	0.657±0.098	0.614±0.086	0.446
Lipid parameters (mmol l^{-1})						
TCH	1.93±0.109	2.05±0.078	0.481	2.02±0.055	1.91±0.181	0.598
HDL	1.07±0.087	1.05±0.059	0.897	1.08±0.023	0.95±0.066	0.280
TG	0.170±0.018	0.220±0.037	0.323	0.200±0.025	0.210±0.029	0.820
LDL	0.790±0.044	0.90±0.111	0.545	0.860±0.062	0.860±0.049	0.988
% HDL	57.25±2.89	52.75±3.09	0.429	54.96±2.00	51.83±2.76	0.674
Enzymatic activity (U l^{-1})						
ALP	126.8 b±5.98	168.7 a±6.99	0.037	151.8±7.98	155.7±6.27	0.898
AST	403.2±12.32	378.3±10.09	0.337	389.1±9.17	380.1±8.03	0.791
ALT	5.54±1.01	5.83 ±0.899	0.739	5.41±1.34	7.26±2.06	0.081
LDH	835.7±15.99	804.5±11.03	0.623	792.1±14.76	936.9±8.09	0.068
Macroelements (mmol l^{-1})						
Phosphorus	1.12 b±0.010	1.49a±0.019	0.047	1.32±0.027	1.36±0.044	0.794
Calcium	2.24 b±0.038	3.14a±0.022	0.019	2.62±0.59	2.80±0.066	0.249
Magnesium	0.78±0.099	0.72±0.105	0.210	0.73±0.039	0.79±0.072	0.460

a, b – values within a row with different letters differ at P≤0.05 (where “a” is a higher value).
A, B – values within a row with different letters differ at P≤0.01 (where “A” is a higher value).
*see Table 1.

WBC, NEU, and MID counts, as well as the NEU/LYM ratio, were significantly higher in the oldest horses (10–16 years), while the LYM count was significantly lower in this group (Table 1). Horses used for sport had significantly higher WBC (P=0.023) and LYM (P=0.035) counts and a lower NEU/LYM ratio (P=0.039) than the recreational horses. Gender had no significant effect on the leukocyte indices (Table 2). There was, however, an interaction between A × U for WBC (P=0.047) and NEU (P=0.050) and for NEU/LYM (P=0.0.32), as well as between U × G for WBC (P=0.049) and between U × G for WBC (P=0.049) (Table 3).

The content of triacylglycerols was significantly higher (P≤0.05) in the blood plasma of the youngest horses (2–6 years), while the remaining lipid indices were similar in all age groups (Table 1). Neither type of use nor gender significantly affected the lipid indices in the plasma (Table 2). An interaction was noted between age and gender for TG (P=0.025) (Table 3).

Table 3. Interactions between age (A), gender (G) and type of use (U) of Małopolski horses

Item*	A×G	A×U	U×G
Erythrocyte indices		P-value	
Hb	0.027	0.555	0.031
Ht	0.012	0.387	0.022
RBC	0.019	0.289	0.039
MCV	0.078	0.438	0.067
MCH	0.059	0.371	0.077
MCHC	0.644	0.729	0.505
Leukocyte indices			
WBC	0.288	0.047	0.049
NEU	0.491	0.050	0.068
LYM	0.067	0.368	0.099
MID	0.052	0.055	0.057
NEU/LYM	0.071	0.032	0.065
Lipid parameters			
TCH	0.123	0.402	0.152
HDL	0.324	0.525	0.094
TG	0.025	0.422	0.061
LDL	0.348	0.328	0.089
% HDL	0.418	0.482	0.107
Enzymatic activity			
ALP	0.164	0.436	0.073
AST	0.206	0.451	0.327
ALT	0.066	0.420	0.022
LDH	0.061	0.288	0.055
Macroelements			
Phosphorus	0.104	0.507	0.173
Calcium	0.100	0.439	0.431
Magnesium	0.281	0.471	0.238

*see Table 1.

Age had no significant effect on activity of most of the liver enzymes, i.e. AST, ALT and LDH. One exception is ALP activity, which was significantly higher in the blood plasma of the youngest horses (2–6 years) than in older horses (Table 1). Significantly higher (by about 33%) activity of this enzyme was also noted in the plasma of sport horses as compared to recreational horses (Table 2). An interaction was shown between use and gender for ALT activity (P=0.022) (Table 3).

Age and sex had no significant effect on mineral content (Table 1). Horses used for sport had significantly higher serum content of calcium and phosphorus than recreational horses (Table 2).

