

**SEASONAL VARIATIONS OF HEMATOLOGICAL INDICES
IN EQUINES INVOLVED IN RECREATIONAL HORSE RIDING**

Anastasiia Andriichuk¹, Halyna Tkachenko²

¹ *Institute of Animal Science, National Academy of Agrarian Sciences of Ukraine,
Gvardeyskoi Armii St. 3, p.d. Kulynychy, Kharkiv, Ukraine*

e-mail: anastasia.pohlyad@gmail.com

² *Institute of Biology and Environmental Protection, Pomeranian University in Słupsk,
ul. Arciszewskiego 22B, 76-200 Słupsk, Poland*

e-mail: tkachenko@apsl.edu.pl, biology.apsl@gmail.com

Abstract

Blood is an important and reliable medium for assessing the health status and performance level of horses. Variations in hematological parameters in horses are associated with several factors such as exercise and training, feeding, age, sex, breed, diurnal and seasonal variation, temperature and the physiological status etc. The objective of this study was to determine the influence of seasonality on hematological indices and osmotic resistance of erythrocytes in horses involved in recreational horseback ride before and after exercise. Thirteen healthy adult horses from Pomeranian regions in Poland (aged 9.5 ± 2.4 years) were used. All horses participated in recreational horseback riding. Blood samples were taken once per season for one year – in spring (April) and summer (July). Blood was drawn from jugular veins of the animals in the morning, 90 minutes after feeding, and immediately after exercise. The blood samples were assessed for haematocrit (HCT) value, haemoglobin concentration (HGB), the red blood cells (RBC) amount, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), the platelets (PLT) amount, platelets distribution width (PDW) and osmotic resistance of erythrocytes before and after exercise. Our results confirm that hematological parameters in equines involved in recreational horse riding are subjected to seasonal variations. Significant increase of RBC amount and hemoglobin level after exercise both in spring and summer seasons were observed ($p < 0.05$). In spring, exercise caused enhancement of the hematocrit level, the mean corpuscular volume and the mean corpuscular hemoglobin levels ($p < 0.05$). At the same time, post-exercise MCH were higher in the spring ($p < 0.05$) compared to summer season. However, pre-exercise MCHC level in the summer was lower ($p < 0.05$) compared to the spring season. Higher percent of hemolyzed erythrocytes in spring season probably caused by the liberation of red blood cells from spleen and/or the increase of oxygen consumption.

Key words: horses, hematological parameters, seasonal variations, exercises

INTRODUCTION

Recreational horseback riding widely used today as a non-competitive physical activity and allows to improve some health aspects both of horses and riders (Newsome et al. 2004, Andriichuk et al. 2014c). Horse-riding is a major tourist/recreational activity and takes place in a wide spectrum of environmental conditions in various countries (Newsome et al. 2004). Horse-riding tours and treks, for example, are widely marketed and available in Australia, USA, Canada, Spain, United Kingdom, Poland, Ukraine etc. (Newsome et al. 2004, Andriichuk et al. 2014c). Some geographic areas are more closely associated with the use of horses than others, e.g. Pomeranian and Carpathian regions in Poland. In addition, recreational horseback riding helps to develop health and promote to improve physical fitness, as a tourists horse-riders and horses too. Although recreational riding is a non-competitive activity, but physical fitness is necessary for a horses, especially if these horses used for long or difficult rides (Kimball 2005, Andriichuk et al. 2014c). Proper conditions and feeding help horses to meet the physical demands of recreational riding, but training and mental readiness also play important roles in preparing horses for ride (Kimball 2005, Andriichuk et al. 2014c).

Normal cell physiological processes, body composition, water and food intake, organ function, and diseases are subjected to periodic variations related to biological rhythms in a number of species, including human beings and horses (Maes et al. 1995, Piccione et al. 2002, Satué et al. 2013). Knowledge of hematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological conditions in horses. Previous study in horse hematology have revealed that interpretation of blood parameters is quite difficult, since variation in the blood is caused by internal and external factors (Satué et al. 2012).

