

Haematological and biochemical blood parameters in horses used for sport and recreation

Hematologiczne i biochemiczne parametry krwi koni użytkowanych sportowo i rekreacyjnie

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Abstract

The study was conducted to determine and compare the resting values of haematological and biochemical blood indices in clinically healthy horses competing in show jumping and used for leisure activities. The experiment included twenty adult horses of Polish half-bred and Wielkopolska breed which were subjected to a daily training programme typical of their type of use. Blood samples were collected at rest before feeding from the jugular external vein. Show jumpers had higher ($P < 0.05$) red blood cell count, haematocrit value and haemoglobin concentration as well as the serum level of total protein, albumin, α_2 - and γ -globulin, HDL and iron. The level of β -globulin, magnesium and activity of creatine kinase were significantly higher in recreationally used horses. There were no differences in the values of red blood cell parameters (MCV, MCH, MCHC), number of leucocytes and the percentage of their respective kinds, platelets, concentration of urea, creatinine, glucose, triglycerides, total cholesterol, LDL, activity of AST, ALT, LDH and in the content of Na, K, Cl, P, Ca between the tested groups. The results of the present study indicate that the type of training influences some of the resting haematological and biochemical indices in adult horses. Horses used for show jumping have higher aerobic capacity and show a more beneficial lipid profile compared to horses used for recreation.

Keywords: horse, recreation, resting blood indices, show jumping

Streszczenie

Celem badań było określenie i porównanie spoczynkowych wartości wskaźników hematologicznych i biochemicznych krwi u koni biorących udział w zawodach skokowych i koni użytkowanych rekreacyjnie. Badania przeprowadzono na 20 dorosłych, klinicznie zdrowych koniach rasy wielkopolskiej oraz polskich koniach szlachejnych półkrwi. Konie poddawano codziennemu programowi treningowemu, odpowiadającemu rodzajowi ich użytkowania. Krew do badań pobrano w spoczynku w godzinach rannych przed karmieniem, z żyły szyjnej zewnętrznej. U koni użytkowanych sportowo w pełnej krwi stwierdzono wyższą ($P < 0,05$) liczbę krwinek czerwonych, wartość hematokrytu i koncentrację hemoglobiny, jak również wyższy poziom białka całkowitego, albumin, α_2 - i γ -globulin, HDL i żelaza w surowicy krwi. Poziom β -globulin, magnezu i aktywność kinazy kreatynowej były istotnie wyższe u koni użytkowanych rekreacyjnie. Nie odnotowano istotnych różnic w wartościach parametrów czerwonych (MCV, MCH, MCHC), liczbie leukocytów i udziale procentowym poszczególnych form krwinek białych w ogólnej ich ilości oraz liczbie płytek krwi jak również w koncentracji mocznika, kreatyniny, glukozy, triglicerydów, cholesterolu całkowitego, frakcji LDL oraz aktywności AST, ALT, LDH i zawartości Na, K, Cl, P, Ca pomiędzy badanymi grupami. Wyniki badań wskazują, że rodzaj stosowanego treningu wpływa na spoczynkowe wartości hematologiczne i biochemiczne krwi dorosłych koni. Konie wykorzystywane w skokach przez przeszkody charakteryzują się lepszą wydolnością tlenową i korzystniejszym profilem lipidowym krwi w porównaniu do koni użytkowanych rekreacyjnie.

Keywords: koń, spoczynkowe wskaźniki krwi, użytkowanie rekreacyjne, użytkowanie sportowe

Detailed abstract

Celem badań było określenie i porównanie spoczynkowych wartości wskaźników hematologicznych i biochemicznych krwi u koni sportowych, biorących udział w zawodach skokowych i koni użytkowanych rekreacyjnie. Badania przeprowadzono na 20 dorosłych, klinicznie zdrowych koniach rasy wielkopolskiej oraz polskich koniach szlachejnych półkrwi, pochodzących ze stajni sportowej i ośrodka rekreacji konnej woj. kujawsko-pomorskiego. W zależności od typu użytkowania i typu treningu zwierzęta podzielono na 2 grupy ($n=10$): konie sportowe, biorące czynny udział w zawodach w konkurencji skoków przez przeszkody (8 klaczy i 2 wałachy; średni wiek konia: 10 lat) i konie rekreacyjne, użytkowane do jazd spacerowych (7 klaczy i 3 wałachy; średni wiek konia: 11 lat). Wszystkie konie poddawane były codziennemu programowi szkolenia, którego natężenie odpowiadało rodzajowi ich użytkowania. Badania przeprowadzono w okresie zimowym, w połowie sezonu intensywnego użytkowania koni sportowych na zawodach halowych. Zwierzęta, utrzymywane w indywidualnych boksach, karmione były 3 razy dziennie paszami tradycyjnymi: owsem, sianem, lucerną i marchwią, z uwzględnieniem ich indywidualnych potrzeb.

