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Hematological parameters in endurance horses pre and post 120 km race

Parâmetros hematológicos em cavalos de enduro pré e pós prova de 120 km

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ABSTRACT

The endurance discipline has the shortest history in Bulgaria compared with other disciplines of equestrian sports. Endurance competitions are held over distances from 40 km to 160 km. The present study focused on the effect of exercise on horses over a distance of 120 km. Changes in the following hematological parameters were investigated: Leukocytes (WBC, g/l); Erythrocytes (RBC, T/l); Platelets (PLT, g/l); Hemoglobin (Hb, g/l); Hematocrit (HCT,%); Mean corpuscular volume (MCV, fl); Mean corpuscular hemoglobin (MCH, pg); Mean cell hemoglobin concentration (MCHC, g/l), Leukogram (Eos; Bas; Neu; Lym; Mon,%) as well as some electrolytes and hormone concentration. The study aimed to establish the changes in these indicators' values before and after the competition. Analysis of the haemogram showed a significant increase in the mean values of 4 of the indicators under study. No significant differences were established in MCV, MCH and MCHC. A decrease was found in the number of Lym, Eos, and Mon and a significant increase in Neu after the competition. The concentration of electrolytes (Ca, Na and K) in the blood serum decreased, and the levels of CK and LDH significantly increased.

KEYWORDS: horse; electrolytes; hematology; endurance.

RESUMO

A disciplina de resistência tem a história mais curta na Bulgária em comparação com outras disciplinas de esportes equestres. As competições de resistência são realizadas em distâncias de 40 km a 160 km. O presente estudo focou no efeito do exercício em cavalos em uma distância de 120 km. Foram investigadas alterações nos seguintes parâmetros hematológicos: Leucócitos (g/l); Eritrócitos (T/l); Plaquetas (g/l); Hemoglobina (g/l); Hematócrito (%); Volume corpuscular médio (VCM, fl); Hemoglobina corpuscular média (HCM, pg); Concentração de hemoglobina corpuscular média (CHCM, g/l), Leucograma (Eos; Bas; Neu; Lin; Mon,%), bem como alguns eletrólitos e concentração hormonal. O estudo teve como objetivo estabelecer as mudanças nos valores desses indicadores antes e depois da competição. A análise do hemograma mostrou um aumento significativo nos valores médios de 4 dos indicadores estudados. Não foram encontradas diferenças significativas no VMC, HCM e CHCM. Foi encontrada uma diminuição no número de Lin, Eos e Mon e um aumento significativo em Neu após a competição. A concentração de eletrólitos (Ca, Na e K) no soro sanguíneo diminuiu e os níveis de Creatina Quinase (CK) e Lactato Desidrogenase (LDH) aumentaram significativamente.

PALAVRAS-CHAVE: cavalo; eletrólitos; hematologia; resistência.

INTRODUCTION

In the endurance discipline, the condition of the horses is strictly monitored before, during and after the end of the race, which guarantees their well-being. In Bulgaria, the studies conducted on horses in the endurance discipline are relatively few. SABEV (2010), studied the morphological indicators of blood in horses participating at different distances, observed statistically significant changes in all indicators under study. VALEV et al. (2019), studied the psychophysical preparation of the rider and its impact on him in a stressful situation, such as an endurance competition.

Developing training methods to achieve high sports performance in endurance events was also a subject of interest (NEDKOVA-IVANOVA & VALEV 2020). The hemogram is particularly important when working with sport horses subjected to heavy workloads. It allows tracking of the function of blood

components, workability and performance of horses. On the other hand, it will establish the effectiveness of the applied training and, if necessary, introduce improvements to achieve higher results (GUTIÉRREZ & JARAMILLO 2006). It was reported that endurance-galloping exercises on sandy tracks change PCV, hemoglobin and RBC (WAKIL et al. 2022). A significant increase of 36% for WBC was described in endurance horses immediately after exercise during the training season, as for the RBC, Hb and HTC, no significant changes were noted (MAŚKO et al. 2021).

