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HEMATOLOGICAL CHANGES AND RESISTANCE OF ERYTHROCYTES OF HORSES IN RESPONSE TO 32 KM RACES

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The objective of the present study was to investigate the alterations of some hematological parameters (hematocrit (HCT), hemoglobin concentration (HGB), the count of red blood cells (RBC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), as well as resistance of erythrocytes to urea and hydrogen peroxide in horses after 32 km endurance race. Seven horses from Crimea region (Bilohirsk, Crimean region) were involved in this study. Endurance horses used in this study are trained and conditioned to perform over long distances at moderate speeds. The prolonged exercises were used in endurance race. The walk about 3 km/h for 20 min, the trot about 7 km/h for 15 min, and the canter about 5 km/h for 15 min) and the walk about 1 km were repeated for 1 h (phase I); rest in an outdoor paddock without access to water for 30 min. And phase II: the walk about 3 km/h for 20 min, the trot about 7 km/h for 15 min, and the canter about 5 km/h for 15 min) and the walk about 1 km was repeated for 1 h.

The results of the present study showed that adequate endurance race of low intensity could improve oxygen-dependent respiratory function in horses from Crimean region. Furthermore, the non-significant increase of red blood cells indices in endurance horses indicate about good athletic level after 32 km endurance ride. Statistically significant differences in the percentage of hemolyzed erythrocytes between pre- and post-ride period were observed and thereby signifying an oxidative stress-dependent impairment of erythrocyte stability.

Keywords: ENDURANCE RIDE, ENDURANCE HORSES, HEMATOLOGICAL PARAMETERS, RESISTANCE OF ERYTHROCYTES, EXERCISE

ПОКАЗНИКИ ЧЕРВОНОЇ КРОВІ ТА РЕЗИСТЕНТНІСТЬ ЕРИТРОЦИТІВ У КОНЕЙ, ЯКІ БЕРУТЬ УЧАСТЬ У ДИСТАНЦІЙНИХ ПРОБІГАХ

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Однією з прикладних галузей конярства, які активно розвиваються сьогодні, є дистанційні пробіги та кінний туризм. В Україні, враховуючи регіональні ландшафтні особливості, дистанційні пробіги найбільш поширені в Криму і на заході країни. До коней, які використовуються у дистанційних пробігах, існують певні вимоги, але, на жаль, у наукових роботах небагато досліджень, пов'язаних з аналізом кінського складу, гематологічних та біохімічних показників. Зважаючи на актуальність цієї проблеми, ми поставили собі за мету проаналізувати кількісні зміни показників червоної крові та резистентності еритроцитів у коней, які брали участь в дистанційному пробігу на 32 км.

Об'єктом досліджень було 7 коней кримського типу. Вивчали такі показники крові: кількість еритроцитів (RBC), середній об'єм еритроцитів (MCV), індекс анізоцитозу (RDWc), вміст гемоглобіну

(HGB), середній вміст гемоглобіну в еритроциті (MCH), середню концентрацію гемоглобіну в еритроцитах (MCHC), гематокрит (HCT), а також пероксидну та осмотичну резистентність еритроцитів.

Результати досліджень не показали вірогідних змін в показниках червоної крові у коней після 32-кілометрового пробігу, що свідчить про хороший фізичний стан та адаптованість коней до довготривалої їзди. Натомість осмотична резистентність еритроцитів була вірогідно нижчою після пробігу, що свідчить про порушення цілісності еритроцитарних мембран внаслідок фізичних навантажень. Вивчення динаміки показників крові у поєднанні з біохімічними параметрами та резистентністю еритроцитів дає можливість оцінити процеси адаптації до фізичних навантажень на витривалість в організмі коней, а також проаналізувати рівень їх фізіологічного резерву.

Ключові слова: ДИСТАНЦІЙНІ КІННІ ПРОБІГИ, КОНІ, ГЕМАТОЛОГІЧНІ ПОКАЗНИКИ, РЕЗИСТЕНТНІСТЬ ЕРИТРОЦИТІВ, ФІЗИЧНІ НАВАНТАЖЕННЯ

ПОКАЗАТЕЛИ КРАСНОЙ КРОВИ И РЕЗИСТЕНТНОСТЬ ЭРИТРОЦИТОВ У ЛОШАДЕЙ, УЧАСТВУЮЩИХ В ДИСТАНЦИОННЫХ ПРОБЕГАХ

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Одной из прикладных отраслей коневодства, которые активно развиваются сегодня — это дистанционные пробеги и конный туризм. В Украине, учитывая региональные ландшафтные особенности, дистанционные пробеги наиболее распространены в Крыму и на западе страны. К лошадям, используемых в дистанционных пробегах, предъявляются определенные требования, но, к сожалению, в научных работах немного исследований, связанных с анализом конского состава, гематологических и биохимических показателей. Учитывая актуальность данной проблемы, мы поставили перед собой за цель проанализировать количественные изменения показателей красной крови и резистентности эритроцитов у лошадей, участвовавших в дистанционном пробеге на 32 км.