Dieta koni sportowych uzupełniana była dodatkowo mieszankami mineralno-witaminowymi. Skład diet pokrywał dzienne zapotrzebowanie na energię, białko, składniki mineralne i witaminy, zgodnie z normami żywienia koni (Chachułowa et al., 1997). Zwierzęta miały nieograniczony dostęp do wody. Krew do badań pobierano w spoczynku, w godzinach rannych (7:00-8:00) przed karmieniem, z żyły szyjnej zewnętrznej. W próbkach pełnej krwi, przy użyciu analizatora hematologicznego, oznaczono: liczbę krwinek czerwonych (RBC), hematokryt (HCT), koncentrację hemoglobiny (HGB), wskaźniki czerwonekrwinkowe (MCV, MCH, MCHC), ilość płytek krwi (PLT), liczbę krwinek białych (WBC) oraz udział procentowy poszczególnych form krwinek białych w ogólnej ich ilości. W surowicy krwi, przy użyciu analizatora biochemicznego, oznaczono: białko całkowite (TP) i jego frakcje, mocznik, kreatyninę, triglicerydy, cholesterol całkowity, frakcje HDL i LDL cholesterolu, zawartość składników mineralnych (Na, K, Cl, P, Ca, Mg i Fe) oraz aktywność kinazy kreatynowej (CK), dehydrogenazy mleczanowej (LDH), aminotransferazy asparaginowej (AST) i alaninowej (ALT). W osoczu krwi oznaczono zawartość glukozy. Wyniki poddano analizie statystycznej przy użyciu programu STATISTICA 8 software (StatSoft, Inc.®), z zastosowaniem testu t-Studenta dla prób niezależnych ($P < 0,05$).

U koni użytkowanych sportowo stwierdzono wyższą ($P < 0,05$), spoczynkową liczbę krwinek czerwonych, wartość hematokrytu i koncentrację hemoglobiny ($8,46 \text{ T} \cdot \text{l}^{-1}$; $0,37 \text{ l} \cdot \text{l}^{-1}$ i $8,75 \text{ mmol} \cdot \text{l}^{-1}$, odpowiednio) w porównaniu do koni użytkowanych rekreacyjnie ($7,49 \text{ T} \cdot \text{l}^{-1}$; $0,33 \text{ l} \cdot \text{l}^{-1}$ i $7,90 \text{ mmol} \cdot \text{l}^{-1}$, odpowiednio). Nie stwierdzono istotnych różnic w wartościach MCV, MCH i MCHC oraz w liczbie PLT, WBC i udziale procentowym poszczególnych form krwinek białych w ogólnej ich ilości. U koni intensywnie użytkowanych sportowo, w porównaniu do koni rekreacyjnych, odnotowano również istotnie wyższy poziom TP, albumin, α_2 - i γ -globulin w surowicy krwi ($64,07 \text{ g} \cdot \text{l}^{-1}$; $31,63 \text{ g} \cdot \text{l}^{-1}$; $10,46 \text{ g} \cdot \text{l}^{-1}$ i $12,17 \text{ g} \cdot \text{l}^{-1}$ oraz $60,82 \text{ g} \cdot \text{l}^{-1}$; $29,44 \text{ g} \cdot \text{l}^{-1}$; $8,62 \text{ g} \cdot \text{l}^{-1}$ i $8,82 \text{ g} \cdot \text{l}^{-1}$, odpowiednio). Koncentracja β -globulin była istotnie wyższa w krwi koni rekreacyjnych ($12,61 \text{ g} \cdot \text{l}^{-1}$ i $8,56 \text{ g} \cdot \text{l}^{-1}$, odpowiednio u koni użytkowanych rekreacyjnie i sportowo). Nie odnotowano istotnych różnic pomiędzy grupami koni w koncentracji kreatyniny, mocznika jak również w aktywności badanych enzymów w surowicy krwi, za wyjątkiem aktywności CK. Spoczynkowa zawartość glukozy, triglicerydów oraz cholesterolu całkowitego i frakcji LDL była wyrównana w krwi koni sportowych i rekreacyjnych, natomiast poziom frakcji HDL cholesterolu był wyższy ($P < 0,05$) u koni sportowych ($1,41 \text{ mmol} \cdot \text{l}^{-1}$ i $1,30 \text{ mmol} \cdot \text{l}^{-1}$, odpowiednio). U koni użytkowanych sportowo stwierdzono również w surowicy krwi istotnie wyższą spoczynkową koncentrację Fe i niższy ($P < 0,05$) poziom Mg w porównaniu do koni rekreacyjnych. Nie odnotowano wpływu typu użytkowania koni na zawartość pozostałych elektrolitów (Na, K, Cl, P i Ca) w surowicy krwi. Wyniki badań wskazują, że rodzaj stosowanego treningu wpływa na spoczynkowe wartości hematologiczne i biochemiczne krwi dorosłych koni. Konie wykorzystywane w skokach przez przeszkody charakteryzują się lepszą wydolnością tlenową i korzystniejszym profilem lipidowym krwi w porównaniu do koni użytkowanych rekreacyjnie.