It is well known that laboratory techniques of blood sampling, genetic properties, sex, population density, degree, physical effort and exercises, environmental stress, lack of food supply, seasonal variations and transportation could affect on hematological data (Krumrych 2006, Andriichuk et al. 2012, Satué et al. 2012, Satué et al. 2014, Andriichuk et al. 2016). The seasonal rhythms reflect the ability of endogenous adaptative mechanisms of animals to react in advance to environmental changes associated with seasons. Season is an exogenous factor that modulates the dynamic of blood components in horses (Satué et al. 2012, 2014). Recent studies of equine exercise physiology have focused mainly on determining the usefulness of biochemical and haematological parameters for evaluating physiological capacity and adaptation to increasing loads in horses of different breeds (Krumrych 2006, Satué et al. 2014). Therefore, the objective of this study is to determine the seasonal-induced variations of exercise impact on hematological indices and osmotic resistance of erythrocytes in horses involved in recreational horseback ride.

MATERIALS AND METHODS

Horses. Thirteen healthy adult horses from central Pomeranian region in Poland (village Strzelinko, N54°30'48.0" E16°57'44.9", Fig. 1), aged 9.5±2.4 years old, in-

cluding 5 Hucul pony, 2 Thoroughbred horses, 2 Anglo-Arabian horses and 4 horses of unknown breed, were used in this study. All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 8 AM and 6 PM, and water available *ad libitum*. All horses were thoroughly examined clinically and screened for hematological, biochemical and vital parameters, which were within reference ranges. The females were non-pregnant.

Exercise test. Training started at 10 AM, lasted 1 hour and consisted from a ride of cross country by walking (5 min), trotting (15 min), walking (10 min), trotting (10 min), walking (5 min), galloping (5 min), and walking (10 min).



Fig. 1. Map of Pomeranian voivodeship in northern Poland. Marked village Strzelinko (N54°30'48.0" E16°57'44.9") where blood samples of horses were collected

Blood samples. Blood was drawn from jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8.30 and 10 AM), and immediately after exercise test (between 11 AM and 2 PM). Blood samples were taken once per a season for one year: spring (April), summer (July). Blood was stored into tubes with K-EDTA and held on the ice until centrifugation at 3,000 g for 15 minutes. The plasma was removed. The erythrocytes' suspension (one volume) was washed with five volumes of saline solution three times and centrifuged at 3,000 g for 15 minutes. Plasma aliquots were frozen and stored at -25°C until assays.

Hematological assays. Routine hematological parameters (haematocrit (HCT), haemoglobin concentration (HGB), the count of red blood cells (RBC), platelets (PLT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW) and platelets distribution width (PDW)) were measured and counted with an automated hematology analyzer (Abakus Junior Vet, Austria).

Assays of osmotic resistance of erythrocytes. The osmotic resistance of erythrocytes in solutions with different NaCl concentration was measured spectrophotometrically at the wavelength of 540 nm as described by Mariańska et al. (2003). The method is based on the determination of differences between osmotic resistance of erythrocytes to a mixture containing different concentration of sodium chloride (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%). Absorbance of mixture contained erythrocytes and distilled water was determined as 100% hemolysis (blank). The degree of hemolysis in every test tube (%) was calculated in accordance to the absorbance of blank. Hemolysis of erythrocytes (%) in every test tube with different sodium chloride concentration was expressed as curve (Mariańska et al. 2003).

Statistical analysis. Results are expressed as mean \pm S.E.M. All variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p > 0.05$). In order to find significant differences (significance level, $p < 0.05$) between states at before and after exercise, Wilcoxon signed-rank test was applied to the data (Zar 1999). Statistical significance between mean in groups of horses in spring and summer both before and after exercise was evaluated by Mann-Whitney U test (Zar 1999). All statistical analyses were performed using Statistica 10.0 software (StatSoft, Poland).

RESULTS AND DISCUSSION

During the experimental period, the animals got exposed to marked seasonal changes in ambient temperature and humidity. The variation in climatic variables like temperature, humidity and radiation are recognized as the potential hazards for the growth and production of all domestic livestock species (West 2003). Table 1 indicates the seasonal variation in mean values of erythrocytes' indices and thrombocytes count in the blood of horses.