Discussion

Knowledge of the haematological and biochemical parameters of horse blood have significance for preparing an individual training plan (Lacerda et al., 2006; Burlikowska et al., 2015). Red blood cells and the utilization of oxygen in working muscles are responsible for how quickly oxygen is distributed in the body, which is a significant matter during exercise (Vergara and Tadich, 2015). Months of training can cause an increase in the erythrocyte count in the blood of horses (Satué et al., 2012). This is confirmed by research by Burlikowska et al. (2015), which indicates that horses used for sport exhibit significantly higher RBC, Ht and Hb than recreational horses. This demonstrates that they are better prepared for increased physical activity (Burlikowska et al., 2015). In our investigation, only a slight increase in these parameters was observed in the horses used for sport, which was consistent with research by Neuberg and Geringer de Oedenberg (2007).

Our study shows that the red blood cell indices in horses were significantly influenced by gender. Stallions, due to the presence of testosterone (a hormone stimulating erythropoietin secretion) (Neuberg-Zuchowicz and Geringer de Oedenberg, 2011), or, as shown by Czech et al. (2016), the significantly higher plasma content of iron (an essential element in haemoglobin synthesis), have significantly higher values for erythrocyte parameters. This is confirmed not only in the present study but in research by other authors as well (Neuberg and Geringer de Oedenberg, 2007). The mares, on the other hand, had significantly higher MCH and MCV, which was in agreement with a study by Bis-Wencel et al. (2011).

Our investigation showed no interaction between the type of use and the age of the horses for erythrocyte indices. However, it should be noted that as horses age numerous changes in the body affect erythrocyte indices (Yaqub et al., 2013), which was confirmed in our study. Older horses had significantly higher haemoglobin content, mean corpuscular volume and mean corpuscular haemoglobin, while the RBC count decreased slightly. The reduction in the erythrocyte count may be due to ageing of the body, which is associated with reduced capacity for bone marrow regeneration (Satué et al., 2012; Yaqub et al., 2013; Neuberg and Geringer de Oedenberg, 2007). The interaction between gender and type of use and between gender and age for red blood cell parameters clearly indicate that the level of exercise must be adjusted to the sex of the horses. This is essential for maintaining the body's homeostasis and is probably linked to the level of androgens, as mentioned above. However, the mechanisms through which androgens (primarily testosterone) stimulate erythropoiesis are not fully understood (Bachman et al., 2010).

Age, sex or physical exertion can also contribute to changes in the white blood cell system, responsible for proper immune function (Satué et al., 2012). Physical exertion in horses (as in humans) can exert an immunostimulatory effect (Neuberg and Geringer de Oedenberg, 2007). This has been confirmed by research by Vergara and Tadich (2015) and by Wanderley et al. (2015), who demonstrated an increase in leukocyte count caused by neutrophilia and lymphocytosis following exercise (as a stressor), as well as an increase in phagocytic and bactericidal activity in neutrophils. Similar correlations were noted in the present study, despite the fact that blood was taken from the horses in the morning. The increased leukocyte count correlated with an increased lymphocyte count in the horses undergoing physical exercise can be explained as a normal response to the release of adrenocorticotropic hormone and cortisol, which stimulate the migration of leukocytes from the peripheral lymphoid organs to larger blood vessels (Welles, 2000). Following exercise, the pool of marginal neutrophils and/or lymphocytes is mobilized, which is associated with decreased neutrophil adhesion, as well as an increase in the speed of microcirculation and splenic contraction (Latimer, 1999).

The leukocyte counts in training horses were also determined by their age, as indicated by the interaction. In a study by Jawor et al. (2007), the greatest difference in leukocyte counts was observed between young horses with outstanding sport potential and older horses with average sport potential. The study also showed that older horses have significantly higher numbers of leukocytes, including neutrophils, than younger horses, which is consistent with our results. Furthermore, lymphocyte counts are significantly reduced with age, leading to a higher NEU/LYM ratio in older horses than in foals (Smith et al., 2002; Satué et al., 2010).

Neither the type of use nor gender significantly influenced lipid parameters in the horses, which is in agreement with studies by other authors (Grela et al., 2003; Burlikowska et al., 2015). In contrast, according to Bis-Wencel et al. (2012) training increases the HDL cholesterol level, which is accompanied by a reduction in total cholesterol and in LDL cholesterol and VLDL cholesterol. It should be emphasized here that lipid metabolism in horses differs from that of other species and the effect of physical activity on lipid concentration in the blood has not been fully clarified. The slight increase in TG values in the horses used for sport was a confirmation of results obtained by Tateo et al. (2008), who reported that TG levels were positively correlated with intensity of exercise.