Introduction

Haematological and biochemical blood analysis provides significant information about the health status of an animal, metabolic changes in its organism and it is often helpful in revealing health disorders already in the preclinical stage. In horses, one of the factors influencing the blood parameters is the type of exercise, its intensity, duration and frequency (Szarska, 2003; Krumrych, 2006; Neuberg-Zuchowicz and Geringer de Oedenberg, 2011; Bis-Wencel et al., 2012; Cieřła et al., 2013). Regular blood testing performed at rest allows for the assessment of horse response to the activity and evaluation of the applied training methods, whereas a single examination of resting blood indices is used mainly for comparing the values with physiological norms. A horse having all the blood parameters in accordance with reference values has a much greater chance of achieving satisfactory sport results and it regenerates much quicker after physical effort (Szarska, 2003). Horses used for sport as well as recreation are subjected to physical effort but the type of the training and its intensification are different. Show jumping is a relatively new but currently the most popular equestrian sport in which, besides proper jump technique, flexibility, speed, endurance and high fitness level are also required of a horse (Jawor et al., 2007).

Compared to sport horses, physical effort of recreationally used horses is generally less intensive but these animals must satisfy various needs of different people (often unskilled) which can be a source of stress and psychic burden (Bis-Wencel et al., 2009). Despite the fact that nowadays there is a growing interest in recreational and therapeutic use of horses, only a few studies presenting blood parameters in those groups of animals have been performed so far (Bis-Wencel et al., 2009; Cieřła et al., 2013). Thus, the aim of the present study was to determine and compare the values of resting selected haematological and biochemical parameters in horses used for show jumping and recreation.

Materials and Methods

Experimental animals

The experiment was carried out with the permission from the Local Ethical Committee on Animal Testing at the University of Technology and Life Sciences in Bydgoszcz (Resolution No. 1 dated January 24, 2013). The study was conducted in a private horse riding centre on 20 adult horses of Polish half-bred and Wielkopolska breed. Depending on the type of use and the applied training methods, the horses were divided into two groups (n=10): sport horses actively competing in show-jumping discipline (8 mares and 2 geldings, mean age 10 years) and recreational horses used for pleasure riding (7 mares and 3 geldings, mean age 11 years). The horses were subjected to a daily training programme typical of their type of use. The experiment was conducted in the winter period in the middle of an intensive indoor season. The sport horses were trained every day for about one hour with a conventional programme, consisting of, on a weekly basis: 2 light dressage days, 1 light jumping day or 1 day off, 1 intense jumping day and they participated in indoor show jumping competitions at the weekends. The training for the second group of horses lasted for one hour from Tuesday to Sunday and included walking, trotting and galloping. Monday was the day off for the group of recreational horses. The

experimental animals were clinically healthy and remained under constant veterinary supervision. All horses were kept in a stable, in individual boxes on straw bedding, under appropriate welfare standards recommended for this animal species. Diets were formulated to meet the energy, protein, mineral, and vitamin requirements stated in horse feeding standards (Chachulowa et al., 1997). The animals were fed according to their individual needs, three times a day, traditionally with oat, hay, lucerne, and carrot. The diet for sport horses was additionally supplemented with mineral-vitamin mixture. Drinking water was given ad libitum.

