HEMATOLOGICAL ALTERATIONS AND OSMOTIC RESISTANCE OF ERYTHROCYTES IN ENDURANCE HORSES DURING 32 KM RACES

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ABSTRACT

Exhausting and chronic exercise, have been associated with changes of hematological, biochemical, immune and hormonal parameters. Physical activity has variable effects on the hematological parameters, depending on exercise duration and intensity (short-term high intensity or maximal exercise and long-term low intensity or sub-maximal prolonged exercise), fitness and training levels and environmental conditions. The aim of this study was to appraise the alterations in hematological indices and osmotic resistance of erythrocytes of endurance horses during 32 km races in Crimean region. The prolonged exercise were used in endurance race. Walk about 3 km/h for 20 min, the trot about 7 km/h for 23 min, the canter about 5 km/h for 10 min), and the walk about 1 km were repeated for 1 h (phase I); the resting period in an outdoor paddock without access to water was 30 min. Phase II was consisted of the walk about 3 km/h for 20 min, the trot about 7 km/h for 23 min, the canter about 5 km/h for 10 min), and the walk about 1 km. It was repeated for 1 h. Blood was drawn from jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables, and immediately after endurance race. The hematological indices [haematocrit (HCT), haemoglobin concentration (HGB), the count of red blood cells (RBC), white blood cells (WBC), platelets (PLT), leucogram, mean cor-
puscular 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). The content of glycated hemoglobin in red blood cells was determined by calorimetric method based on acid hydrolysis of keto-aminic group with oxalo-acetic acid. The osmotic resistance of erythrocytes in solutions with different NaCl concentration was measured spectrophotometrically. Adequate endurance race of low intensity could improve oxygen-induced hematological function in Crimean horses. Furthermore, the non-significant increase of red blood cells indices in endurance horses indicates on good athletic level after 32 km ride. There were no significant differences between values of white blood cell indices in endurance horses during 32 km race. Platelets count was significantly higher in post-ride period by 46% (p < 0.05), and mean platelet volume (MPV) was significantly decreased after exercise (by 8%, p < 0.05). Statistically significant differences in the percentage of haemolyzed erythrocytes between pre- and post-ride period were observed. The haematological alterations caused by various physical efforts reflect changes in the functions of different systems and can be used for health control and diagnosis of diseases. It also allow the evaluating the level of sport performance, the accuracy of training, and physiological condition of horses.

**Key words:** endurance horses, endurance race, hematological parameters, glycated hemoglobin, resistance of erythrocytes

**INTRODUCTION**

Endurance horses compete in races that can be classified as low-intensity long-lasting trials (Bargero et al. 2005). The first modern endurance ride was held in 1955 in California, from Lake Tahoe to Auburn (100 miles). Today, most endurance rides range from 30 to 160 km, or 100 + 100 km to be run over 2 days, or 500 km to be run over 5 days (Duren 2000, Bargero et al. 2005). Endurance horses undergo severe stress during the course of a competitive ride. These horses are trained and conditioned to perform over long distances at moderate speeds (Adamu et al. 2010). When conditioning a horse for long distance competitions, the training program must be designed and monitored to match the specific exercise type and intensity of competitive endurance riding (Bargero et al. 2005, Adamu et al. 2012). Horses are superb athletes in comparison to other athletic species. Exceptionally high maximal oxygen uptake, a splenic reserve of red blood cells released into the circulation during exercise, and a high amount of energy stored as glycogen in the muscles contribute to the high performance capacity of a horse (Pösö 2002, Hinchcliff and Geor 2008). The major physiological adaptations that can directly influence exercise capacity and stamina of endurance horses include the efficiency of gas exchange, oxygen uptake and delivery to the exercising muscles. The working muscle of endurance horses depends on aerobic metabolism of its glycogen stores, blood fatty acids and volatile fatty acids from hindgut fermentation, heart size and capacity to deliver large volumes of blood to the tissue (Bargero et al. 2005, Adamu et al. 2010).
Most breeds have been tested and used for endurance races; the most competitive are Arabian or Arabian crosses due to their muscle fibre composition, but other breeds, including Thoroughbred, Quarter Horses, Mustangs, Appaloosas, Morgans, Standardbred (Duren 2000, Bargero et al. 2005, Williams et al. 2005, Adamu et al. 2012). Arabian horses, compared to Thoroughbreds, are better adapted to endurance work because of their superior oxidative capacity (Prince et al. 2001). Arabians and Anglo-Arabians seem to have lower lactate concentrations, compared to Andalusians, at speeds up to 25 km/h (Castejon et al. 1994), thus, showing a better adaptation to long-distance, low-intensity work. For endurance race, often use horses of local breeds, which a bred directly in recreational areas. For example, Hucul horses which widespread in Carpathians – in Poland and Ukraine, or horses from Crimean Mountains (Andriichuk et al. 2014b). The basis of Crimean horses was formed from the Bashkir breed horses imported to the Crimea in the 60s of the last century. As a result of crosses under the influence of harsh conditions of maintenance of herd, it was appeared the heterogeneous for genotype and phenotype horse. The horses from Crimea are small, about 145 cm at the withers. They are wide in the body and deep-chested, with a thoracic circumference (girth) averaging about 180 cm; they have a large head and a short neck, low withers and a flat back. The legs are short with heavy bone; cannon bone diameter may reach 20 cm. The commonest coat colours are bay, red, brown, chestnut, mouse grey. The mane and tail are thick and the coat is also thick (Photo 1). Nowadays, horses in Crimean mountains are widely used in endurance race and recreational riding (Andriichuk et al. 2014a).

