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Comparative Evaluation of Hemato-biochemical and Oxidative Parameters of Racing Horses

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Abstract

Racing horses are prone to alterations in hemato-biochemical and oxidative stress parameters. The aim of this study was to determine the effect of racing on hemato-biochemical, and oxidative stress parameters of racing horses. Blood samples were collected from 15 control and 15 racing horses at four intervals (before, immediately after, 30 minutes after, and 24 hours after) to assay for hematological, serum biochemical, and oxidative stress markers. The result revealed significant changes ($P < 0.05$) in vital parameters such as erythrocyte count, hematocrit index, hemoglobin concentration, leucocyte count, lymphocyte, neutrophil, neutrophil: lymphocyte ratio, total protein, albumin, urea, creatinine, cortisol, lactate, lactate dehydrogenase, creatinine kinase, aspartate aminotransferase, while glucose, cholesterol, triglyceride, gamma-glutamyl transferase, malondialdehyde, glutathione peroxidase, and catalase remained unaltered ($P > 0.05$). The alteration in hemato-biochemical parameters reported in this study only lasted for about 30 minutes after racing, with all values returning to the baseline range by 24 hours post-race. Interestingly, the comparable activities of antioxidant stress markers reported suggests that the horses have developed adaptive mechanisms that have conferred them with increased resistance to oxidative stress and reduced oxidative damage. This study demonstrates the importance of schematic monitoring of racing horses peri-racing to prevent racing-induced pathophysiological alterations and promote their general wellbeing.

Keywords

Biochemical indices, Equine athletes, Hematological parameters, Oxidative stress parameters.

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Introduction

Horse racing is an equestrian performance sport that typically involves two or more horses ridden by jockeys over a specified distance for competition (Richardson and Williams, 2020). It is one of the most ancient of all sports, with the main intent of identifying the fastest among the competing horses (McIntire, 2003). Just like their human counterparts, equine athletes need routine and adequate training to achieve good performance (Murphy *et al*., 2009; Nagy *et al*., 2012). The gambling aspect of horse racing is estimated to generate about US$115 billion annually (Gainsbury *et al*., 2015). In Nigeria, the past few decades have witnessed a tremendous increase in the growth of equestrian activities, with horse racing now taking place in almost all northern cities across the federation. However, several studies have reported racing-related fatalities with an overall fatality rate in racehorses ranging from 0.06 to 1.9% deaths (McLean and McGreevy, 2010; Lyle *et al*., 2012; Georgopoulos and Parkin, 2016; Hellings *et al*., 2022), thereby subjecting the sport disciplines to increasing criticism (McLean and McGreevy, 2010).

Researchers have long recognized the potential detrimental effects of racing-induced stress on body functions (Shephard and Shek, 1995; Cywinska *et al*., 2010; Mellor and Beausoleil, 2017). During racing, more than 80% of the energy produced by the catabolism of stored substrates is converted to heat, which is mainly dissipated through evaporation and hence promotes fluid losses of up to 12 liters per hour (Lyle *et al*., 2012). When this occurs, racehorses are at risk of potentially life-threatening metabolic illnesses secondary to dehydration, electrolyte and acid-base derangements, heat accumulation, and substrate depletion (Klobucar, 2020). In addition, equestrian racing is believed to be an aerobic discipline where elite horses are required to perform at increasingly higher speeds, making this sport a potential inducer of oxidative stress (Fielding and Dechant, 2012; Siqueira *et al*., 2014).

The idea of blood-based assessment of the effect of racing on hemato-biochemical alterations and performance is certainly not new (Casella *et al*., 2016). Hematological and biochemical parameters are crucial aspects of clinical diagnostics and sensitive indicators of pathophysiological alterations. Notably, numerous studies have been conducted to determine the alterations in hematological and serum biochemical parameters in horses (Larsson *et al*., 2013; Mami *et al*., 2019; Ko *et al*., 2020), but there is still relatively little evidence-based information available on the comparative study between racing and control groups of horses to determine the impact of racing on these parameters. Also, the literature available on the oxidative stress alteration is scant and often controversial, hence the need for this study. This study aimed to assess the hemato-biochemical parameters and oxidative stress status in control and racing horses in order to gain valuable insights into their responses to racing and provide a guide to better informed decision-making on proper management practices and the institution of injury prevention strategies.

