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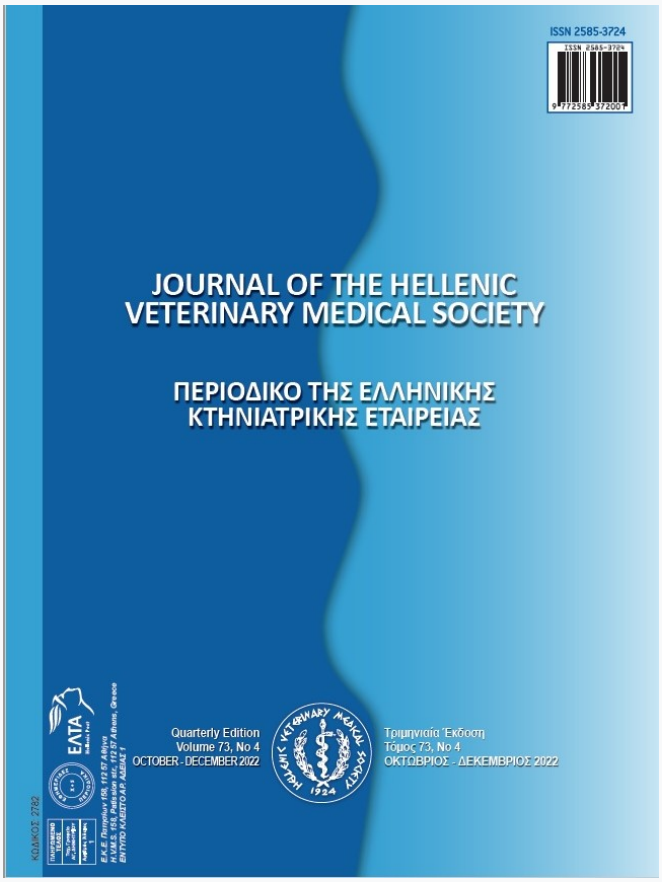

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Research article
Ερευνητικό άρθρο

Pre- and post -exercise variation of blood parameters on performance endurance horses: a first race study from Algeria

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ABSTRACT: This paper presents the first study conducted in Algeria to determine the pre and post-race hematological and biochemical parameters of horses of a 90 km endurance race for eliminated horses' category and those who completed the races with good performance. This study was conducted on 15 endurance horses (10 Arabian Thouroughbred, 4 Arabian-Barb and 1 Barb). Nine (9) horses successfully completed the endurance race, whereas the other 6 were eliminated. Blood samples were collected from each animal before and after the end of the race. Whole blood samples were used for measuring the blood-biochemical parameters. Our results showed a significant increase of the hematological parameters (RBC, HB, Ht and MCV), the total leucocyte count, neutrophils and platelets for the group of horses who finished the race successfully. A significant decrease was noticed for the lymphocytes and eosinophils numbers while serum biochemical parameters showed a significant increase of Ca, TG, urea, U-A, AST and CK and a decrease of Mg, CHE, GLU and ɣGT. This study showed that, for the eliminated horses, there were significant changes in values of the total leucocytic count, lymphocytes, neutrophils, HB and Ht as compared to good performance horses. Serum biochemical parameters showed a significant increase of TG, U-A, AST and CK and a significant decrease of Ca, Mg, CHE and ɣGT. Most of the hemato-biochemical parameters of both the eliminated horses and those who completed the race successfully depicted significant differences. The results of this study suggested that biochemical findings could help identify horses with an increased risk of developing metabolic alterations before their elimination during competitive endurance races of different distances. Therefore, proper management protocols, standard feeding and watering with proper preparation of animals before the race could prevent electrolytes imbalance and pathological affections during racing.

Key words: endurance horses; hematology; biochemical; performance

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INTRODUCTION

The first endurance race took place in Algeria in 1984 at the Tiaret horse show. Tiaret is one of the main cities that holds endurance horse championships and qualifications and is home to the national championships organized annually by the Fédération Équestre Algerienne (FEA). This is because the city brings together several equine institutions, notably the Chaouchaoua National Stud founded in 1877 and dedicated to the conservation and improvement of the Algerian horse breeds especially the Arabian Thoroughbred and the Barb breed.