Photo 1. The horses in Crimean mountains
Source: photo by I. Tkachova

The endurance competitions are extremely difficult from a metabolic point of view, and for this reason, they are subjected to very strict veterinary controls to spare the horse’s health (Bargero et al. 2005, Kinnunen et al. 2005, Al-Qudah and Al-Majali 2008, Gondim et al. 2009). In overview of 7117 starts in international (Eldric) races,
only 50% of the subjects completed the ride, and 30% were eliminated: 63% because of lameness, 24% for metabolic reasons, and 13% for other causes (Bargero et al. 2005). Metabolic problems, therefore, cause the elimination of 7.2% of horses starting international races, but some are retired for the same reasons between two veterinary gates during the race, while others have problems after the final veterinary examination (Bargero et al. 2005). For this reason, the correct metabolic management of the endurance horse is of the utmost importance, together with the correct prevention and treatment of disease (Bargero et al. 2005).

Several studies reported, that exhausting and chronic exercise, have been associated with changes of hematological, biochemical, immune and hormonal parameters (Hyyppä 2005, Piccione et al. 2007, Satue et al. 2012, Andriichuk et al. 2014a, b). Physical activity has variable effects on the hematological parameters, depending on exercise duration and intensity (short-term high intensity or maximal exercise and long-term low intensity or submaximal prolonged exercise), fitness and training levels and environmental conditions (Satue et al. 2012). Our previous study has shown significant differences of hematological parameters in mares of different breeds (Andriichuk et al. 2012). Values of hematological parameters determined can be used for health control and diagnosis of diseases and allow the evaluating the level of performance potential, the accuracy of training and physiological condition of horses (Andriichuk et al. 2012, Satue et al. 2012). For this reason, the correct interpretation of the hematologic and metabolic changes after prolonged or endurance exercise in horses is required, because it can help the veterinarian, trainer, or owner to choose appropriate training and post-exercise recovery. Thus, the aim of this study was to appraise the alterations in hematological indices and osmotic resistance of erythrocytes of endurance horses during 32 km races in Crimean region.

**MATERIALS AND METHODS**

**Horses.** Total seven horses from Crimean region (Bilohirsk) were involved in our study (Fig. 1). All horses participate in endurance race. Horses were used for herd maintenance and fed (hay and oat) twice a day and water available *ad libitum.*

![Maps of Crimean region in Ukraine, marked are Bilohirsk (Crimean region, Ukraine)](image)

Source: own research
All horses were thoroughly examined clinically and screened for hematological, biochemical and vital parameters, which were within reference ranges. The females were non-pregnant. Owners were allowed to provide supplemental feed and salts to their horses. Information on supplementation of horse diets with antioxidant compounds, such as vitamin E or selenium, was not available. Information regarding levels of training and previous activities was not available. A comprehensive physical examination was performed on all horses. The physical examination included monitoring horses’ vital clinical signs (heart rhythm, respiratory rhythm and gut sounds). In addition, the hydration status, gait of the animal, and presence of any injuries, especially in the legs, girth, withers, and back, were noted. Only horses that had normal clinical parameters were allowed to participate in the endurance race.