TEIXEIRA-NETO et al. (2012), observed an increase in RBC count, Hb and HTC in endurance horses competing at a distance from 30 km to 100 km. In horses participating in different distances was stated increased RBC and WBC counts both in short-distance and long-distance races (CYWINSKA et al. 2012). At a distance of 120 km, was noted an increase in the values at the end of the race by 3% for hemoglobin and RBC and 36% for WBC. The most significant increases were estimated in the studied enzymes' levels after the race's completion, one of which was CK (LARSSON et al. 2013). Significant changes were observed during different length races (80 to 160 km) in levels of CK and LDH by TRIGO et al. (2010). During 91 km endurance race significant increase was noted in CK and LDH levels, and decreases in K and Na concentration. Upon completion of 166 km competition, the concentration of electrolytes K and Ca dropped down, but Potassium remained unchanged (MUÑOZ et al. 2010).

Due to the few studies, regarding the endurance discipline in racehorses, carried out in Bulgaria, the present study aimed to monitor hematological parameters in the blood of horses competing in the discipline of endurance at a distance of 120 km. Likewise, identifying changes in the values of CK, LDH and electrolytes Ca, K and Na before the start and after the end of the competition is also important. Horses competing in CEI** undergo comprehensive training to develop fitness and stamina for an intensive workload. Therefore, relatively constant values of the studied parameters may be retained.

MATERIAL AND METHODS

Animal welfare and ethical statements

All the experimental procedures were carried out under art. 153 of the Law on Veterinary Activity, Bulgaria, following the EU Directive 86/609.

Competition sites and animals

The study was conducted on the territory of the “Kabiyuk” national stud farm near Shumen, Bulgaria, where a CEI** endurance competition was held during the summer season period (26.06.2021). The altitude of the competition site is 269 m, the course consisted of grassy, hilly and agricultural roads. The average daily temperature was 30 °C and the humidity was 60% in the day of competition. The total number of registered horses in the starting list was eight. Consent from the owners to be included in the study was obtained for five horses. The animals (four mares and one stallion) were aged between 7 and 12 years, four horses were purebred Arabians, and one was Anglo-Arabian.

Sample collection and examination

Before the start of competition, all horses underwent a compulsory pre-ride medical examination, as well as after each stage. Food and water were offered to the animals 1.5 h prior to the beginning of competition and water was available throughout all stages of the endurance test. Blood samples (whole blood and serum) were collected by jugular vein puncture in 4 ml vacutainers 30 minutes before the start from the horses and immediately after the race in the order of arrival of the horses. Samples before the race were collected inside the stables and after the race on a shaded location near the finishing point. The serum intended for biochemical testing was obtained on-site by centrifugation (Hettich EBA 8S centrifuge) at 3500 rpm for 5 min. The serum was stored under refrigerated conditions until its examination in the Clinical Laboratory at the Faculty of Veterinary Medicine of Trakia University. Biochemical analysis (Creatine Kinase (CK), Lactate Dehydrogenase (LDH), and electrolytes (Ca, K and Na) was performed using an automatic biochemical analyzer (Mindray BS - 120).

The hematological indicators included in the study were as follow: Leukocytes (WBC, g/l); Erythrocytes (RBC, T/l); Platelets (PLT, g/l); Hemoglobin (Hb, g/l); Hematocrit (HCT, %); Mean cell volume (MCV, fl); Mean cell hemoglobin (MCH, pg); Mean cell hemoglobin concentration (MCHC, g/l) and leukogram (Eos; Bas; Neu; Lym; Mon, %). The described indicators were examined with an automatic hematological analyzer (Mindray BC - 5000 Vet).

Statistical analysis

Statistical data processing was performed using IBM SPSS Statistics, version 26. A Shapiro-Wilk test for normal distribution was carried out. Means and standard deviations were computed performing a Paired-

Samples T-test to determine whether the differences between the two samples were significant. Values were considered as statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The hemogram results before and after the completion of the 120 km race showed a statistically significant increase in the average value of leukocytes after the competition (20.96±3.83 g/l) compared to the average level before the race (11.82 ±0.81 g/l). A statistically significant increase was also found in the level of erythrocytes, where the mean value recorded before the start was 18% lower (8.49±0.32 T/l) compared to the mean value after finishing (10.08±0.58 T/l). The platelet levels before and after the race remained almost unchanged. The mean hemoglobin and hematocrit values obtained before and after the completion showed an increase of just over 19%. Before the start, hemoglobin had an average value of 142.00±7.68 g/l, and at the finish, an average value of 169.60±19.39 g/l. Hematocrit values before and after the end of the competition were 38.88±2.30% and 46.32±6.34%, respectively (Table 1).

Table 1. Estimated values for the hematological parameters pre- and post-race.