Our results showed the significant increase of erythrocytes amount (RBC) and hemoglobin level (HGB) after exercise test both in the spring and summer seasons. In particular, RBC amount was increased by 23% ($p < 0.05$) after exercise test in the spring and by 41% ($p < 0.05$) in the summer. Our results shown the increased HGB level after exercise test in the spring and summer seasons by 31% and 37% ($p < 0.05$), respectively. In the spring, exercise test caused enhancement of hematocrit level (HCT), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels by 30%, 7%, and 8% ($p < 0.05$), respectively. A large number of studies investigated the response of the hemogram to various types of training (Krumrych 2006,

Table 1

Seasonal variations of hematological indices of horses before and after exercise (n = 13)

Hematological indices	Spring		Summer		Reference values
	before exercise	after exercise	before exercise	after exercise	
Erythrocyte count, RBC [$\cdot 10^{12}/L$]:	8.04 \pm 0.26*	9.90 \pm 0.42*	7.52 \pm 0.54**	10.60 \pm 0.56**	6.8-12.9• 5.5-10.0••
Hemoglobin level, HGB [g/dL]	11.65 \pm 0.52*	15.23 \pm 0.75*	11.29 \pm 0.56**	15.49 \pm 0.71**	11-19• 8-18••
Hematocrit, HCT [%]	35.55 \pm 1.06*	46.26 \pm 1.86*	39.11 \pm 2.55	45.20 \pm 2.09	32-53• 24-52••
Mean Corpuscular Volume, MCV [fL]	44.54 \pm 0.66*	47.85 \pm 1.10*	47.0 \pm 0.78	47.69 \pm 1.08	34-58• 35-58••
Mean Corpuscular Hemoglobin, MCH [pg]	15.17 \pm 0.27*	16.47 \pm 0.19* ^b	15.82 \pm 0.18	15.83 \pm 0.21 ^b	12.3-19.7• 10-20••
Mean Corpuscular Hemoglobin Concentration, MCHC [g/dL]	35.37 \pm 0.44* ^a	32.71 \pm 0.44*	33.73 \pm 0.49 ^a	33.78 \pm 0.35	31-39• 31-37••
Red Blood Cell Distribution Width, RDW [%]	19.76 \pm 0.27	20.87 \pm 0.49	20.54 \pm 0.39	21.08 \pm 0.39	11-17•
Platelet count, PLT [$\cdot 10^9/L$]	116.46 \pm 4.24*	152.0 \pm 5.02*	113.73 \pm 3.94**	136.55 \pm 8.72**	100-400• 150-400••
Mean platelet volume, MPV	7.34 \pm 0.23 ^a	7.50 \pm 0.09 ^b	8.13 \pm 0.18 ^a	8.62 \pm 0.13 ^b	9.7-12.8•
Platelet distribution width, PDW [%]	32.31 \pm 1.16	34.0 \pm 0.64	32.70 \pm 1.46	33.80 \pm 0.31	

Data are represented as mean \pm S.E.M.

- – reference values according to the Operating Instructions Hematology Analyzer Abacus Junior Vet;
- – reference values according to A. Winnicka (2004); *
- * – statistical significance ($p < 0.05$) between mean in groups of horses before and after exercise in spring season, ** – statistical significance ($p < 0.05$) between mean in groups of horses before and after exercise in summer season,
- ^a – statistical significance ($p < 0.05$) between seasonal mean in groups of horses before exercise,
- ^b – statistical significance ($p < 0.05$) between seasonal mean in groups of horses after exercise

Piccione et al. 2007). The most common findings for horses undergoing high-intensity training has been significant increases in the resting red blood cell count, hematocrit, and hemoglobin concentration (Krumrych 2006, Piccione et al. 2007, Andriichuk et al. 2014c). At the onset of the exercise, this rise derives from the mobilization of splenic RBC under the influence of the catecholamines (Krumrych 2006, Piccione et al. 2007, Satué et al. 2012). The increase of hematocrit is result of enhancement in the

erythrocyte count and hemoglobin concentration. The increase in hemoglobin concentration causes an enhance of oxygen transport capacity, an important factor in the horse's high aerobic capacity (Krumrych 2006, Piccione et al. 2007, Andriichuk et al. 2013). Cardiovascular changes linked to exercise are mediated by catecholamines and the recruitment of the sympathetic nervous system causes the contraction of the spleen. This organ in horses act as a reservoir of blood, with the release of 4-12 L of red blood cells into the bloodstream at the beginning of the exercise (McKeever et al. 1993, Muñoz et al. 2008). In our study, elevated amounts of red blood cells, hemoglobin, as well as hematocrit after exercise test were noted (Table 1). As a consequence of the increase in hemoglobin concentration, there is increased mean corpuscular volume and mean corpuscular hemoglobin concentration, that leads to improve of oxygen transport capacity, an important factor in the horse's high aerobic capacity (Table 1). Our results confirm our previous studies, which established that the exercise caused the elevation of red blood cells indices. The influence of catecholamines and mobilization of splenic erythrocytes play a pivotal role in adaptation to exercise-induced stress in horses (Krumrych 2006, Piccione et al. 2007, Andriichuk et al. 2016).