Endurance race. The prolonged exercise were used in endurance race. Walk about 3 km/h for 20 min, the trot about 7 km/h for 23 min, the canter about 5 km/h for 10 min, and the walk about 1 km were repeated for 1 h (phase I); the resting period in an outdoor paddock without access to water was 30 min. Phase II was consisted of the walk about 3 km/h for 20 min, the trot about 7 km/h for 23 min, the canter about 5 km/h for 10 min), and the walk about 1 km. It was repeated for 1 h (Photo 2).

Photo 2. The Crimean horses participated in 32 km endurance race
Source: photo by I. Tkachova

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 endurance race (between 11:00 AM and 2:00 PM). Blood were stored into tubes with K-EDTA and held on ice until centrifugation at 3,000 g for 15 minutes. The plasma was removed. The erythrocyte's 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 analyzed.

Hematological assays. Routine hematological indices [haematocrit (HCT), haemoglobin concentration (HGB), the count of red blood cells (RBC), white blood cells (WBC), platelets (PLT), leucogram, 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).
**The glycated hemoglobin assay.** The content of glycated hemoglobin in red blood cells was determined by calorimetric method based on acid hydrolysis of ketoaminoic group with oxalo-acetic acid (Dormandy and Lorday 1965). The resulting product, 5-hydroxymethylfurfural, interacts with 2-thiobarbituric acid and forms a colored complex. The color intensity is determined on a spectrophotometer at a wavelength 443 nm. The washed red blood cells were hemolysed with distilled water in the ratio 1:3 and centrifuged at 14,700 g for 15 min. To 2 ml of hemoglobin was added 1 ml of 0.3 M oxalo-acetic acid and incubated at 100°C for one hour. After incubation, to chilled mixture was added 40% trichloroacetic acid, stirred and centrifuged for 10 minutes at 6000 g. To 2 ml of the supernatant was added 0.05M 2-thiobarbituric acid and incubated for 40 min at 40°C. After 20 minutes of the incubation, the optical density was measured in 1 cm cuvette. The content of glycated hemoglobin was calculated in %. The value of \( E = 0.029 \) corresponds to 1% content of glycated hemoglobin.

**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% (standard). The degree of hemolysis in every test tubes (%) was calculated in accordance to the absorbance of standard. Haemolysis 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 ± 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 before and after riding, Wilcoxon signed-rank test was applied to the data (Zar 1999). All statistical analyses were performed using Statistica 10.0 software (StatSoft, Poland). In addition, the relationships between values of hematological indices of all individuals were evaluated using Spearman's correlation analysis (Zar 1999).

**RESULTS AND DISCUSSION**

In our study, all hematological indices of horses were within the reference values (Tab. 1-3). There were no significant differences between values of white blood cell indices in endurance horses during 32 km race (Tab. 1).

During endurance events, leukocytosis is due to neutrophilia and lymphopenia is a result of redistribution of neutrophils from the peripheral pool in to the circulation and splenic contraction this depend on the aerobic or anaerobic nature of the exercise and the associated stress factors (Rose and Hodgson 1994, Piccione et al. 2010, Adamu et al. 2012). Adamu and coauthors (2012) observed metabolic crisis in endurance
Table 1

Values of white blood cell indices of horses during 32 km riding (M ± m, n = 7)

<table>
<thead>
<tr>
<th>White blood cell indices</th>
<th>Before riding</th>
<th>After riding</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte count, WBC [-10^9/l]</td>
<td>8.40 ± 0.80</td>
<td>9.23 ± 0.91</td>
<td>5.4-14.3•</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.5-12.0••</td>
</tr>
<tr>
<td>Lymphocyte count, LYM [-10^9/l]</td>
<td>3.84 ± 0.68</td>
<td>2.53 ± 0.57</td>
<td>1.5-7.7•</td>
</tr>
<tr>
<td>Monocytes and some eosinophils count, MID [-10^9/l]</td>
<td>0.21 ± 0.07</td>
<td>0.31 ± 0.10</td>
<td>0-1.5•</td>
</tr>
<tr>
<td>Granulocytes (neutrophils, eosinophils and basophils) count, GRA [-10^9/l]</td>
<td>4.34 ± 0.59</td>
<td>6.40 ± 0.54</td>
<td>2.3-9.5•</td>
</tr>
<tr>
<td>Percentage of lymphocytes, LY%</td>
<td>43.66 ± 5.11</td>
<td>25.63 ± 3.87</td>
<td>17-68•</td>
</tr>
<tr>
<td>Percentage of monocytes and some eosinophils, MID%</td>
<td>2.37 ± 0.80</td>
<td>3.63 ± 1.24</td>
<td>0-14•</td>
</tr>
<tr>
<td>Percentage of granulocytes, GR%</td>
<td>52.53 ± 5.82</td>
<td>70.74 ± 4.31</td>
<td>22-80•</td>
</tr>
</tbody>
</table>