Blood sampling, haematological and biochemical analyses

Blood samples (10 ml) were collected from each horse at rest in the morning (7:00-8:00) before feeding from the external jugular external vein by the veterinarian, which ensured stress-free course of the treatment. The following haematological parameters were determined in the whole blood (anticoagulant: dipotassium ethylene diamine tetraacetic acid, K₂EDTA) with the use of the haematology analyser ADVIA 2120 (Siemens AG, Munich, Germany): red blood cell count (RBC), haematocrit (HCT), haemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), number of platelets (PLT), white blood cell count (WBC) and the percentage of particular kinds of WBC in the total number of leucocytes. To obtain serum, the blood was collected into plain tubes without anticoagulant, whereas to obtain plasma the test tubes containing sodium fluoride (NaF) as a stabilizer were used. Blood samples were centrifuged at 3,000 g for 15 min, and serum and plasma were obtained immediately. In the blood serum, the following parameters were determined: total protein (TP) and its fractions (albumin, α -, β - and γ - globulin), urea, creatinine, triglycerides, total cholesterol, high-density lipoproteins (HDL), sodium (Na), potassium (K), chloride (Cl), phosphorus (P), calcium (Ca), magnesium (Mg), iron (Fe) and activity of creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH). The content of glucose was determined in the blood plasma. All the above mentioned biochemical parameters were determined with the use of the OLYMPUS AU 680 analyzer (Beckman Coulter). The content of low-density lipoproteins (LDL) in the blood serum was calculated on the basis of Friedewald equation (Friedewald et al., 1972).

Statistical analysis

Statistical analysis of the data were performed using the STATISTICA 8 software (StatSoft, Inc.®). The results obtained were verified using Student's t-test for independent samples and the level of significance was set at $P < 0.05$. The presented results are expressed as mean and standard error of the mean (SEM).

Results

The haematological parameters determined in the present study are presented in Table 1. Show jumping horses had significantly higher resting RBC count, HCT value and HGB concentration compared to horses used for recreation. The values of MCV,

MCH and MCHC did not differ between groups of the tested animals. Moreover, there were no significant differences in the number of platelets, leucocytes and their types (lymphocytes, neutrophils, eosinophils and monocytes).

Table 1. Haematological parameters of show jumping and recreationally used horses (mean \pm SEM)

| Parameter | Group of horses | | Significance level |
|-----------------------------------|--------------------|--------------------|--------------------|
| | Show jumping | Recreational | |
| Red blood cells, $T \cdot l^{-1}$ | 8.46 \pm 0.32 | 7.49 \pm 0.17 | P<0.05 |
| Haematocrit, $l \cdot l^{-1}$ | 0.37 \pm 0.01 | 0.33 \pm 0.01 | P<0.05 |
| Haemoglobin, $mmol \cdot l^{-1}$ | 8.75 \pm 0.24 | 7.90 \pm 0.23 | P<0.05 |
| MCV, fl | 44.17 \pm 1.05 | 44.46 \pm 1.00 | NS ^a |
| MCH, pg | 16.75 \pm 0.43 | 17.02 \pm 0.40 | NS |
| MCHC, $g \cdot dl^{-1}$ | 37.88 \pm 0.20 | 38.28 \pm 0.17 | NS |
| Leucocytes, $G \cdot l^{-1}$ | 5.89 \pm 0.27 | 6.65 \pm 0.33 | NS |
| Lymphocytes, % | 45.60 \pm 2.66 | 37.30 \pm 3.86 | NS |
| Neutrophils, % | 52.40 \pm 2.70 | 59.10 \pm 3.78 | NS |
| Eosinophils, % | 1.10 \pm 0.28 | 2.10 \pm 0.55 | NS |
| Monocytes, % | 0.90 \pm 0.31 | 1.50 \pm 0.48 | NS |
| Platelets, $G \cdot l^{-1}$ | 129.90 \pm 13.75 | 117.20 \pm 11.95 | NS |

^anot statistically significant, MCV-mean corpuscular volume, MCH-mean corpuscular haemoglobin, MCHC-mean corpuscular haemoglobin concentration