During an endurance race, horses must undergo veterinary checks before and during the race. After each loop, a medical examination is carried out in order to assess the general condition of the horse, heart and respiratory rate (Bachir and Rasedee, 2009) (Cottin, et al., 2006), dehydration rate and examination of gaits to guarantee the safety of the horse and its rider.

Of all equine competitions, endurance races have the greatest metabolic demands for sport horses. Long distance endurance races are a big challenge for the body of the horse requiring substantial energy production for many hours (Treiber, et al., 2006). Such sustained energy demands cause the cardiorespiratory, endocrine and neuromuscular systems to operate at an elevated level for a considerable length of time (Flaminio and Rush, 1998; Schott, et al., 2006).

Blood biochemical parameters are considered to be indicative of health status of athletic horses affecting performance and presence of any disorder (Hodgson, et al., 2014).

The results of various blood analyses on horses competing in different distances have been reported (Castejon, et al., 2006; Schott, et al., 2006) and a reasonably clear picture can be obtained from the changes that occur in horses performing in endurance exercises. However, the thin line between a horse eliminated for metabolic reasons and a fit horse is not always clear and disputes occasionally arise. The information concerning horses eliminated due to metabolic conditions is not so extensive (Fielding, et al., 2009). This leads to unnecessary medical treatments in some cases and the development of life-threatening diseases that could have been detected earlier in others (Rezazadeh, et al., 2016).

Endurance riding is a growing sport in Algeria, and its popularity has led to an increasing number of participating horses and riders at competitions. This interest in this type of sport makes it even more important to ensure that any adverse effects on the horses as a result of such a physically demanding race are minimized. Consequently to improve performance, there is a need for further studies to best manage endurance horses.

To this end, the objective of this study is to detect the variations in some hematological and biochemical parameters in endurance horses just before and after completing the race. The differences, in the levels of these parameters, between horses that completed the race with good performance and those who did not successfully complete the race were determined. These parameters obtained from these two categorized groups at the start of the race were also compared in order to estimate the capability of an endurance horse to complete the race successfully.

MATERIAL AND METHODS

Animals

Fifty (50) horses participated in the 90 km endurance ride, held in the city of Tiaret in west Algeria in November 2018 (temperature 10-15°C). Only 15 male and female horses (10 Arabian Thoroughbred, 4 Arabian-Barb and 1 Barb) were enlisted for this study. The age and the body weight of these horses ranged between 4 and 11 years and 350-450kg, respectively. They were clinically healthy and admitted to take part in the race as per FEI rules. The additional 35 horses in the competition were not included in this study due to the lack of owners' consents.

Out of the 15 horses, 6 (2 Arabian Thoroughbred, 3 Arabian-Barb and 1 Barb) developed, during the course of the race, metabolic syndrome and lameness and were subsequently eliminated from the race and only 9 (8 Arabian Thoroughbred and 1 Arabian-Barb) completed the race successfully. The race was conducted in accordance with FEI rules.

Clinical Examination

A pre-race check was conducted for every horse. Veterinary inspections were also conducted after each phase of the race on all competing horses and their physical parameters (heart rate, mucous membrane, skin recoil, gut motility or sound and gait) were recorded. The horses were also observed for soreness or injuries on the back, withers, girth area, body or distal extremities (Khaled, and Ahmed, 2008; Lawan, et al., 2010).

Horses were categorized as successfully completing the race based on their ability to maintain normal gastrointestinal, respiratory, cardiac, or musculoskeletal status (Khaled, and Ahmed, 2008).

Samples

Venous blood from the jugular vein was collected using an 18 gauge needle mounted on a 10 CC sterile syringe (KD-JECT® III, KDM®, Germany) one day before the race, then stored in K_3EDTA x4ml (FL medical-ITALY) test tubes for hematological tests, and into heparinized vacutainer tubes for biochemical analysis. Blood sample collection was performed immediately after a 20-minute recovery period and samples were analyzed immediately in the veterinary institute laboratory before and after the race. The tubes with no anticoagulant were centrifuged at 3000 g for 15 minutes. Serum was aspirated, immediately frozen, and stored at -20oC. Since certain biochemical values can be modified by freezing, we took care to analyze them in the two weeks following the sample.