Legend: • – reference values according to the Operating Instructions of Hematology Analyzer Abacus Junior Vet; •• – reference values according to Winnicka (2008); * – statistical significance between means before and after 32 km riding

Source: own research

horses which accompanied by the significant increases in WBC and neutrophil and monocytes. However, in other study, Adamu and coauthors (2010) have observed non-significant alterations of white blood cell indices in endurance horses and made the assumption that it points to the good performance and the fitness of horses. Different cell type in the immune system is provided to perform a particular function and work together to create the integrated immune response (Kadowaki et al. 2000). The mechanism responsible for change of leukocyte values and function following exercise are believed to be multifactorial and complex; although, alterations in neuroendocrine hormones, in particular, catecholamines and corticosteroids, in response to an exercise challenge have been associated with transient immunosuppression (Mackinnon 2000, Pedersen and Hoffman-Goetz 2000, Satué et al. 2012). Furthermore, changes in levels β-endorphins, cytokines and sex steroids have been linked to alter of leukocyte values and function after acute exercise (Mackinnon 2000, Pedersen and Hoffman-Goetz 2000). In general, moderate training is considered to have beneficial effects on host defense mechanism (Hines et al. 2008), whereas prolonged periods of high-intensity training may lead to slight impairment of several immune parameters (Mackinnon 2000, Satué et al. 2012). Results observed in our study are in agreement with previous studies that have suggested that adequate endurance race low-intensity could improve immune function in endurance horses (Adamu et al. 2010, Andriichuk et al. 2012, 2014a).

Certain cardiovascular and hematological adaptations are necessary to guarantee the correct oxygen and blood-borne substrates supply to active muscles during exercise and the release of metabolites (Piccione et al. 2009). In the present study, post-
ride values of red blood cell indices non-significantly changed compared to pre-ride period (Tab. 2).

<table>
<thead>
<tr>
<th>Red blood cell indices</th>
<th>Before riding</th>
<th>After riding</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte count, RBC ([10^{12}/l]):</td>
<td>9.63 ± 1.22</td>
<td>10.60 ± 0.70</td>
<td>6.8-12.9*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.5-10.0**</td>
</tr>
<tr>
<td>Hemoglobin level, HGB ([g/dl])</td>
<td>15.06 ± 1.85</td>
<td>16.71 ± 1.01</td>
<td>11-19•</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8-18••</td>
</tr>
<tr>
<td>Hematocrit, HCT</td>
<td>43.36 ± 4.70</td>
<td>48.61 ± 2.08</td>
<td>32-53•</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24-52••</td>
</tr>
<tr>
<td>Mean Corpuscular Volume, MCV ([fl])</td>
<td>45.57 ± 1.43</td>
<td>46.86 ± 1.34</td>
<td>34-58••</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin, MCH ([pg])</td>
<td>15.73 ± 0.41</td>
<td>15.88 ± 0.24</td>
<td>12.3-19.7•</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin Concentration, MCHC ([g/dl])</td>
<td>34.56 ± 0.38</td>
<td>34.03 ± 0.55</td>
<td>31-39•</td>
</tr>
<tr>
<td>Red Blood Cell Distribution Width, RDW [%]</td>
<td>21.50 ± 0.68</td>
<td>22.15 ± 0.68</td>
<td>11-17•</td>
</tr>
</tbody>
</table>