The blood biochemical parameters determined in the tested animals are shown in Table 2. Significantly higher levels of TP, albumin, α_2 - and γ -globulin were determined in the serum of sport horses, whereas β -globulin fractions were higher (P<0.05) in horses used in recreation. The concentrations of creatinine, urea as well as the activities of the determined enzymes (except for CK) did not differ between the tested groups. The content of blood glucose, triglycerides, total and LDL cholesterol were similar in both groups of horses but the HDL fraction was significantly higher in show jumping horses. The sport horses were characterised also by higher Fe and lower Mg concentrations compared to the recreationally used horses. There was no effect of the type of horse use on the level of Na, K, Cl, P and Ca.

Table 2. Blood biochemical parameters of show jumping and recreationally used horses (mean \pm SEM)

| Parameter | Group of horses | | Significance level |
|---|--------------------|--------------------|--------------------|
| | Show jumping | Recreational | |
| Total protein, g \cdot l $^{-1}$ | 64.07 \pm 1.01 | 60.82 \pm 0.80 | P<0.05 |
| Albumin, g \cdot l $^{-1}$ | 31.63 \pm 0.40 | 29.44 \pm 0.66 | P<0.05 |
| Globulin, g \cdot l $^{-1}$ | 32.44 \pm 1.08 | 31.38 \pm 0.84 | NS ^a |
| - α_1 -globulin, g \cdot l $^{-1}$ | 1.26 \pm 0.06 | 1.33 \pm 0.04 | NS |
| - α_2 -globulin, g \cdot l $^{-1}$ | 10.46 \pm 0.51 | 8.62 \pm 0.26 | P<0.05 |
| - β_1 -globulin, g \cdot l $^{-1}$ | 5.66 \pm 0.34 | 7.61 \pm 0.53 | P<0.05 |
| - β_2 -globulin, g \cdot l $^{-1}$ | 2.90 \pm 0.44 | 5.00 \pm 0.38 | P<0.05 |
| - γ -globulin, g \cdot l $^{-1}$ | 12.17 \pm 1.06 | 8.82 \pm 0.57 | P<0.05 |
| Albumin: Globulin | 0.99 \pm 0.04 | 0.95 \pm 0.04 | NS |
| Urea, mmol \cdot l $^{-1}$ | 5.26 \pm 0.20 | 5.26 \pm 0.12 | NS |
| Creatinine, μ mol \cdot l $^{-1}$ | 101.84 \pm 2.38 | 105.16 \pm 2.62 | NS |
| Glucose, mmol \cdot l $^{-1}$ | 4.45 \pm 0.07 | 4.49 \pm 0.07 | NS |
| Triglycerides, mmol \cdot l $^{-1}$ | 0.28 \pm 0.03 | 0.25 \pm 0.02 | NS |
| Total cholesterol, mmol \cdot l $^{-1}$ | 2.39 \pm 0.09 | 2.12 \pm 0.10 | NS |
| HDL, mmol \cdot l $^{-1}$ | 1.41 \pm 0.04 | 1.30 \pm 0.03 | P<0.05 |
| LDL, mmol \cdot l $^{-1}$ | 0.92 \pm 0.06 | 0.77 \pm 0.07 | NS |
| CK, IU \cdot l $^{-1}$ | 197.20 \pm 8.58 | 288.00 \pm 12.69 | P<0.05 |
| AST, IU \cdot l $^{-1}$ | 325.80 \pm 15.10 | 378.80 \pm 29.90 | NS |
| ALT, IU \cdot l $^{-1}$ | 8.80 \pm 0.61 | 8.10 \pm 0.62 | NS |
| LDH, IU \cdot l $^{-1}$ | 335.60 \pm 24.80 | 363.30 \pm 21.74 | NS |
| Sodium, mmol \cdot l $^{-1}$ | 138.30 \pm 0.40 | 137.30 \pm 0.34 | NS |
| Potassium, mmol \cdot l $^{-1}$ | 3.76 \pm 0.08 | 3.96 \pm 0.08 | NS |
| Chloride, mmol \cdot l $^{-1}$ | 101.90 \pm 0.67 | 100.60 \pm 0.37 | NS |
| Phosphorus, mmol \cdot l $^{-1}$ | 1.11 \pm 0.05 | 1.02 \pm 0.09 | NS |
| Calcium, mmol \cdot l $^{-1}$ | 3.03 \pm 0.03 | 3.10 \pm 0.03 | NS |
| Magnesium, mmol \cdot l $^{-1}$ | 0.70 \pm 0.02 | 0.83 \pm 0.03 | P<0.05 |
| Iron, μ mol \cdot l $^{-1}$ | 32.17 \pm 1.79 | 24.88 \pm 1.69 | P<0.05 |