Material and Methods

The study was conducted within the Ilorin metropolis, the capital city of Kwara State, north central Nigeria. Ilorin lies in the Guinea Savannah Belt; the city is located at latitude 8° 30'N and longitude 4° 33'E, with a mean annual temperature of 26.5°C. However, the monthly temperature varies between 21°C and 37°C in

the two (wet and dry) different seasons (RCE, Ilorin).

Experimental horses

Thirty healthy Arabian breed horses weighing 340–410 kg were used for this study. The horses were divided into two groups: the racing group (n = 15) and the control group (n = 15). The horses were housed and managed under the same housing facility, and their selection was not based on sex discrimination. The horses used in the present study comprised three stallions and 27 mares of the Arabian breed, which are most prevalent in Nigeria. Horses in the racing group competed in a high-intensity race of 14,000 meters that was covered within an hour. Physical examination revealed that all the experimental horses had normal body conformation and body condition scores (BCS) ranged from 4-6. Vital parameters, including temperature, respiration, and heart rate, were measured before and after racing. Racing was conducted on sandy terrain during dry and sunny weather.

The study protocol was approved by University of Ilorin Animal Care and Use Committee (Approval number, URE/FVM/2022/039; 15th, March, 2022). The consent of the horse owner and/or rider was obtained prior to the sampling. Blood samples (6 ml) were collected from the left jugular vein of all 30 horses at four intervals (before, immediately after, 30 minutes after and 24 hours after racing) via venipuncture, with a total of 120 samples collected and analyzed. Two (2) mls of blood were dispensed into a tube containing EDTA and was used to determine the blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), white blood cell count (WBC), and neutrophil and lymphocytes counts by the means of an automated hematology analyzer (Mindray Biomedical, China). The Neutrophil/lymphocyte ratio was calculated by dividing the neutrophil count by the lymphocyte count.

The remaining 4 ml of blood was transferred into a plain tube and centrifuged at 2000 rpm for 15 minutes to separate the serum from the blood cells. The serum was carefully harvested and transferred into a new tube, labelled appropriately and stored at -20^{o}C until required. The serum was used to assay for total protein, albumin, blood urea nitrogen (BUN), creatinine, glucose, lactate, triglyceride, cholesterol, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), gamma-glutamyl-transferase (GGT), and creatinine kinase (CK) by means of an automated biochemistry analyzer (Roche, India) using commercial kit (Randox, UK) following the manufacturer's instructions.

The serum concentration of cortisol was determined using a commercially bought cortisol enzyme-linked immunosorbent assay (ELISA) kit (Abcam, UK) following the manufacturer's instructions. The major markers of oxidative stress (malondialdehyde, MDA), and endogenous antioxidant enzymes (glutathione peroxidase, GPx, and catalase, CAT) were measured by enzyme-linked immunosorbent assay using assay kits (Elabscience, USA) following the manufacturer's instructions. The measurement was conducted using a Biobase-Elisa microplate reader (Biobase, China)

The statistical package "Graph Pad Prism Version 9.0" was used to express the data as mean± SEM (standard error of mean). The difference in hemato-biochemical and oxidative stress parameters between racing and control horses was compared using a student t-test.

Results

The vital parameters - temperature, respiratory rate, and heart rate - showed significant changes in racing compared to control horses (Figure 1).

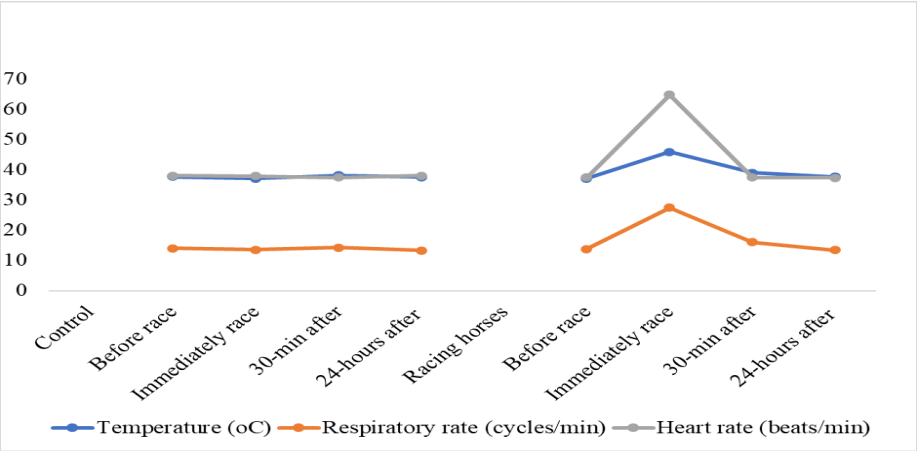

Figure 1. Line graph showing the mean vital parameters of control and racing horses sampled before, immediately after, 30-minutes after, and 24-hours after racing.