Legend: •, •• and * see Table 1
Source: own research

Exercises have variable effects on the erythrocyte indices depending on work intensity, fitness and training levels, environmental conditions and breed of horses (Rose and Hodgson 1994, Piccione et al. 2010, Satue et al. 2012, Vazzana et al. 2014). The increase in the value of RBC indices in horses is caused, most of all, by a release of erythrocytes from the spleen, where about 50-60% of the general number of these blood cells are located (Rose and Hodgson 1994, Satue et al. 2012). However, the increase of hematocrit could also be attributable to changes in plasma volume, in relation to thermoregulatory processes, mainly sweating and evaporation from the respiratory mucosa and to fluid shift derived from physical activity (Muñoz et al. 2008). Adamu and coauthors (2012) have reported significant increases in RBC, HGB and HCT at \((p < 0.0001)\) which could be indicate of metabolic crisis and poor performance in endurance horses. However, numerous studies have shown that horses subjected to high altitude possessed significantly higher RBC, HGB and platelet corpuscular volume (PCV) values, compared to animals that lived at less altitude (Wickler and Anderson 2000, Satue et al. 2012). It is considered a compensatory mechanism for the lower content of oxygen in the atmospheric air, which is proportionally reduced to the altitude (Wickler and Anderson 2000). Given that fact that horses of Crimean region were housed under high altitude of the Crimean Mountains, our results are consistent with studies of other researchers (Wickler and Anderson 2000). Moreover, one of reason the increase of red blood cells indices in the blood of horses of Crimean Mountains can be inhabitation on the altitude. Long term hypoxic exposure and/or stress to altitude can lead to an increment of red blood cells, hemoglobin, density of capillary blood vessels, and myoglobin density in skeletal muscle (Laitinen et al. 1995, Rodriguez et al. 2000), resulting in enhancement of oxygen delivery capacity. In the cellular level, hypoxic exposure can accelerate the proliferation of mitochondria in the muscles (Desplanches et al. 1993), increase the buffering capacity for lactic acid (Favier et al. 1995), and
subsequently enhance endurance capacity in the high altitude environments. In spite of these theoretical rationale, the majority of studies investigating athletes who returned to the sea level from high altitude training reported no changes or even reduction of the level of physical performance. And few studies demonstrated an improvement of physical performance after the altitude training (Bailey and Davis 1997). This statement can be meaningful in connection with evidence of Adamu and coauthors (2010). They have shown that in endurance horses with good performance level, non-significant changes of red blood cells indices in post-ride period were observed.

Platelets count was significantly higher in post-ride period by 46% (p < 0.05), and mean platelet volume (MPV) was significantly decreased after exercise (by 8%, p < 0.05) (Tab. 3).

<table>
<thead>
<tr>
<th>Platelet indices</th>
<th>Before riding</th>
<th>After riding</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count, PLT [10^9/l]</td>
<td>103.86 ± 6.68</td>
<td>152.14 ± 6.84*</td>
<td>100-400•, 150-400••</td>
</tr>
<tr>
<td>Mean platelet volume, MPV [fl]</td>
<td>8.18 ± 0.25</td>
<td>7.53 ± 0.12*</td>
<td>9.7-12.8•</td>
</tr>
<tr>
<td>Platelet distribution width, PDW [%]</td>
<td>33.83 ± 1.23</td>
<td>34.04 ± 0.87</td>
<td>24-72•</td>
</tr>
</tbody>
</table>

Legend: •, •• and * see Table 1
Source: own research

Circulating platelets are heterogeneous in size density and reactivity (Satue et al. 2012). It was demonstrated that platelet age and size are independent determinants of platelet function (Yilmaz et al. 2004). Platelets produced under condition of stimulated platelet production, called “stress” platelets by Penington et al. (1976), show an increase in the MPV, which is the most accurate measure of platelet size compared with the normal circulating platelets (Penington et al. 1976). Platelet count can be increased by exercise and this might also be associated with fresh release of platelets from the spleen, bone marrow and other reservoir (Yilmaz et al. 2004). In acute states of platelet activation, the increase in platelet volume might be a result of a change in the fragmentation pattern of megakaryocyte cytoplasm (Yilmaz et al. 2004). It was suggested that acute exercise could play an important role in the enhancing fibrinolytic activity together with other factors associated with clot breakdown in post exercise period, as vasopressin and catecholamines (McKeever 2011). Platelet size has been shown to reflect platelet activity (Yilmaz et al. 2004). Activation of platelets by acute vigorous exercise has been demonstrated by various parameters (Satue et al. 2012). The measurement of MPV can reflect changes in platelet stimulation and hence might be a simple marker of activation of platelets (Yilmaz et al. 2004). Our results have showed the decreased MPV after exercise in horses. Strenuous exercise commonly causes thrombocytosis but there is dispute as to whether the mean platelet volume (MPV) also increases. No significant changes in the MPV of trained runners after running race of 10-26.2 miles were observed (Watts 1990).