As in the case of lipid indicators, age, gender, and type of use had no significant effect on the activity of most of the analysed enzymes indicative of liver function (AST, ALT and LDH). However, ALP activity was significantly higher in the youngest horses, indicating normal growth processes, as this enzyme is primarily involved in the development of bone matrix and skeletal growth, and its activity is highest in animals during the growth stage (Bosco et al., 2014). The type of use was also a factor influencing ALP activity, as significantly higher activity of this enzyme was noted in training horses than in recreational horses. This may be due to the exertion that the body is subjected to during exercise, which stimulates changes in the skeletal system and thereby stimulates ALP secretion (Padalino et al., 2007; Bosco et al., 2014). According to Trigo et al. (2010), Vergara and Tadich (2015) and Padalino et al. (2007), over-training leading to skeletal muscle damage may also be evidenced by an increase in AST and LDH activity. This was not observed in our experiment, which may indicate that the training process was appropriate (Winnicka, 2015).

The increase in activity of ALP, an enzyme that plays an important role in bone tissue production (Padalino et al., 2007; Bosco et al., 2014), was accompanied by an increase in the plasma content of calcium and phosphorus in the sport horses. This may be linked to the fact that in the first stage of bone mineralization ALP catalyses hydrolysis of organic phosphatase esters such as beta-glycerophosphate, increasing the local concentration of inorganic phosphate required for bone mineralization (Anderson et al., 2005; Bosco et al., 2014).

To sum up, the gender of horses significantly affects erythrocyte indices, as evidenced by their significantly higher values in the stallions as compared to the mares, as well as by the interactions between gender and type of use and between the gender and age of the horse.

The leukocyte and neutrophil counts increase with the age of horses, and the interaction between age and type of use (A×U) indicates that the leukocyte and neutrophil counts in training horses are closely linked to their age. Systematic physical exercise in sport horses increases leukocyte and lymphocyte counts and contributes to osteogenesis (increase in ALP activity and plasma content of calcium and phosphorus), which has a beneficial effect on their health. The correlations obtained may improve breeders' awareness of the effect of various factors, such as age, gender or type of use, on blood indices in horses and can be helpful in evaluating the health of Małopolski horses.

References

Anderson H.C., Garimella R., Tague S.E. (2005). The role of matrix vesicles in growth plate development and biomineralization. Front. Biosci., 10: 822–837.

Bachman E., Feng R., Travison T., Li M., Olbina G., Ostland V., Ulloor J., Zhang A., Basaria S., Ganz T., Westerman M., Bhasin S. (2010). Testosterone suppresses hepcidin in men: a potential mechanism for testosterone-induced erythrocytosis. J. Clin. Endocrinol. Metab., 95: 4743–4747.

Bis-Wencel H., Lutnicki K., Rowicka A.Z., Bryl M. (2011). Long-term exercise and its effect on selected hematological parameters of blood in horses. Med. Weter., 67: 418–421.

Bis-Wencel H., Lutnicki K., Rowicka A.Z., Nowakowicz-Dębek B., Bryl M. (2012). Effort of varying intensity as a factor influencing the variability of selected biochemical blood parameters of jumping horses. Bull. Vet. Inst. Pulawy, 56: 225–229.

Bosco R., Leeuwenburgh S.C.G., Jansen J.A., van den Beucken J.J.J.P. (2014). Configurational effects of collagen/ALP coatings on enzyme immobilization and surface mineralization. Appl. Surf. Sci., 311: 292–299.

Brzóska F., Strzetelski J.A., Borowiec F., Jamroz D. (2015). Feeding recommendations for horses and feed tables (in Polish). Kraków, Poland, 2015.

Burlikowska K., Bogusławska-Tryk M., Szymeczko R., Piotrowska A. (2015). Hematological and biochemical blood parameters in horses used for sport and recreation. J. Cent. Eur. Agric., 16: 370–382.

Czech A., Kiesz M., Ognik K., Różański P. (2016). Influence of the manner of use, age and sex on the biochemical and antioxidant blood parameters of Malopolski horses. Med. Weter., 72: 652–655.