Hematological Examination

In whole blood, the following parameters were examined: red blood cells (RBC), hematocrit (Ht), hemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC) and platelets (PLT). A hematology automaton was used, calibrated for the equine species (Orphee Mythic 18 Hematology Analyzer®). Differential blood count including neutrophils (NEUT), eosinophils (EOS), basophils (BASO), monocytes (MONO), lymphocytes (LYMP), was determined on blood smears using a light microscope at x400 magnification. Those blood smears were ethanol-fixed and stained according to May-Grünwald-Giemsa technique.

Biochemical Analysis

In blood serum, the following parameters were determined: calcium (Ca), magnesium (Mg), total cholesterol (CHE), triglycerides (TG), urea, uric-acid (U-A), glucose (GLU), activity of creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (ɣGT). These biochemical parameters were determined using a split beam UV/Vis spectrophotometer (OPTIZEN 2120UV Plus).

Statistical Analysis

Statistical data analysis was carried out using Statistica StatSoft software (version 6.1, Statsoft, Tulsa, OK). One-way ANOVA test was used to compare the means between two groups. This analysis was followed by the Tukey post-hoc test to determine the significant differences and compare the means between two groups. The differences were considered to be statistically significant for a p-value lower than 0.05 in all the statistical analyzes.

RESULTS

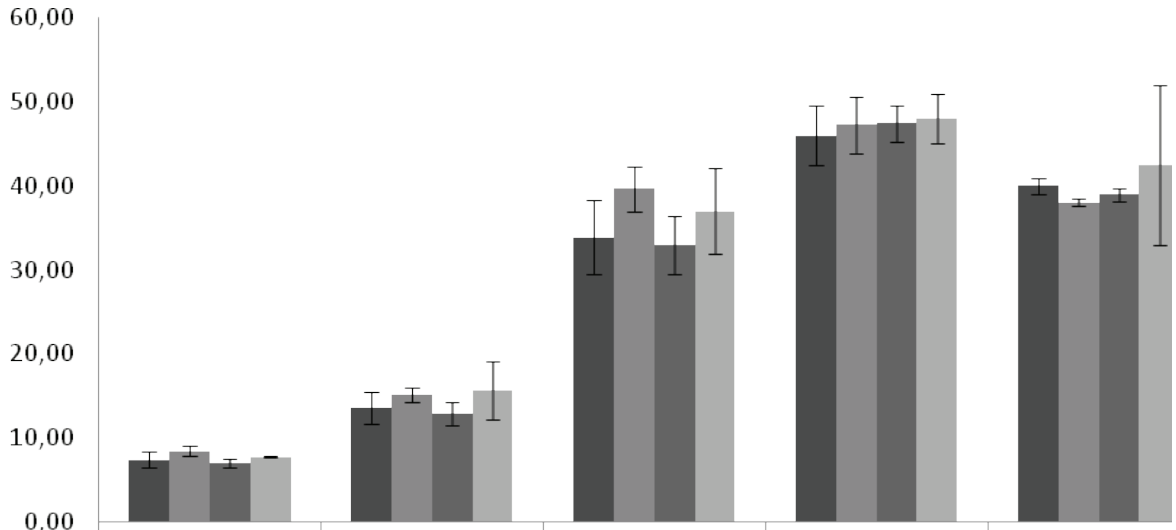
From 15 examined horses (10 Arabian Thoroughbred, 4 Arabian-Barb and 1 Barb), 6 were eliminated from the endurance competition and 9 horses completed the race. All the eliminated horses were able to complete between 20 to 30 km of the 90 km race, due to the onset of metabolic disorder or physical lameness. Considering the reduced number of horses eliminated, we could not divide them into two groups, those eliminated for metabolic cause and those eliminated for lameness. In this study, the performance of horses that completed the race successfully is referred to as good performance (GP) and that of eliminated horses is referred to as poor performance (PP).

Pre and post GP and PP hematology, plasma/serum electrolyte and biochemical parameters of endurance horses are presented in Figure 1, Figure 2 Figure 3 and Figure 4.