The relative percentage utilization of the two main energy sources (carbohydrates and fat) varies according to the intensity and duration of the exercise (Bargero et al. 2005). Muscle glycogen is another important energy source; a dramatic decrease in
its muscle content has been reported in horses competing in endurance rides (Snow et al. 1981). The reduction of muscle glycogen is linked, together with an increase in lactic acid (LA) and a decrease in glucose blood levels (glycaemia), to the onset of peripheral fatigue (Newsholm et al. 1992). Glycated hemoglobin (HbG) is used in humans as a stable indicator of glucose status of healthy and diabetic patients (Carral et al. 2013). Among the various proteins that are known to undergo nonenzymatic glycation in vivo, hemoglobin has been the most thoroughly investigated (Selvaraj et al. 2008, Shahbazkia et al. 2010, Carral et al. 2013). Glycated hemoglobin (HbA1C) is the product of a slow and largely irreversible reaction that occurs throughout the life span of the erythrocyte (Selvaraj et al. 2008, Lyons and Basu 2012). The interindividual heterogeneity in RBC life span and in glucose gradient across RBC membranes have also been shown to be strong determinants of hemoglobin glycation in diabetic and nondiabetic subjects (Lyons and Basu 2012). Determination of HbA1C in diabetic patients is currently acknowledged as the most reliable indicator for assessment of retrospective glycemic control and the planning of clinical management (Selvaraj et al. 2008). Furthermore, hemoglobin has been considered a model protein that has provided insights into the nonenzymatic glycation of other more complex tissue proteins (Selvaraj et al. 2008). Blood glucose can readily diffuse into erythrocytes and glucose in erythrocytes can react nonenzymatically with amino groups of hemoglobin (hemoglobin N-terminal or lysine side chain amino groups) to form HbGs (Shahbazkia et al. 2010, Lyons and Basu 2012). Formation of HbGs is essentially irreversible, and this activity depends on both the lifespan of the erythrocytes and the blood glucose concentration over the previous several weeks and is not affected by transient factors. The average lifespan of erythrocytes in horses is 145 days (Shahbazkia et al. 2010), hence, HbG concentration is a measurement of the mean blood glucose level over the previous several weeks and not affected by recent stresses, drug use, or estrous cycle and can be a better indicator of glucose metabolism in comparison with other tests (Shahbazkia et al. 2010, Lyons and Basu 2012). Determination of HbG in horses has been done previously (Shahbazkia and Nazifi 2005, Shahbazkia et al. 2010) but its relation with fasting plasma glucose and its value as a metabolic biomarker of blood glucose status has not been studied. So, in the next part of our study we assessed the level of glycated hemoglobin (HbA1c) after endurance ride (Fig. 2).

![Glycated hemoglobin content (% of total hemoglobin content) of horses during 32 km riding (M ± m, n = 7)](source: own research)
Non-significant changes in glycated hemoglobin level in post-riding period were observed. Theoretically, percent of glycated hemoglobin (HbA1c) is dependent on blood glucose concentration over the previous weeks (Shahbazkia et al. 2010), erythrocyte life span (Burtis and Ashwood 1999), permeability of erythrocyte to blood glucose, and potential of hemoglobin to condense with glucose (Higgins et al. 1982).

In researches of diabetes diseases have demonstrated that resistance training leads to reduction of glycated hemoglobin in the blood and improving insulin sensitivity (Pereira et al. 2011, Carral et al. 2013). Regarding intensity, one study has shown that low- to moderate-intensity exercise training is as effective as moderate- to high-intensity exercise training (Hansen et al. 2009), whereas another study reported that high-intensity training was more effective in improving glycemic control (Dunstan et al. 2002). Carral and coauthors (2013) did not observe any differences in HbA1c levels in relation to time dedicated to moderate physical activities. However, patients who dedicated more than 150 min to intense physical activity per week had lower levels of HbA1c. It has been suggested that physical activity commits beneficial effects on glycemic control in patients with type 1 diabetes (Carral et al. 2013). In this regard, we can assume that low-intensity race favorably affect glycemic parameters in endurance horses. In conclusion, we suggest that the glycated hemoglobin can be a potentially good indicator of blood glucose status in horses (Shahbazkia et al. 2010).