Fielding C.L., Magdesian K.G., Rhodes D.M., Meier C.A., Higgins J.C. (2009). Clinical and biochemical abnormalities in endurance horses eliminated from competition for medical complications and requiring emergency medical treatment: 30 cases (2005–2006). J. Vet. Emerg. Crit. Car., 19: 473–478.

Friedewald W.T., Levy R.I., Fredrickson D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499–502.

Górecka-Bruzda A., Suwała M., Kamińska B., Jaworski Z., Jezierski T. (2015). Profiles of oestradiol, testosterone and androstenedione in stable vs. forest born young Konik Polski horses. Anim. Sci. Pap. Rep., 33: 137–146.

Grela R., Pastuszak J., Czech A., Kempa J. (2003). Influence of sex and feeding season on the hematological and biochemical parameters of Thoroughbred blood foals (in Polish). Ann. UMCS section EE, 21: 199–205.

Jawor P., Stefaniak P., Borkowski J., Przewoźny M., Wierzbicki H., Swagemakers J.H. (2007). Utility of selected blood parameters in monitoring the accuracy of show jump-horse training. Med. Weter., 63: 213–218.

Lacerda L., Campos R., Sperb M., Soares E., Barbosa E., Rerreira R., Santos V., González F.H. (2006). Hematological and biochemical parameters in three high performance horse breeds from southern Brazil. Arch. Vet. Sci., 11: 40–44.

Latimer K.S. (1999). Leukocytic hematopoiesis. In: Equine medicine and surgery. Mosby, St. Louis, USA, 1992–2001.

Neuberg K., Geringer de Oedenberg H. (2007). The influence of training on the hematological parameters of sport horses (in Polish). Acta. Sci. Pol. Zootechn., 6: 59–68.

Neuberg-Zuchowicz K., Geringer de Oedenberg H. (2011). Changes in hematological parameters of show jumping horses during early training cycle. Med. Weter., 67: 765–769.

Padalino B., Rubino G., Centoducati P., Petazzi F. (2007). Training versus overtraining: evaluation of protocols. J. Equine Vet. Sci., 27: 28–31.

Satué K., Hernández A., Lorente C., O'Coonor J.E. (2010). Immunophenotypical characterization in Andalusian horse: variations with age and gender. Vet. Immunol. Immunop., 133: 219–227.

Satué K., Hernández A., Muñoz A. (2012). Physiological factors in the interpretation of equine hematological profile, Hematology – Science and Practice. Available from: http://www.intechopen.com/books/hematology-science-and-practice/haematologicalprofile-of-the-horse-phisiological-factors-influencing-equine-haematology, Intech.

Smith R., Chaffin K., Cohen N., Martens R.J. (2002). Age-related changes in lymphocyte subset in Quarter Horse foals. Am. J. Vet. Res., 63: 531–536.

Tateo A., Valle E., Padalino B., Centoducati P., Bergero D. (2008). Change in some physiologic variables induced by Italian traditional conditioning in standardbred yearling. J. Equine Vet. Sci., 28: 743–750.

Tomczyk-Wrona I. (2014). Contribution of founding breeds to Małopolski stallions qualified for inclusion in the genetic resources conservation programme for Małopolski horses (in Polish). Wiad. Zoot., 4: 125–135.

Trigo P., Castejón F.M., Riber C., Muñoz A. (2010). Use of biochemical parameters to predict metabolic elimination in endurance rides. Equine Vet. J., 42: 142–146.

Vergara F., Tadich T.A. (2015). Effect of the work performed by tourism carriage horses on physiological and blood parameters. J. Equine Vet. Sci., 35: 213–218.

Wanderley E.K., Bem B.S.C., Melo S.K.M., Gonzales J.C., Manso H.E.C.C.C., Manso Filho H.C. (2015). Hematological and biochemical changes in Mangalarga Marchador horses after a four-beat gait challenge in three different distances. J. Equine Vet. Sci., 35: 259–263.

W e l l e s E. (2000). Clinical interpretation of equine leucograms. In: Schalm's Veterinary Hematology. Williams & Wilkins, Philadelphia, UK, pp. 405–410.
W i n n i c k a A. (2015). Reference values in basic laboratory analyses in veterinary medicine (in Polish). Warsaw Agricultural University Publishing, Warsaw, Poland.
Y a q u b L.S., K a w u M.U., A y o J.O. (2013). Influence of reproductive cycle, sex, age and season on haematologic parameters in domestic animals: a review. J. Cell. Anim. Biol., 7: 37–43.

Received: 29 XII 2017
Accepted: 11 VI 2018