Объектом исследований было 7 лошадей крымского типа. Изучали следующие показатели крови: количество эритроцитов (RBC), средний объем эритроцитов (MCV), индекс анизоцитоза (RDWc), содержание гемоглобина (HGB), среднее содержание гемоглобина в эритроците (MCH), среднюю концентрацию гемоглобина в эритроцитах (MCHC), гематокрит (HCT), а также перекисную и осмотическую резистентность эритроцитов.

В результате исследований не выявлено достоверных изменений в показателях красной крови у лошадей после пробега в 32 километра, что свидетельствует о хорошем физическом состоянии и адаптации лошадей к долговременной езде. Зато осмотическая резистентность эритроцитов была достоверно ниже после пробега, что свидетельствует о нарушении целостности эритроцитарных мембран в результате физических нагрузок. Изучение динамики показателей крови в сочетании с биохимическими параметрами и резистентностью эритроцитов дает возможность оценить процессы адаптации к физическим нагрузкам на выносливость в организме лошадей, а также проанализировать уровень их физиологического резерва.

Ключевые слова: ДИСТАНЦИОННЫЕ КОННЫЕ ПРОБЕГИ, ЛОШАДИ, ГЕМАТОЛОГИЧЕСКИЕ ПОКАЗАТЕЛИ, РЕЗИСТЕНТНОСТЬ ЭРИТРОЦИТОВ, ФИЗИЧЕСКИЕ НАГРУЗКИ

Equine endurance races have become an important field of competition in recent years but unfortunately have received little attention from scientists [2, 14]. The training of endurance horses and athletic longevity is comparable to that of a human marathoner [8].

Most breeds have been tested and used for endurance races; the most competitive are Arabian or Arabian crosses due to their muscle fiber composition, but other breeds, including Thoroughbred, Quarter Horses, Mustangs, Appaloosas, Morgans, Standardbred [1, 7]. For endurance race, horses of local breeds, which a bred directly in recreational areas are often used. For example, Hucul horses which are widespread in Carpathians — in Poland and Ukraine, or horses from Crimean Mountains [5]. The basis of Crimean horses was formed from the Bashkir breed horses which were 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 of horses was appeared too heterogeneous for genotype and phenotype horses. The horses from Crimea are small, about 145 cm at the withers. It is wide in the body and deep-chested, with a thoracic circumference (girth) averaging about 180 cm; it has 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 colors are bay, red, brown, chestnut, mouse grey. The mane and tail are thick and the coat is also thick. Nowadays, horses in Crimea mountains are widely used is endurance race and recreational riding [5].

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 [3, 7, 11]. The major physiological adaptations to endurance training which may directly influence exercise capacity and stamina of 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 [2, 7]. Certain cardiovascular and haematological adaptations are necessary to guarantee the correct oxygen and blood borne substrates supply to active muscles during exercise and the release of metabolites. These systems could act as limiting factors to the aerobic potential and thereby, could limit the physical performance [23].

Endurance exercise in horses leads to variety of physiological changes including an increase in haematocrit (Hct), cardiac output, increase in mean pulmonary arterial blood pressure, and in the arterial hypoxemia [2, 7, 23]. In addition, during an exercise, muscle temperature increases significantly, which can also affect the reduction of erythrocyte resistance. Also acidosis, elevated catecholamines, dehydration, and compression of erythrocytes in capillaries within the contracting muscle are some important mechanisms that play a role in intravascular haemolysis during regular physical activity [27]. Intravascular hemolysis is one of the most emphasized mechanisms for destruction of erythrocytes during physical activity in horses [4, 5, 10, 21]. In endurance races, stress and fatigue are clearly expressed by changes in hematological and biochemical parameters of horses [2]. In additional blood hematological parameters can be good indicators of the response to treatment, the severity and the systemic effects of a disease, as well as horse welfare, health and fitness levels of horses [2, 3, 7, 14]. Therefore, the objective of the present study was to investigate the alterations of some hematological parameters (haematocrit (HCT), haemoglobin concentration (HGB), the count of red blood cells (RBC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), as well as, resistance of erythrocytes to hemolytic agents in endurance horses after 32 km endurance race.

Materials and methods

Horses. Seven horses from Crimea region (Bilohirsk, Crimean region, Ukraine) were involved in our study (fig. 1) were used in this study. All horses participate in endurance race. Horses were subjected of herd maintenance with feeding (hay and oat) provided twice a day 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. 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 recorded. Only horses that had normal clinical parameters were allowed to participate in the endurance race.