Intravascular hemolysis is one of the most emphasized mechanisms for destruction of erythrocytes during physical activity in horses (Murakami 1974, Cywinska et al. 2011). Exercise-induced hemolysis has been confirmed under various conditions in stallions (Inoue et al. 2005), mares (Schott et al. 1995) and mixed population (Hanzawa et al. 2002). In our study, we also observed that erythrocytes exposed to the different concentrations of sodium chloride had a lower level of haemolysis after endurance race compared to the pre-ride period (Fig. 3). Moreover, increased osmotic resistance at 0.8% NaCl by 35% (p = 0.027) was observed in horses after endurance race (Fig. 3).

![Fig. 3. Osmotic resistance of erythrocytes (% of hemolyed erythrocytes in solutions with different NaCl concentration) of horses during 32 km riding](image)

* statistical significance (p < 0.05) between means before and after 32 km riding

Source: own research
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 (Smith 1995, Petibois and Deleris 2005, Burak Çimen 2008). On the other hand, the erythrocytes contain cellular defense mechanisms against free radical-induced lipid peroxidation including both enzymatic and non-enzymatic antioxidants (Petibois and Deleris 2005, Burak Çimen 2008). Recent evidence indicates that endurance training reduces erythrocyte susceptibility to oxidative stress and increases erythrocyte antioxidant defense against oxidative stress (Petibois and Deleris 2005, Burak Çimen 2008). Numerous studies strongly indicate that ROS generation during exercise is associated with the adaptation processes involving redox-sensitive transcription, upregulation of antioxidant enzymes and more effective repair of ROS-related molecular attacks (Radak et al. 2008, Burak Çimen 2008). Results of our study showed established effect of resistance erythrocytes to low-intensity physical activity. This could be due to exercise-induced adaptations and some breed features of Crimean horses.

CONCLUSION

Adequate endurance race of low intensity could improve oxygen-induced hematological function in Crimean horses. Furthermore, the non-significant increase of red blood cells indices in endurance horses indicates on good athletic level after 32 km ride. There were no significant differences between values of white blood cell indices in endurance horses during 32 km race. Platelets count was significantly higher in post-ride period by 46% (p < 0.05), and mean platelet volume (MPV) was significantly decreased after exercise (by 8%, p < 0.05). Statistically significant differences in the percentage of hemolyzed erythrocytes between pre- and post-ride period were observed. The value of glycated hemoglobin can be used a potentially good indicator of blood glucose status in horses. Statistically significant differences in the percentage of hemolyzed erythrocytes between pre- and post-ride period showed of exercise-induced adaptation of Crimean horses. The haematological alterations caused by various physical efforts reflect changes in the functions of different systems and can be used for health control and diagnosis of diseases. It also allow the evaluating the level of sport performance, the accuracy of training, and physiological condition of horses.

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**SUMMARY**

Physical activity has variable effects on the hematological parameters, depending on exercise duration and intensity (short-term high intensity or maximal exercise and long-term low intensity or submaximal prolonged exercise), fitness and training levels and environmental conditions. The purpose of this study was to appraise the
alterations in hematologic indices and osmotic resistance of erythrocytes of endurance horses during 32 km races in Crimean region. Walk about 3 km/h for 20 min, the trot about 7 km/h for 15 min, the canter about 5 km/h for 15 min), and the walk about 1 km were repeated for 1 h (phase I); the resting period in an outdoor paddock without access to water was 30 min. Phase II was consisted of the walk about 3 km/h for 20 min, the trot about 7 km/h for 15 min, the canter about 5 km/h for 15 min), and the walk about 1 km. It was repeated for 1 h. Blood was drawn from jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables, and immediately after endurance race. The hematological indices were measured and counted with an automated hematology analyzer (Abakus Junior Vet, Austria). Platelets count was significantly higher in post-ride period by 46% (p < 0.05), and mean platelet volume (MPV) was significantly decreased after exercise (by 8%, p < 0.05). Statistically significant differences in the percentage of haemolyzed erythrocytes between pre- and post-ride period were observed. The haematological alterations caused by various physical efforts reflect changes in the functions of different systems and can be used for health control and diagnosis of diseases. It also allow the evaluating the level of sport performance, the accuracy of training, and physiological condition of horses.