Our results showed a significant increase in the hematological parameters (RBC, HB, Ht and MCV) of GP horses after completing the race. Total leucocytic count, monocytes, neutrophils and platelets also showed such a significant increase. Lymphocytes and eosinophils showed a significant decrease while the serum biochemical parameters showed a significant increase of Ca, TG, urea, U-A, AST, ALT and a decrease of Mg, CHE, GLU, ɣGT, and CK.

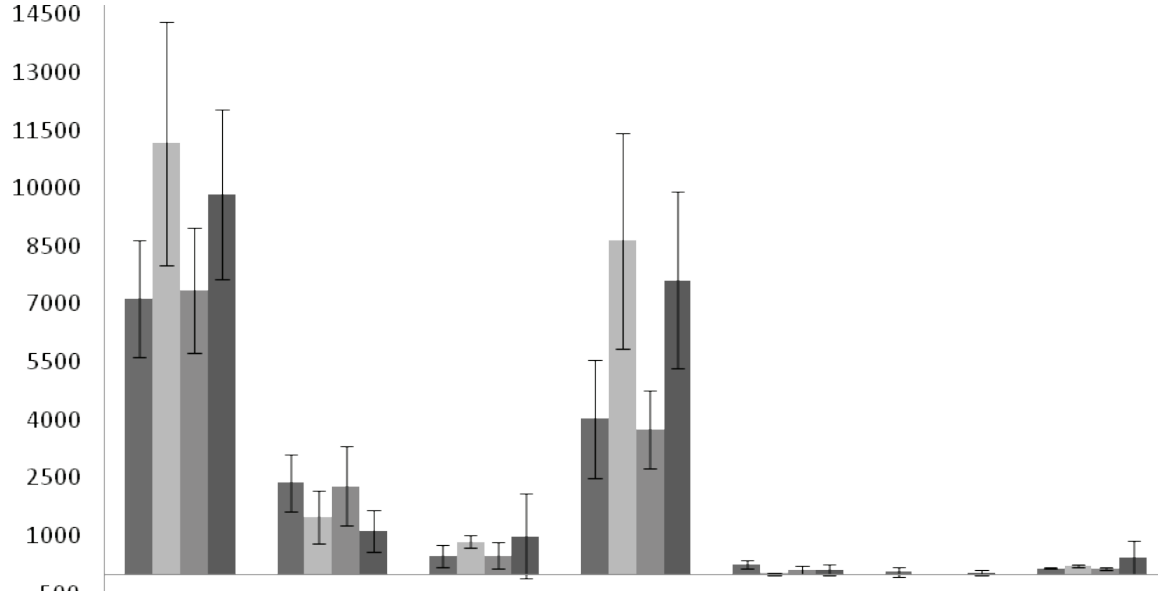
This study showed that the eliminated horses had significant changes in post-race values for total leucocytic count, lymphocytes, neutrophils, HB and Ht than in GP horses. Serum biochemical parameters showed a significant increase in triglyceride, U-A, and AST and a significant decrease in Ca, Mg, CHE, ɣGT and CK post-race.

The two groups (GP and PP) showed a significant difference in the total leucocytic count, neutrophils, eosinophils, RBC, HB, Ht, MCV, MHC, platelets, Ca, CHE, TG, urea, U-A, AST and ɣGT pre-race.