In spring season, decreased level of mean corpuscular hemoglobin concentration (MCHC) by 7.5% ($p < 0.05$) after exercise was observed. Kupczyński and Špitalniak (2015) noted the decrease of MCHC in eight Polish Halfbred driving horses after training. According to Hanzawa et al. (1995), a decrease in MCV and MCHC levels usually accompanies light exercise. Greater physical effort in turn causes an increase in the level of these parameters (Satué et al. 2012). Ghosh (2013) observed the declining of HGB, MCH and MCHC parameters during change from anaerobic to aerobic exercise. Atan and Alacam (2015) examined the chronic effects of exercise and found out a decrease in red blood cell parameters. Ricci et al. (1988) also found that hematological parameters were decreased after chronic exercises. Decrease of MCHC value was most probably caused by decrease of blood circulation volume (Ricci et al. 1988).

In spring, water intake with grazing might have limited the effect of hemoconcentration induced by physical activity and/or thermolysis (Satué et al. 2013). Generally, the haematological profile is an important indicator of the physiological changes in animals (Yaqub et al. 2013). Seasonal changes in the environment influence the physiological responses of animals. Changes in haematological parameters such as total RBC count, hematocrit and RBC indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) are of value in determining the adaptation of animals to the environment (Yaqub et al. 2013, Satué et al. 2013, 2014). Satué and coauthors (2014) observed increase of erythrocytes, hematocrit and platelets counts in Carthusian broodmares in summer. During the summer months, the higher temperatures could develop an adaptative response to heat stress (Satué et al. 2013). These high environmental temperatures activate thermoregulatory mechanisms, with loss of water through sweating and evaporation through respiratory mucoses. These facts could lead to a decrease in plasma volume and increase in hematocrit level (Donoghue et al. 2000, Satué et al. 2013, 2014).

However, in our case non-significant differences between erythrocytes, hemoglobin and hematocrit values in spring and summer seasons were observed (Table 1). Nevertheless, in our study, calculated characteristics of erythrocytes were affected by the season (Table 1). Particularly, pre-exercise mean corpuscular hemoglobin (MCHC) concentration in the summer was lower by 4.6% ($p < 0.05$) compared to the spring season. At the same time, post-exercise MCH were higher by 4% ($p < 0.05$) in the spring compared to summer. MCHC plays an important role in detecting the influence of heat stress, which decreases by the elevation of ambient temperature (Okab et al. 2008). Pre-exercise lower level of MCHC in summer observed in our study is in agreement with those obtained by Satué et al. (2013, 2014), who reported that the decline of MCH and MCHC during the hottest months of the year (i.e., May, June, July and August) in broodmares associated with a reduced efficiency in the transport of oxygen from the erythrocyte. This fact could result in a compensatory increase in the number of red blood cells (Gill and Wanska 1978). Our results showed lower post-exercise level of MCH in summer compared to the spring season. The lowest values of MCH in summer has been justified as a compensatory mechanism in face of a less efficient oxygen transport in the erythrocytes (Satué et al. 2014, Gill and Wanska 1978).

After exercise test, platelet count were higher by 30% and 20% ($p < 0.05$), both in the spring and summer seasons. In the summer, mean platelet volume was higher by 10% and 15% ($p < 0.05$) compared to the spring period (Table 1). Platelet count can increase in exercise and this might also be associated with fresh release of platelets from the spleen, bone marrow and other reservoir (Yilmaz et al. 2004). It was suggested that acute exercise could play an important role in enhancing fibrinolytic activity, together with other factors associated with clot breakdown post exercise, as vasopressin and catecholamines (Satué et al. 2012). It has also been shown that platelet response to exercise depends on several factors such as the relative intensity of exercise, mode of exercise and physical fitness (Yilmaz et al. 2004). Mild exercise does not alter and may even suppress platelet activation while strenuous exercise causes intensity-dependent platelet activation (Singh et al. 2006). Activation of platelets by acute vigorous exercise has been demonstrated by various parameters (Satué et al. 2012). Mean platelet volume was significantly higher in summer (Table 1). Our results are in agreement with the results provided for research of horses (Satué et al. 2014) and human beings (Crawford et al. 2003). In laboratory animals, it has been shown that the release of platelets from bone marrow to peripheral blood is lower during winter and spring (Satué et al. 2014). Other studies in dogs have shown no seasonal variations of PLT amount (Sothorn et al. 1993).