^anot statistically significant

HDL-high density lipoproteins, LDL-low density lipoproteins, CK-creatine kinase, AST-aspartate aminotransferase, ALT- alanine aminotransferase, LDH- lactate dehydrogenase

Discussion

The haematological parameters constitute the first blood indices applied for the assessment of horse health, performance and a training degree. Majority of haematological indices determined in the present study were within the wide range of reference values specified for horses (Winnicka, 2011), which proves good health and proper condition of the tested animals. The values of RBC count, HCT value and HGB concentration determined in the current experiment for sport horses were close to those obtained in earlier studies. The resting values of red blood cells, haematocrit and haemoglobin in jumping horses of different breeds and age cited by other authors were within the ranges: $7.32-8.42 \text{ T}^* \text{I}^{-1}$, $0.33-0.39 \text{ l}^* \text{I}^{-1}$ and $6.11-8.27 \text{ mmol}^* \text{I}^{-1}$, respectively (Szarska, 2003; Krumrych, 2006; Jawor et al., 2007; Neuberg and Geringer de Oedenberg, 2007; Neuberg-Zuchowicz and Geringer de Oedenberg, 2011). However, the values of red blood cell indices for recreationally used horses were generally lower than values obtained in the previous studies (RBC $8.22-9.54 \text{ T}^* \text{I}^{-1}$, HCT $0.39-0.48 \text{ l}^* \text{I}^{-1}$ and HGB $8.02-14.86 \text{ mmol}^* \text{I}^{-1}$) (Bis-Wencel et al., 2009; Cieřła et al., 2013). The resting HCT value and HGB concentration give information on blood oxygen capacity. Significant increase of the above mentioned parameters measured at rest are noted in sport horses (especially mares and geldings) under the influence of an intensive training process as a result of regular repeated hypoxia stimulating erythropoiesis and causing increase in the number of circulating erythrocytes (Neuberg-Zuchowicz and Geringer de Oedenberg, 2011). Oxygen transport, in turn, is improved and buffer capacity of the blood is enhanced, which results in the body's adaptation to increased exercise workload (Neuberg and Geringer de Oedenberg, 2007). This could be the explanation for the differences in RBC, HCT and HGB values between sport and recreational horses noted in the current study.

Red blood cell parameters (MCV, MCH, MCHC) indicate efficiency of haemoglobin synthesis and its oxygen transport capacity. Neuberg-Zuchowicz and Geringer de Oedenberg (2011) determined, in mares and geldings of show jumping horses, higher resting values of MCV ($46.30-47.40 \text{ fl}$), similar MCH ($16.30-16.50 \text{ pg}$) and lower MCHC ($33.70-35.30 \text{ g}^* \text{dl}^{-1}$) compared to our results obtained for sport horses. The values obtained for recreationally used horses in this study differed considerably from those presented by Bis-Wencel et al. (2009) (MCV 50.37 fl ; MCH 15.60 pg ; MCHC $30.90 \text{ g}^* \text{dl}^{-1}$).

The present study did not show significant differences in the resting WBC count and their kinds between sport and recreational horses. Both groups of the tested animals had lower WBC count than results presented by other authors ($6.30-8.00 \text{ G}^* \text{I}^{-1}$ for show jumping horses and $7.00-7.50 \text{ G}^* \text{I}^{-1}$ for recreational horses) (Krumrych, 2006; Jawor et al., 2007; Neuberg and Geringer de Oedenberg, 2007; Bis-Wencel et al., 2009; Cieřła et al., 2013). According to Dmoch et al. (2008), the total number of leucocytes depends on the season and the lowest values are noted in the winter period which corresponds to the relatively low WBC count determined in our experiment. In the present study, neutrophils constituted more than 50% of WBC in both groups of horses, which agrees with the results obtained by Krumrych (2006) and Bis-Wencel et al. (2009). PLT count in both groups of animals was lower than the reference values given by Winnicka (2011) ($150-400 \text{ G}^* \text{I}^{-1}$) and it was similar to the resting data presented by Jawor et al. (2007) ($112-115 \text{ G}^* \text{I}^{-1}$). Cieřła et al. (2013)

reported that horses used recreationally were characterized by quite broad PLT count range (from 99 to 563 $G \cdot l^{-1}$) and no external symptoms of disease were observed in these animals.