The hematological parameters revealed significant differences in red blood cell count, hemoglobin concentration, packed cell volume, total leucocyte count, neutrophil, lymphocyte, and neutrophil: lymphocyte ratio between the racing and control groups after the racing event ($P < 0.05$) (Figure 2).

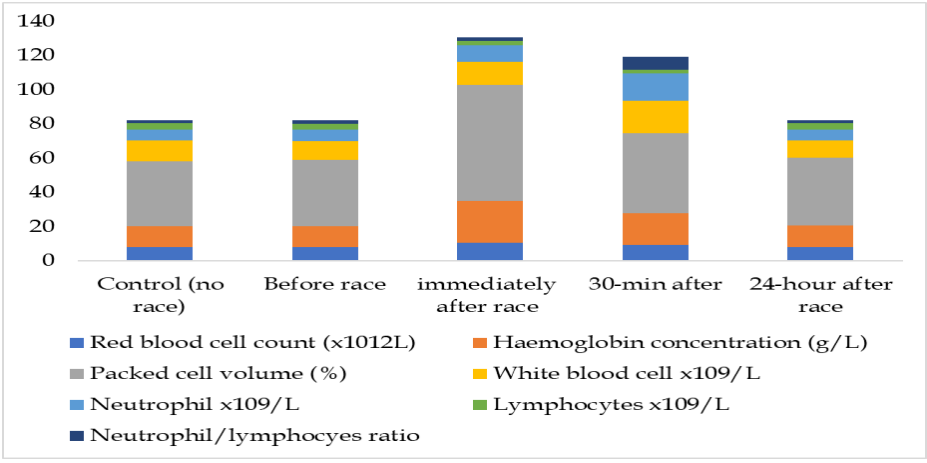

Figure 2. Stacked column showing the mean haemogram and leucogram of control and racing horses sampled before, immediately after, 30-minutes after, and 24-hours after racing.

Biochemical parameters including total protein, albumin, BUN, creatinine, lactate, lactate LDH, AST, GGT, and CK differed considerably between racing and control horses, while the concentrations of glucose, triglyceride, and cholesterol remained altered throughout the experiment (Figure 3).

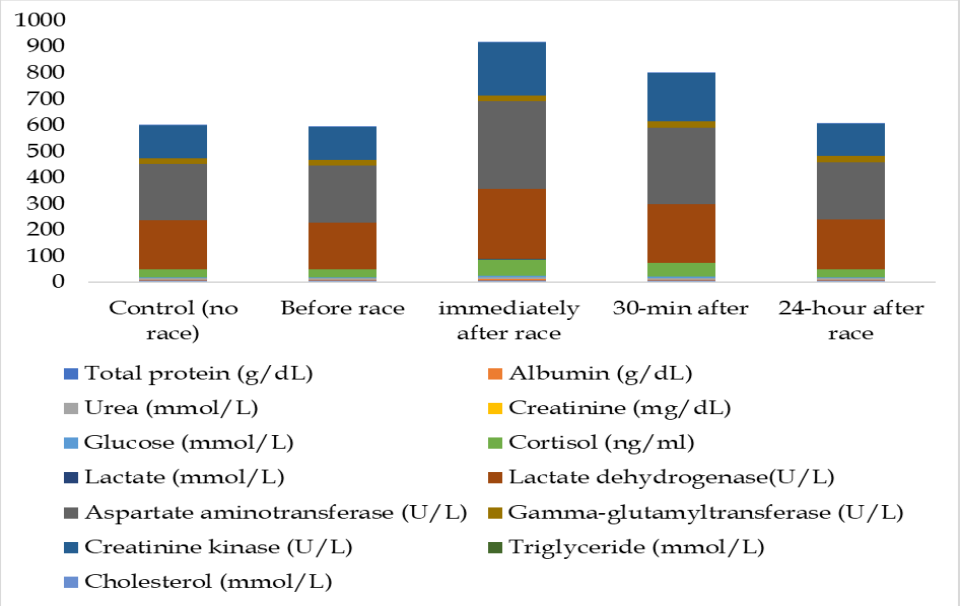

Figure 3. Stacked column showing the mean protein, energy, hepatic, muscular, lipid, and electrolyte profiles of control and racing horses sampled before, immediately after, 30-minutes after, and 24-hours after racing.