Osmotic resistance of erythrocytes (percent of hemolyzed erythrocytes in solutions with different NaCl concentration) of horses before and after exercise in spring and summer seasons are present in Fig. 2. There were more significant differences in percentage of hemolyzed erythrocytes both in spring and summer season. In particular, pre-exercise percent of hemolyzed erythrocytes in solutions with 0.3-0.6% NaCl were lower in the summer by 8%, 12%, 56%, and 80% ($p < 0.05$), respectively, compared to the spring season. After exercise, the percent of hemolyzed erythrocytes in solutions with 0.2-0.7% NaCl were lower in the summer by

3%, 6%, 10%, 71%, 88%, and 42% ($p < 0.05$), respectively in comparing to the spring season (Fig. 2).

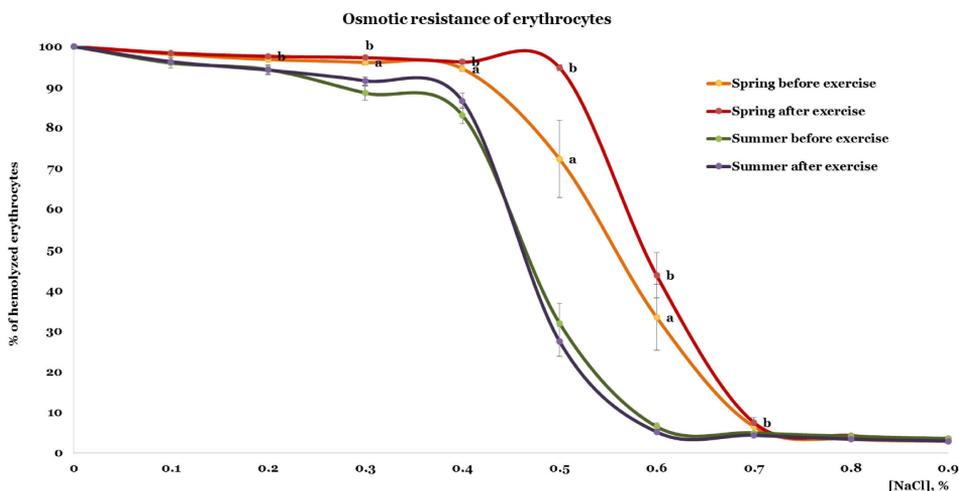


Fig. 2. Percent of hemolyzed erythrocytes in solutions with different NaCl concentration of horses before and after exercise in spring and summer seasons. Data are represented as mean \pm S.E.M. ($n = 13$)

^a – statistical significance ($p < 0.05$) between seasonal mean in groups of horses before exercise,
^b – statistical significance ($p < 0.05$) between seasonal mean in groups of horses after exercise

Intravascular hemolysis is one of the most emphasized mechanisms for destruction of erythrocytes during and after physical activity (Andriichuk et al. 2016, Andriichuk et al. 2014c, Sentürk et al. 2005). Common environmental factors have an important influence on red blood cell count and haematocrit. The low ambient temperature requires a higher metabolic rate for body temperature regulation and could be a stimulus for erythropoiesis which would be of great advantage in oxygen transport (Satué et al. 2013). Additionally, enhanced sympathetic activity in winter could lead to increased spleen mobilization, with the release of blood into the bloodstream (Hata et al. 1982, Satué et al. 2013). Higher percents of hemolyzed erythrocytes in spring observed in our results probably caused by the liberation of red blood cells from spleen or the increase in oxygen consumption. Moreover, exercise-induced oxidative stress has been proposed among other factors as an explanation of exercise-induced hemolysis (Andriichuk et al. 2014a, b, c, Andriichuk et al. 2016, Sentürk et al. 2005). Erythrocytes appear much more vulnerable to oxidative damage during intense exercise because of their continuous exposure to high oxygen fluxes and their high concentrations of polyunsaturated fatty acids (PUFAs) and heme iron (Sentürk et al. 2005). Our previous studies have shown that physical effort in horses involved in recreational horse riding leads to significant increase of carbonyl derivatives level of protein oxidation in the plasma associated with exercise-induced oxidative stress (Andriichuk et al. 2014c). In this study, the highest pre- and post-exercise changes in the resistance of erythrocytes in solutions with different

NaCl concentration probably indicate to oxidative stress-dependent impairment of erythrocyte stability in the spring season.