Similarly to haematological indices, also biochemical parameters obtained in the present study were generally in accordance with physiological norms for horses (Winnicka, 2011). The TP concentration is one of more steady blood parameters and the analysis of serum protein fractions is a useful screening test in equine medicine used as a sensitive indicator to assess changes or pathological states in liver function and the immune system (Riond et al., 2009; Abeni et al., 2013). Albumin is the main (48-76% of TP) and the most osmotically active protein fraction of a horse serum (Winnicka, 2011) whereas a globulin fraction is a heterogeneous group of blood proteins including carrier proteins, enzymes, immunoglobulins and other inflammatory molecules (Abeni et al., 2013). Previous studies demonstrate that the resting values of TP obtained in healthy show jumping horses of different age, sex and breed range from 62.5 to 75.0 $g \cdot l^{-1}$ (Lekeux et al., 1991; Aguilera-Tejero et al., 2000; Szarska, 2003; Carapeto et al., 2006; Jawor et al., 2007; Soares et al., 2011). According to Szarska (2003), show jumping horses have a higher content of TP measured at rest (67.1 $g \cdot l^{-1}$) compared to race horses (60.2-64.2 $g \cdot l^{-1}$) but there are no significant differences among jumpers and horses used in other equestrian sports. In the current study, significantly higher levels of TP and albumin were determined in the blood of sport horses compared to recreationally used horses. The content of γ -globulin in sport horses was at the lower limits of the standards while the other group of animals was characterized by too low concentration of γ -globulin concerning the limits given by Winnicka (2011) as normal (12.0-40.0 $g \cdot l^{-1}$). In the study by Aguilera-Tejero et al. (2000) on show jumping horses, the higher, compared to our results, resting levels of serum albumin and globulin were determined (35.0 and 40.0 $g \cdot l^{-1}$, respectively). According to Carapeto et al. (2006), the resting levels of albumin, β_1 - and β_2 -globulins can increase as a consequence of different disorders of the locomotor system to which especially sport horses are exposed. Despite the significant differences in concentrations of protein fractions, no symptoms of any disorders were observed in the experimental horses used in our study. In the available literature, there is no information on the level of total protein and its fractions in recreationally used horses. In the experiment by Abeni et al. (2013), healthy, mature horses used in slight exercise activities (on average 1 hour per day) had considerably higher, compared to our results, levels of total protein, albumin and γ -globulin, similar content of α_1 - and α_2 -globulins and almost twice lower level of β -globulin.

Serum urea and creatinine concentrations are common indices reflecting protein metabolism and efficiency of renal functions. Moreover, creatinine is a breakdown product of muscular creatine phosphate and its level depends directly on muscle mass and its activity. In the present study, the concentrations of these indices did not differ between the tested groups. In the experiment on jumping horses within the spring-winter training season, values of resting blood creatinine, generally higher (98.12-126.41 $\mu mol \cdot l^{-1}$) in comparison to our results, were determined and no negative impact of different workload was found (Bis-Wencel et al., 2012).

Creatine kinase, lactate dehydrogenase and, to a lesser extent, aspartate aminotransferase are the most muscle-specific enzymes. Under normal conditions,