Endurance race. The prolonged exercises were used in endurance race. 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 was repeated for 1 h (phase I); the rest in an outdoor paddock without access to water for 30 min. Phase II consisted with 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 was repeated for 1 h.

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 10:00 AM), and immediately after endurance race (between 11:00 AM and 2:00 PM). Blood was stored into tubes with K-EDTA and held on ice until centrifugation at 3,000g 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 3,000g for 15 minutes. Plasma aliquots were frozen and stored at –25 °C until analyzed.

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

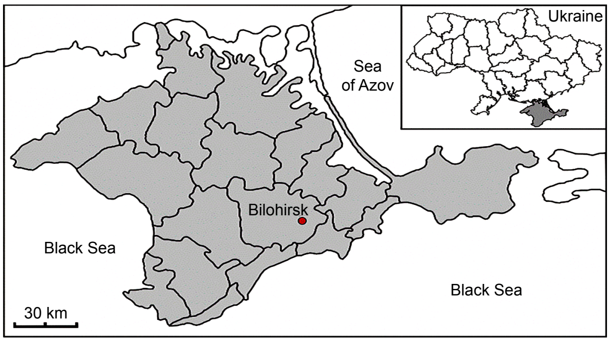

Fig. 1. Maps of Crimean region in Ukraine, Bilohirsk is marked

Assays of Osmotic Resistance of Erythrocytes. The osmotic resistance of erythrocytes was measured spectrophotometrically at the wave length of 540 nm as described by Kamyshnikov (2004) [13]. The method is based on the determination of differences between osmotic resistance of erythrocytes to a mixture containing different concentration of sodium chloride and urea. Absorbance of mixture contained erythrocytes and 0.3M urea solution was determined as 100 % hemolysis (standard). The level of hemolysis in every test tube (%) was calculated in respect to the absorbance of standard. Hemolysis of erythrocytes (%) in every test tube with different urea concentration was expressed as curve [18].

Assay of Resistance of erythrocytes to hydrogen peroxide. The peroxide resistance of erythrocytes was determined spectrophotometrically at 540 nm by monitoring the rate of erythrocytes disintegration by hydrogen peroxide as described by Gzhegotskyi et al. (2004) [15]. The mixture contained 0.25 mL of washed erythrocytes, 0.08 mL of 4 mM phosphate buffer (pH 7.4) with 4 mM sodium azide for catalase activity inhibition, and 0.17 mL of 30 μM hydrogen peroxide dissolved in phosphate buffer (pH 7.4). In the blank, hydrogen peroxide was substituted by phosphate buffer. Absorbance of mixture containing erythrocytes, distilled water and hydrogen peroxide was determined as 100 %. The peroxide resistance of erythrocytes (hemolysed erythrocytes fraction) was expressed in % [15].

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 [15]. 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 [30].

Results and discussion

In our study, all hematological parameters of horses from Crimean region were within the reference values. In the present study, post-ride values of red blood cell indices not significantly changed compared to pre-ride period (fig. 2).

Exercises have variable effects on the erythrocyte indices depending on work intensity, fitness and training levels, environmental conditions and breed of horses [5, 23, 25, 28]. 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 [25]. 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 [20]. Adamu and coauthors (2012) 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 [4]. However, numerous studies have shown that horses which subjected to high altitude have significantly higher RBC, HGB and platelet corpuscular volume (PCV) values, compared to animals that live at less altitude [25, 29]. It is considered a compensatory mechanism for the lower content of oxygen in the atmospheric air, which is proportionally reduced to the altitude [29].

Given that fact that horses from Crimean region were housed under high altitude of the Crimean Mountains, our results are consistent with previous studies [29]. Moreover, one of reason slight increase of red blood cells parameters in the blood of horses from 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 [4, 19], resulting in enhancement of oxygen delivery capacity. In the cellular level, hypoxic exposure can accelerate the proliferation of mitochondria in the muscles [11], increase the buffering capacity for lactic acid [13], 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 [6].

This statement can be meaningful in connection with evidence Adamu and coauthors which have shown that in endurance horses in good performance level didn't observed significant changes of red blood cells indices in post-ride period [1, 2].

We also assessed the resistance of erythrocytes to H_2O_2 exposure in horses from Crimean region during 32 km riding. No significant changes in resistance of erythrocytes to H_2O_2 exposure during 32 km riding were observed (fig. 3).