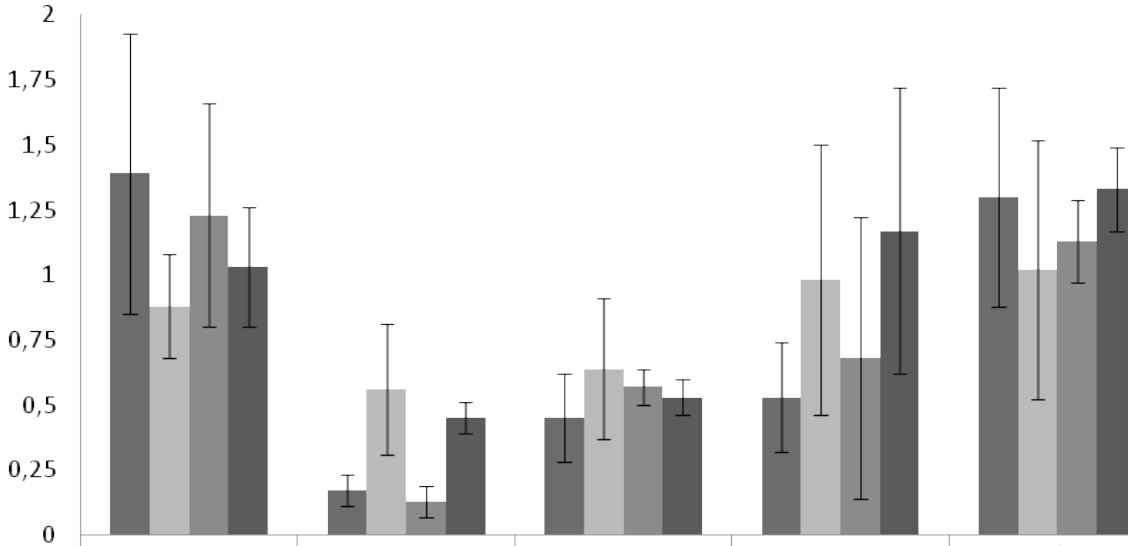
	RBC ($x10^6$ /mm^3)	HB (g/dl)	Ht (%)	MCV (fl)	MCHC (g/dl)
Before race (GP)	7,39	13,54	33,90	45,97	39,99
After race (GP)	8,41	15,08	39,64	47,26	38,06
Before race (PP)	6,92	12,82	32,90	47,47	38,95
After race (PP)	7,71	15,63	37,02	48,06	42,48

Figure 1. Hematological parameters in endurance horses before and after the endurance race (results depicted in terms of mean values ± standard deviations at P*< 0.05, **<0.01, ***<0.001).



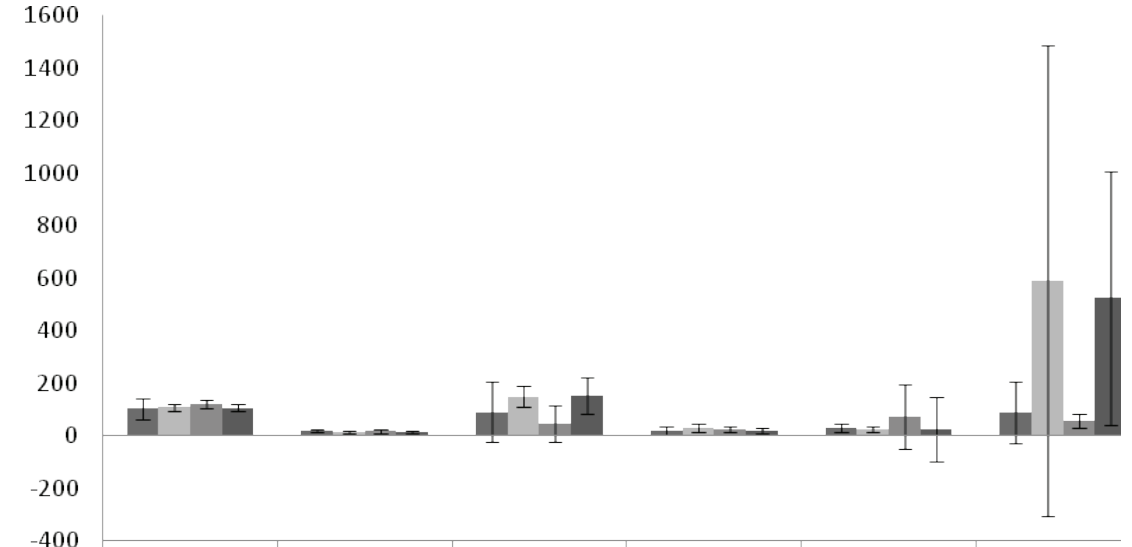
	WBC (/mm^3)	LYMP (/mm^3)	MONO (/mm^3)	NEUT (/mm^3)	EOS (/mm^3)	BASO (/mm^3)	PLT ($x10^3$ /mm^3)
Before race (GP)	7144,44	2376,44	469,89	4027,78	258,33	69,33	165,11
After race (GP)	11166,67	1484,78	845,33	8653	7,56	0	233,11
Before race (PP)	7357,14	2289,66	489,33	3756,17	123,83	0	158,66
After race (PP)	9850	1131,33	993,33	7619,66	125	49,66	434,16