Parameter	Before Race			After Race			*p* value*
	Min	Max	Mean (±Std.D)	Min	Max	Mean (±Std.D)	
WBC (g/l)	10.68	12.72	11.82 (±0.81)	17.36	27.26	20.96 (±3.83)	0.004
RBC (T/l)	7.99	8.80	8.49 (±0.32)	9.60	11.00	10.08 (±0.58)	0.014
PLT (g/l)	133.00	167.00	149.60 (±12.95)	129.00	168.00	149.00 (±16.98)	0.961
Hb (g/l)	130.00	149.00	142.00 (±7.68)	146.00	198.00	169.60(±19.39)	0.011
HCT (%)	35.20	40.60	38.88 (±2.30)	38.80	56.30	46.32 (±6.34)	0.025

*According to the statistical analysis performed. **Reference values (provided by the laboratory): WBC (6.0-12.0 g/l); RBC (6.0-12.0 T/l); PLT (100-360 g/l); Hb (110-160 g/l); HCT (34.0-48.0%).

Several studies have shown significant increases in the number of erythrocytes, lymphocytes, platelets, hemoglobin and hematocrit; the most notable being the high number of platelets that horses present at the end of the 120 km race (CYWINSKA et al. 2012, SIQUEIRA & FERNANDES 2017). Mean values immediately after training were reported to be higher for all parameters except lymphocyte count. Additionally, significant differences were observed between pre-training and 24 h post-training values for RBC count, PCV and segmented neutrophils (LAU et al. 2010). No relation was found between the ranking of horses in the competition and their age with the values of the studied hematological parameters (ADAMU et al. 2013).

For horses competing at a distance of 30 km, no significant differences in hemoglobin levels and in the number of red and white blood cells before and after the end of the competition were found (RAZAZADEH et al. 2016). An increase was observed in the hematocrit percentage of over 8% at the end of the competition. At a distance of 45 km, an increase of just over 20% was found in hemoglobin and hematocrit values in pure Arabian horses (POŠKIENĖ et al. 2021). After completing races with distances between 60 and 120 km, an average increase in white blood cell count of 36% and a 3% increase in red blood cell count was stated. Hemoglobin and hematocrit increased by 3% and 5%, respectively, after the completion of the race. A decrease was found in the platelet count after finishing (LARSSON et al. 2013).

The increase in hematological parameters is most often explained by activation of the erythrocytes splenic reserve, a process that is dependent on the intensity of the exercise (TEIXEIRA-NETO et al. 2012). Leukocytosis is associated with increased circulation of corticosteroids and splenic contractions during exercise (SATUÉ et al. 2012). The competition season also influences the blood morphological indices in horses compared to the beginning and at the end of the racing season (POŠKIENĖ et al. 2019).

In this study, no significant differences were found in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) (Figure 1). However, similar minimal variations in the volume and concentration of erythrocytes and hemoglobin were also observed in other studies, with a variation of 1% comparing pre- to post-race values even 24 h after the race (LARSSON et al. 2013, SIQUEIRA & FERNANDES 2017).

Regarding the results of the leukogram (Table 2), no significant fluctuations were found in basophils before and after the end of the race. There was an increase in the average values only in the number of neutrophils, as before the start, they were 44.58±5.46%, and at the end of the workload, 77.26±6.86%. In the other three indicators of the leukogram, a decrease in their number was observed at the end of the race, with the highest percentage decrease in the number of eosinophils from 3.00±1.37% before the start to

0.04±0.05% after finishing the horses. Lymphocytes and monocytes averaged 31.44±3.43% and 20.94±5.04%, respectively, before the competition and 8.06±1.79% and 14.64±5.55% after the competition. Another study also reported an increase in the neutrophil count at the end of the race. As for all other leukogram parameters, a decrease in the percentage content was found, with the most significant differences observed in basophils by 21% and monocytes by 16% (LARSSON et al. 2013).