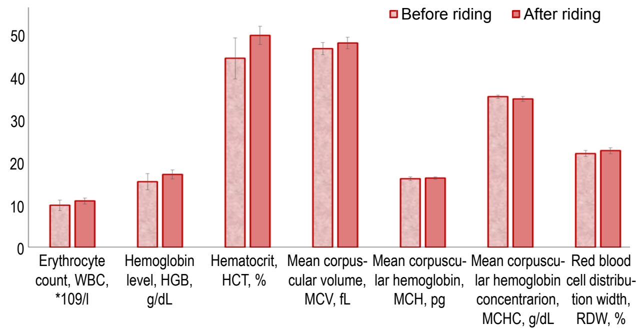

Fig. 2. Values of red blood cell indices of horses from Crimean region during 32 km riding

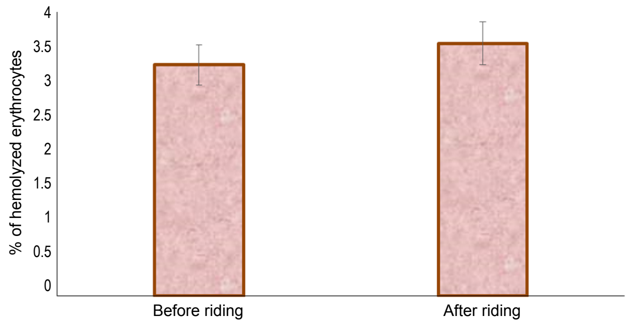

Fig. 3. Resistance of erythrcytes to H_2O_2 exposure in horses from Crimean region during 32 km riding

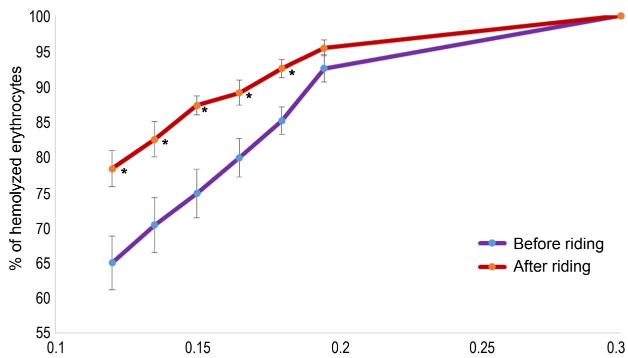

Fig. 4. Osmotic resistance of erythrocytes (% of hemolysed erythrocytes in solutions with different NaCl concentration) in horses from Crimean region during 32 km riding

Note: * — statistical significance ($P<0.05$) between means before and after 32 km riding.

Exercise-induced hemolysis has been confirmed under various conditions in stallions [4, 17], mares [4, 26] and mixed population [15, 16]. In our study, we observed that erythrocytes exposed to the different concentrations of urea after endurance race had a higher level of haemolysis compared to the pre-ride period (fig. 4). Increase of percent of haemolyzed erythrocytes by 20.58 % (P=0.013), by 17.3 % (P=0.023), by 16.75 % (P=0.006), by 11.57 % (P=0.015), and by 8.73 % (P=0.008) at 0.12, 0.135, 0.15, 0.165, and 0.18M urea solutions respectively was observed in horses after endurance race.

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 [9, 22, 27]. Our findings are consistent with data of Devi et al. (2009) which have shown that osmotic stress at 0.3 % and 0.4 % NaCl imposed hemolysis in animals exposed of altitude and thereby signifying an oxidative stress-dependent impairment of erythrocyte stability [12]. It has been shown that lipid peroxidation and oxidation of proteins by free radicals play a major role in many oxidative erythrocytes damage and cause profound alterations in the structural organization and functions of the cell membrane including decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes and loss of essential fatty acids [9, 22].

Based on our results it is possible to affirm that enhanced percent of hemolyzed erythrocytes in horses after endurance ride indicate an oxidative stress-dependent impairment of erythrocyte stability.

Conclusion

In conclusion, we can summarize that adequate endurance race of low intensity could improve oxygen-dependent respiratory function in horses of Crimean region. Furthermore, the non-significant increase of red blood cells indices in endurance horses indicate of good athletic level after 32 km endurance ride.

Statistically significant differences in the percentage of hemolyzed erythrocytes between pre- and post-ride period were observed and thereby signifying an oxidative stress-dependent impairment of erythrocyte stability.

It is important to understand the hematological changes caused by various physical efforts because they reflect changes in the functions of different body systems and can be used for health control and diagnosis of diseases and allow the evaluating the level of sport performance the accuracy of training and physiological condition of horses.

Perspectives of the future investigations. Obtained results concluded that evaluating of blood indexes in horses during different type and periods of training can be illustrative diagnostic parameter for the recommendation of optimal regime of physical loads. Regarding above said in the future investigations authors planning to continue began experiments and enhance the complex of evaluated parameters of blood of horses.

Acknowledgments

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