Figure 2. Total and differential leucocytic counts in endurance horses before and after endurance race (results depicted in terms of mean values ± standard deviations at P*< 0.05, **<0.01, ***<0.001)



	CHE (g/l)	TG (g/l)	Urea (g/l)	U-A (mg/dl)	Glu (g/l)
Before race (GP)	1,39	0,17	0,45	0,53	1,3
After race (GP)	0,88	0,56	0,64	0,98	1,02
Before race (PP)	1,23	0,13	0,57	0,68	1,13
After race (PP)	1,03	0,45	0,53	1,17	1,33

Figure 3. Some serum biochemical parameters in racing horses before and after race (results depicted in terms of mean values ± standard deviations at P*< 0.05, **<0.01, ***<0.001)



	Ca (mg/l)	Mg (mg/l)	AST (U/l)	ALT (U/l)	γGT (U/l)	CK (U/l)
Before race (GP)	102,53	19,97	89,63	18,87	28,55	86,65
After race (GP)	107,09	13,61	148,62	30,72	22,22	589,44
Before race (PP)	118,99	17,28	45,88	22,66	72,58	56,22
After race (PP)	105,65	14,01	151,57	19,75	25,46	524,58

Figure 4. Some other serum biochemical parameters in racing horses before and after race (results depicted in terms of mean values ± standard deviations at P*< 0.05, **<0.01, ***<0.001)

DISCUSSION

The use of laboratorial parameters is required to assess health, nutritional and training status of horses (Gomide, et al., 2006).

The FEI defines endurance race as a long-distance race that tests the speed and endurance of horses across all kinds of terrain and the riders' skills in controlling horse pace using their knowledge to manage their horses' capabilities (FEI, 2019).

During races, some horses show changes in homeostasis results because of evaporative heat loss, with a loss of 10-15 liters primarily as sweat (McConaghy, et al., 1995), energy depletion and alterations in fluids, electrolytes and acid base balance, as a consequence of sustained sweating and electrolyte loss. This is because equine sweat is isotonic or slightly hypertonic in relation to plasma and contains high concentrations of Na, K and Cl and some Ca and Mg (Flaminio, et al., 1998). Prolonged sweating will cause significant electrolyte deficits promoting weakness, muscle cramps, acid-base imbalances, heart arrhythmias, decreased performance and eventually exhaustion (Muñoz, et al., 2010) with negative consequences on both the performance and the health of the horse, which might show significant changes in diverse biochemical parameters (Fielding, et al., 2009).

In the present study, there were significant differences in the hematological and biochemical parameters of pre and post GP and PP endurance horses.

In the post-race sample of the two groups, hemoconcentration was found with a significant increase in RBC, HB and Ht. These findings were confirmed by McKeever et al (1987).

Changes in the values of hematological parameters reflect the adaptation of an organism to prolonged physical exercise. The performance of horses during races is closely associated with the cardiac function and tissue oxygenation. These are dependent on oxygen-carrying capacity of blood, which itself is dependent upon erythrocyte and hemoglobin concentrations (Lawan, et al., 2010). In endurance sports, two physiological events can contribute to RBC, HB and Ht elevation. Splenic contraction is due to an adrenergic stimulus in response to stress caused by taking part in the race (Snow, et al., 1992) and by increased demand for oxygen (Adamu, et al., 2010 ; Piccione, et al., 2007) and sweating leading to extensive body fluid losses in more prolonged exercises. This is because thermoregulation leads to intense sweating with evident body fluid losses and hemoconcentration (Stopyra, et al., 2016 ; Waller, et al., 2009).