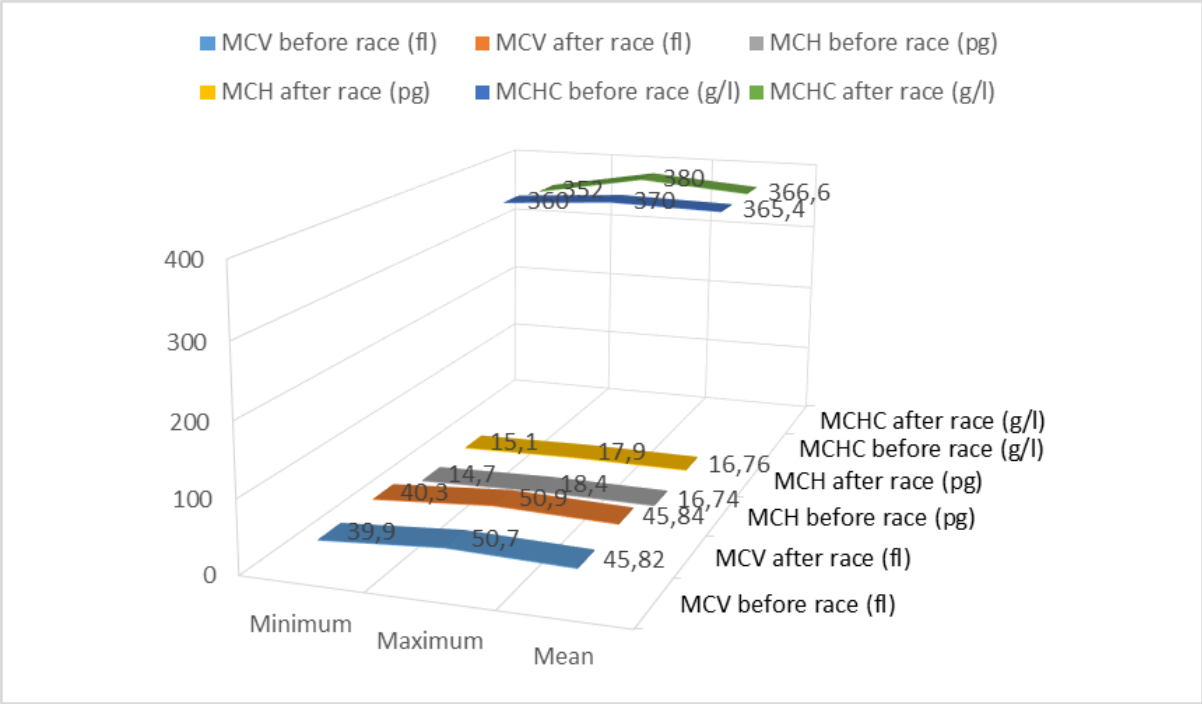

Figure 1. Mean values for RBC volume, hemoglobin volume and hemoglobin concentration.

Table 2. Values of hematological parameters from the leukogram pre- and post-race.

Parameter	Before Race			After Race			*p* value*
	Min	Max	Mean (±Std.D)	Min	Max	Mean (±Std.D)	
Eos (%)	1.10	4.80	3.00 (±1.37)	0.00	0.10	0.04 (±0.05)	0.008
Bas (%)	0.00	0.10	0.04 (±0.05)	0.00	0.00	0.00	0.178
Neu (%)	37.00	51.30	44.58 (±5.46)	66.70	85.40	77.26 (±6.86)	0.001
Lym (%)	27.10	36.40	31.44 (±3.43)	5.40	10.00	8.06 (±1.79)	0.000
Mon (%)	15.50	27.20	20.94 (±5.04)	9.20	24.00	14.64 (±5.55)	0.195

*According to the statistical analysis performed. **Reference values in % (provided by the laboratory): Eos (0-10); Bas (0-3); Neu (35-40); Lym (45-50); Mon (2-6).

Table 3 presents the results for the estimated values of the enzymes Creatine Kinase and Lactate Dehydrogenase, as well as levels of electrolytes, Ca, K and Na, in the blood of the horses before and after the 120 km race. There was a decrease in the concentrations of Ca and Na in the blood serum at the end of the competition by 7.44% and 2.90%, respectively. For K, the concentration decreased by a significantly greater percentage of 46.07% at the end of the competition. An increase was observed in the mean values of biochemical parameters, with CK increasing from 344.00 U/l before the start to 1682 U/l after finishing and LDH from 674.40 U/l before to 1574.80 after finishing the race.

Table 3. Values of biochemical parameters and concentration of electrolytes in the blood serum pre and post-race.