CONCLUSIONS

1. Our results confirm that hematological parameters in horses involved in recreational horse riding are subjected to seasonal variations that should be taken into account when interpreting equine hematology and the applying of training.
2. The enhanced level of red blood cells indices after exercise both in the spring and summer seasons in our study probably caused by release of erythrocytes into the bloodstream from a greater demand for oxygen carriage and increased aerobic capacity.
3. Our results showed the lower post-exercise level of MCH in summer compared to the spring. The lowest values of MCH in summer probably is caused by compensatory mechanism through a less efficient oxygen transport in the erythrocytes.
4. The increase of platelets after exercise in spring and summer seasons could be due to their release from the spleen, bone marrow and other reservoirs.
5. Higher percentage of hemolyzed erythrocytes in spring season probably caused by the liberation of red blood cells from spleen and/or the increase in oxygen consumption. In the future studies it would be interesting to analyze, whether other seasonal variations of hematological indexes in winter and autumn exist in well-trained horses.

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SEZONOWE ZMIANY WSKAŹNIKÓW HEMATOLOGICZNYCH U KONI BIORĄCYCH UDZIAŁ W REKREACYJNYCH JAZDACH KONNYCH

Streszczenie

Analiza krwi obrazuje stan zdrowia i poziom wydajności koni oraz sygnalizuje choroby, które, dzięki wczesnej profilaktyce, jesteśmy w stanie zwalczyć, nim się uaktywnią. Różnice we wskaźnikach hematologicznych u koni są zależne od treningu, żywienia, wieku, płci, rasy, dobowych i sezonowych cykli, temperatury otoczenia, stanu fizjologicznego konia itd. Celem badań było określenie wpływu sezonowości na wskaźniki hematologiczne i odporność osmotyczną erytrocytów u koni biorących udział w rekreacyjnych jeźdździejach przed i po treningu. Trzydzieści zdrowych dorosłych koni z regionu Pomorza w Polsce (w wieku $9,5 \pm 2,4$ roku) wzięło udział w naszych badaniach. Wszystkie konie brały czynny udział w rekreacyjnej jeździe konnej. Próbkę krwi pobrano w dwóch sezonach – wiosną (kwiecień) i latem (lipiec). Krew pobierano z żyły szyjnej zwierząt rano, 90 minut po karmieniu, i natychmiast po treningu. We krwi oceniono wartości hematokrytu (HCT), stężenie hemoglobiny (HGB), liczbę krwinek czerwonych (RBC), średnie stężenie hemoglobiny w krwince (MCHC), średnią objętość krwinki (MCV), średnią masę hemoglobiny w erytrocycie (MCH), rozpiętość rozkładu objętości erytrocytów (RDW), liczbę płytek krwi (PLT), rozpiętość rozkładu objętości płytek krwi (PDW) i odporności osmotycznej erytrocytów przed i po treningu. Nasze wyniki potwierdzają, że parametry hematologiczne u koni biorących udział w rekreacyjnej jeździe konnej są zależne od wahań sezonowych. Znaczący wzrost liczby erytrocytów i poziomu hemoglobiny po wysiłku zaobserwowano zarówno wiosną, jak i latem ($p < 0,05$). Wiosną ćwiczenia spowodowały wzrost poziomu hematokrytu, średniej objętości krwinek czerwonych i średniej masy hemoglobiny

w erytrocycie ($p < 0,05$). Średnia masa hemoglobiny w erytrocycie po wysiłku osiągnęła wyższe wartości wiosną ($p < 0,05$) w porównaniu z sezonem letnim. Jednak średnie stężenie hemoglobiny w krwince u koni przed treningiem w sezonie letnim było niższe ($p < 0,05$) w porównaniu z sezonem wiosennym. Wyższy procent zhemolizowanych erytrocytów w sezonie wiosennym prawdopodobnie spowodowany był wyrzutem czerwonych krwinek ze śledziony i/lub zwiększeniem zużycia tlenu.