Red blood cell parameters (MCV, MCHC) indicate efficiency of hemoglobin synthesis and its oxygen transport capacity. There were significant changes in MCV and MCHC, in examined horses before and after the race of GP horses. Our results agree with the finding of Larsson et al (2013) and Teixeira-Neto et al (2012) who did not link them to pathological changes in the horse body during physical exercise. For PP horses, there was a significant change in MCHC.

A slight increase in MCV observed in subsequent tests may suggest electrolyte imbalance associated with a drop in the osmotic pressure of serum and the migration of free water into red blood cells. These fluctuations, not reaching abnormal levels, may indicate optimal preparation of the horses for the race and their proper performance (Teixeira-Neto, et al., 2012).

A post-exercise increase in white blood cell count was observed in the two groups of this study.

Leukogram alterations in response to training have been reported in horses. Similar to the case of red blood cells, spleen is responsible for the increase in WBC level in peripheral vesicles (Piccione, et al., 2008, 2010 ; Vazzana, et al., 2014). This finding agrees with our result and may be explained by sympathoadrenal responses on the spleen ejecting a reservoir pool of neutrophils as well as the effect of corticosteroids and catecholamines on recruiting marginated neutrophils in circulation (Carlson, 1987).

The significant differences between GP and PP in leucocyte number could be due to the free radicals discharged into the circulation by macrophages, which result in harmful effects on tissues and organs (Adamu, et al., 2012 ; Piccione, et al., 2008).

There was a significant decrease in lymphocytes after the race in our study. According to Trigo et al (2010), this may occur due to exhaustion. In this study, a simultaneous increase in the number of granulocytes (GRA) was defined. According to a study conducted by Art et al (2009), intense exercise induces activation of blood GRA, with a degranulation of NEUT and a release of myeloperoxidase. However, minimal variations in WBC indices are indicative of good levels of performance in horses (Adamu, et al., 2012).

An increase in WBC count may also be associated with the effect of endogeneous adrenalin and cortisol released into blood stream in response to the stress associated with endurance rides (Larsson, et al., 2013).

In this study, eosinophils showed a significant decrease for GP horses only. In a study on endurance horses (Adamu, et al., 2010) found that a lower EOS level was related to a good performance.

Platelets, similar to erythrocytes, are subject to an increase in their number due to the release of catecholamine, splenic contraction, and hemoconcentration (Poskiené, et al., 2019).

This research showed a slight increase in the level of Ca for the GP horses, whereas this level decreased for the PP horse group. This finding was also confirmed by Hassan et al (2015) and Ebrahim et al (2019) who found a decrease in the level of Ca and attributed it to the effect of calcitonin, which may decrease Ca concentration in addition to the loss of Ca through sweating during exercise.

The level of Mg significantly decreased after the race as compared to before racing. Phosphorus and magnesium blood levels are related to diet, physical activity, and environmental conditions (Weiss, et al., 2002).

A significantly high concentration of CHE was recorded in our investigation of the GP and PP horses as agreed with the finding of Bis-Wencel et al (2012). It should be stressed that lipid metabolism in horses differs from that of other animal species and the effect of physical activity on lipid levels in blood has not been fully explained to date (Bis-Wencel, et al., 2012 ; Burliowska, et al., 2015).

Serum glucose concentration decreased significantly during the race, especially for GP horses, while the TG concentration increased significantly. These findings are consistent with those of earlier studies (Spangfors, 1991). Hypoglycaemia is typically seen after endurance races (Stockham, and Scott, 2002), where the energy for endurance racing comes primarily from TG sources (Larsson, et al., 2013).

Our findings showed a significant increase in urea between the beginning and the end of the 90 km race for GP horses. This agrees with Piccione et al (2010) who found that intensive training of athletic horses can lead to kidney dysfunction as indicated by a high level of serum urea. This also agrees with Ebrahim et al (2019) who found that an increased serum urea concentration can be attributed to the massive fluid loss due to sweating and subsequent reduction in the renal blood flow (Ebrahim, et al., 2019 ; Soroko, et al., 2019)

Regarding serum level of uric acid, there was a significant difference between the pre and post GP and PP endurance horses in the present study. Plasma U-A has been considered a non-enzymatic antioxidant defense against oxidation during exercise (Adamu, et al., 2012). This difference could be due to oxidative stress associated with uric acid during strenuous racing.