Parameter	Before Race			After Race			*p* value*
	Min	Max	Mean (±Std.D)	Min	Max	Mean (±Std.D)	
Ca (mmol/l)	3.14	3.55	3.36 (±0.15)	2.75	3.45	3.11 (±0.29)	0.181
K (mmol/l)	3.67	3.91	3.82 (±0.09)	1.16	2.47	2.06 (±0.54)	0.003
Na (mmol/l)	135.60	137.60	136.34 (±0.97)	125.60	138.20	132.38 (±4.49)	0.124
CK (U/l)	318.00	364.00	344.00 (±21.27)	1023.00	3363.00	1682.00 (±956.07)	0.034
LDH (U/l)	557.00	728.00	674.40 (±69.95)	1226.00	2014.00	1574.80 (±399.40)	0.010

* According to the statistical analysis performed. **Reference values (provided by the laboratory): Ca (2.65-3.25 mmol/l); K (2.4-5.2 mmol/l); Na (136-142 mmol/l); CK (113-333 U/l); LDH (140-460 U/l)

A significant increase in CK level at the end of a 120 km competition was reported by other authors as it rose from 210 U/l before to 2242 U/l after the race. The authors reported a 3% increase in Na concentration after the horses finished and a 10% decrease in K concentration compared to pre-race values (FAN et al. 2002). In horses competing at a distance of 80km, categorized by age and sport performance, it was reported that in horses with good performance, Na concentration was on average 134.3-135.8 mmol/l, K concentration on average 4.6 mmol/l and Ca 3.2 - 3.5 mmol/l (ADAMU et al. 2013). Thus, the average values presented slightly exceed the results obtained in the recent study for the concentration of electrolytes in the blood serum of the horses after the completion of the 120 km course.

However, the data obtained in this study for the mean value of CK were within the range of those presented for horses performing well in an 80 km trial (ADAMU et al. 2013). After 30 km workload, the average values estimated for the Na concentration remained almost unchanged. The K concentration dropped from 3.94 mmol/l to 2.69 mmol/l. The mean value of CK increased significantly from 176.00 U/l to 316.86 U/l (LAU et al. 2010). However, these results obtained after the end of 30 km training were significantly lower than those for the mean value of 1682.00 U/l after the 120 km race brought in recent research.

Tracking the levels of CK, as an enzyme actively involved in the cell's metabolic processes, is essential in horses participating in the endurance discipline. In horses participating in a three-day competition at a distance of 210 km, the highest levels of CK were present on the second day of the competition. The enzyme level began to decrease on the third day compared with the previous day of the competition, but its values were higher than those on the first day. This could be explained by the fact that only the best-prepared horses reach the last day of competition (GONDIM et al. 2009).

Maintaining optimal levels of Na, K and Ca is of particular importance for the water-electrolyte balance of the cell, which favors the transmission of nerve impulses and muscle contractions. Prolonged exertion during endurance races is invariably accompanied by loss of electrolytes through sweat, urine, and to some extent with faeces. The loss of electrolytes through sweat leads to fatigue and weakness in horses. Significant fluid and electrolyte deficits were noted overnight post-race in horses competing at 50 and 100 miles (SCHOTT et al. 1997). The recovery of fluid and electrolyte losses should be based on the sweat released for 1 h of exercise, which in most cases equals 2-5 l (BERGERO et al. 2005).

According to other authors, the electrolyte balance does not undergo significant disturbances during races of 120 km. This suggests that endurance horses can compensate for fluid and electrolyte losses through a compensatory mechanism between intracellular and extracellular structures (LARSSON et al. 2013, FLAMINIO & RUSH 1998).

CONCLUSION

The 120 km race significantly influences mean WBC, RBC, Hb and HCT values, which increased between 18.7% and 77%, while PLT levels remained almost unchanged. The increase in indicators was within the reference values, except for WBC and Hb, where the increase exceeded the presented reference values.

No significant differences were established in mean erythrocyte volume (MCV), mean hemoglobin volume (MCH) and mean hemoglobin concentration (MCHC). A decrease was found in the number of Lym, Eos and Mon, and in Neu, a significant increase after the competition, exceeding the upper limit of the reference values. The Ca, Na, and K electrolyte concentrations decreased, and the electrolyte losses observed during the test were not critical and were within or close to the reference values.

The slight lowering of the cited electrolytes can be attributed to the electrolyte supplements that the

athletes use during the race. CK and LDH values increased significantly in response to prolonged exercise during the competition. This increased enzyme activity is logically related to a physiological response of the horse's body to increased muscle activity. Thus, the data presented suggests that horses with adequate training can successfully cover the distance of 120 km without significant deviations in their metabolic status and risk to their health.

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