the activities of muscle enzymes in the blood plasma are maintained on a certain level and their increase is considered a symptom of muscle damage. Moreover, AST and ALT are the enzymes indicating proper functions of liver which plays a vital role in the exercise metabolism (Szarska, 2003). In our experiment, the activities of these enzymes did not differ between jumping and recreational horses. LDH activities were considerably lower than wide reference values given by Winnicka (2011) ($520-1480 \text{ U} \cdot \text{l}^{-1}$) but according to Dmoch et al. (2008), in laboratory diagnostics, the decrease in LDH activity is not of great importance. Lower, compared to standards, activities of LDH measured at rest were also obtained by Soares et al. (2011) in horses submitted to jumping competitions. In the current experiment, a significantly higher activity of CK was determined in recreationally used horses compared to show jumpers but the values were still within the limits considered to be standards (Winnicka, 2011). The earlier studies on horses participating in show jumping revealed lower resting activities of CK ($118.0-169.9 \text{ U} \cdot \text{l}^{-1}$) (Jawor et al., 2007; Soares et al., 2011; Bis-Wencel et al., 2012). It is difficult to explain a considerably high CK activity determined in recreationally used horses of our experiment. To properly interpret CK activity, different factors, such as the horse health state, pathological symptoms as well as type and intensity of training, should be taken into consideration (Bis-Wencel et al., 2012). Increase in CK activity can be the first symptom of overtraining especially when it is accompanied by the increase in ALT activity which was not detected in recreationally used horses in the present experiment (Bis-Wencel et al., 2012). However, gradual increase in resting values of CK can indicate muscle microdamages and should prompt to make a decision to reduce the intensity of workload for sport horses (Jawor et al., 2007).

Glucose and lipid metabolism indices reflect energy economy and its fluctuations in a living organism. The resting level of blood glucose is fairly stable and it generally ranges from 3.1 to $6.2 \text{ mmol} \cdot \text{l}^{-1}$ (Winnicka, 2011) but in sport horses the lower limit is usually higher ($5.0 \text{ mmol} \cdot \text{l}^{-1}$) (Szarska, 2003). In our experiment, the concentrations of blood glucose were similar in both groups of horses but they were slightly below the lower limit established for sport horses and the values reported by other authors in show jumpers at rest (Lekeux et al., 1991; Szarska, 2003). The type of training and intensity of physical effort may influence the resting level of blood glucose which, was confirmed by significant differences between show jumping and race horses (5.6 vs. $6.1 \text{ mmol} \cdot \text{l}^{-1}$) reported by Szarska (2003). A significantly higher concentration of HDL fraction recorded in our experiment in show jumping horses could be a beneficial result of regularly performed more intensive physical activity. According to Bis-Wencel et al. (2012), such an increase is often accompanied by the reduction in total cholesterol, LDL and VLDL fractions. Also, in the experiment on 3-year-old Arabian stallions trained according to the common breaking program, a significant increase in HDL serum concentrations was recorded but the LDL level remained almost unchanged (Kędzierski and Podolak, 2001). It should be stressed that the lipid metabolism in horses differs compared to other animal species and the effect of physical activity on lipid levels in the blood has not been fully explained so far (Kędzierski and Podolak, 2002; Bis-Wencel et al., 2012).

Minerals play a key role in many physiological processes of a living organism including the maintenance of osmotic pressure, blood pH and mineral-water balance. We did not find any effect of the type of horse use on the level of Na, K, Cl, P and

Ca. All the above mentioned minerals were within the reference values (Winnicka, 2011) and similar to the results obtained in sport horses by other authors (Aguilera-Tejero et al., 2000; Szarska, 2003; Pogoda-Sewerniak et al., 2009). The Mg concentration was lower in show jumpers and amounted to $0.7 \text{ mmol} \cdot \text{l}^{-1}$, which is the lower limit of the reference value established for horses (Szarska, 2003; Winnicka, 2011). Compared to the recreationally used horses, show jumpers were characterized also by significantly higher, exceeding the reference values (Winnicka, 2011), level of Fe which was most probably associated with the greater intensification of erythropoiesis reflected in the higher values of RBC, HCT and HGB determined in this group of the tested animals. Similarly to our results, Pogoda-Sewerniak et al. (2009) also determined high, compared to norms, resting concentration of Fe in healthy adult horses ($29.33 \text{ } \mu\text{mol} \cdot \text{l}^{-1}$) while Dmoch et al. (2008) noted the values considerably lower than ours ($12.03\text{-}18.29 \text{ } \mu\text{mol} \cdot \text{l}^{-1}$).

In conclusion, the results of the present study indicate that the type of training influences some of the resting haematological and biochemical indices in adult horses. Compared to recreationally used horses, regular and more intense physical effort of show jumpers results in higher aerobic capacity reflected in significantly higher RBC count, HCT value and HGB and Fe concentrations. Sport horses are characterized also by higher concentration of total protein, albumin and γ -globulin. The lack of differences in total cholesterol concentration and higher level of HDL fraction determined in the blood of show jumpers indicate more beneficial lipid profile in sport horses.

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