The accumulated free radicals (Piccione, et al., 2007) could then attack lipid membranes of muscles as indicated by the increase in TG concentrations leading to an unfavorable environment for U-A antioxidant effects (Muraoka, and Miura, 2003). Increases in U-A as a result of prolonged lopsided energy distribution could lead to metabolic syndromes during endurance events (Castejon, et al., 2006).

In our study, the level of glucose was significantly lower for GP group than PP horses.

The increase in glucose concentration after training in healthy horses is proportional to the intensity of effort and the level of lactate in blood (Bis-Wencel, et al., 2011; Soroko, et al., 2019).

Glucose concentration was lower in our study, which may indicate a more economical management of glucose homeostasis and the use of free fatty acids as a source of energy (Adamu, et al., 2014).

In our study there was an increase in serum enzyme activity post-race for AST for both GP and PP groups. The magnitude of increase of both CK and AST is not related to fatigue or performance (Adamu, et al., 2014). However, these changes are still indicative of muscular injury, which is not inconceivable in connection with a 90 km endurance race.

The increase of muscle enzymes in blood plasma is considered a symptom of muscle damage. Moreover, AST and ALT are enzymes indicating proper functions of liver which plays a vital role in exercise metabolism (Szarska, 2003). In our study ALT increased only for GP horses.

Creatine kinase and lactate dehydrogenase are characteristic enzymes of mammalian muscle tissue

(Zarczynska, et al., 2013). The values of plasma CK observed after exercise depend on the duration and the type of the exercise (Camâra, et al., 2007)

CK is a useful indicator in the assessment of training progress (Szarska, 2002). Janicki et al (2012) reported a higher serum CK activity in untrained horses than trained ones. Serum CK activity is useful to assure that the training intensity is not producing muscle damage or significant changes in muscle membrane permeability (Cywinska, et al., 2012). Exercise, depending on its type, intensity, and duration, can cause an increase in serum CK activity in horses (Castejon, et al., 2006). It is believed that this increase in serum CK is due to an increase in muscle cell membrane permeability because of tissue hypoxia during exercise rather than tissue damage (Buzala, et al., 2015).

ɣGT activity has been evaluated in racehorses where its increase has been associated with both cumulative days in training (Tyler-McGowan, et al., 1999) and poor performance (Snow, et al., 1992). Our results showed no significant change in ɣGT for GP horses, pre and post racing however there was a significant decrease for the PP group. Adamu et al (2014) mentioned that a decreased in ɣGT activity could be associated with a decreased uptake of glucose by the skeletal muscle in endurance horses that showed poor performance. (Ebrahim, et al., 2019).

Hematological and biochemical blood analysis provides significant information about the health status of animal, metabolic changes in its organism and it is often helpful in revealing health disorders already in the preclinical stage. A horse having all the blood parameters in accordance with the reference values has a much greater chance of achieving satisfactory sport results and recover much quicker after physical efforts (Burliowska, et al., 2015) (Szarska, 2003).

In this study, we have compared the pre-race values of both PP and GP horses before racing to evaluate the utility of biochemical analysis in the early diagnosis of metabolic stress and to determine cut-off values of biochemical parameters to assist in the prevention of metabolic alterations in endurance horses. There was a significant difference of the hematological and biochemical values especially for RBC, Ca, Mg, CHE, Urea, U-A, AST, ɣGT and CK.

CONCLUSION

The results of the current study demonstrated that an endurance race induces alterations in hematological and biochemical parameters in racing horses undertaking a 90 km ride and particularly highlighted the significant differences as compared to those horses eliminated from the race for metabolic reasons. Many of the changes in blood biochemistry seem to be grounded on a decreased blood volume, increased energy expenditure, and exercise related muscular damage. The results of this study suggested that biochemical findings could help identify horses with an increased risk of developing metabolic alterations before their elimination during competitive endurance races of different distances. Therefore, proper management protocols, standard feeding and watering with proper preparation of animals before the race could prevent electrolytes imbalance and pathological affections during racing in endurance horses.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

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