The oxidative stress assay revealed unchanged levels of oxidative stress markers (malondialdehyde), and antioxidant enzymes (glutathione peroxidase), and catalase activities (Figure 4).

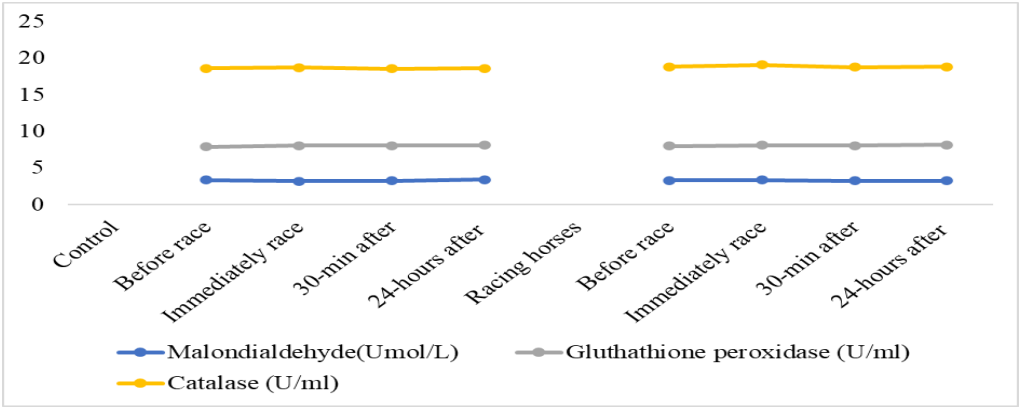

Figure 4. Line graph showing the mean oxidative stress maker of control and racing horses sampled before, immediately after, 30-minutes after, and 24-hours after racing.

Discussion

Temperature regulation is a homeostatic feedback control system that maintains the body's core temperature within a restricted range, even when there are large changes in the surrounding temperature and increased heat production from physical activity (Maśko *et al*., 2021). Hence, there is a need for the dissipation of the body's metabolic heat produced during intense excretion in horses to minimize the potential cell damage that may be initiated due to cascades of pathophysiological alterations (Cramer *et al*., 2022). In this present study, the reported increase in rectal temperature was as a result of the body's protective thermoregulatory mechanism, which was evidenced by the profuse sweating observed in all the horses after racing. The elevated heart rates observed in racing horses immediately after racing compared to other values could be attributable to increased oxygen demand as well as adrenaline-induced myocardium contraction, increased vascular tone, and increased heart perfusion (Brownlow *et al*., 2016), while the considerable increase in the respiratory rate could be chiefly due to increased oxygen consumption by the working muscles with subsequent hyperventilation and resultant dissipation of excessive heat as evidenced by the increased temperature. Interestingly, the increase was correlated with the increase in pulse rate and rectal temperature. The findings in the present study are consistent with those of previous studies (Gandhi and Gunjan, 2009; Adamu *et al*., 2012).

Racing generally results in the mobilization of splenic erythrocytes and, therefore, increases the oxygen transport capacity. The extent of the hematocrit increase is a function of the intensity of the racing. In this present study, the control, pre-race, and 24-hour post-race hematological parameters were within the normal limit (Wannicka, 2011), indicating that the experimental horses were healthy. The elevation in the values of RBC count, HB concentration, and PCV reported in this study was consistent with the findings of another research (Wakil *et al*., 2022; Marichal *et al*., 2023). Both splenic contraction, which is regulated by catecholamine, and exercise-induced fluid shifts from intravascular to interstitial space (Vlaeva *et al*., 2023) could be said to be responsible for this observation. Notably, a clear decrease in RBC parameters was recorded 30 minutes after racing, with the parameters returning to resting values by 24 hours after racing. This finding could be attributed to the backward pool of circulating erythrocytes in the spleen after exertion.

In horses, the mobilization of leukocytes based on excitement and exercise is masked by the concomitant increase in erythrocytes and blood volume as a result of splenic contraction (Soroko *et al*., 2019), and hence racing is regarded as a stress factor that could induce a standard leukocytic reaction (Hurcombe, 2020). The transitory lymphocytic leukocytosis reported in immediately after racing in this study was similar to previous studies in working horses (Zobba *et al*., 2011), post-training in whippets (Miazga *et al*., 2023), and in working buffalo (Pajan-Jimenez *et al*., 2023). This observation could be due to the adrenaline-induced mobilization and release of lymphocytes from the spleen into peripheral circulation, and, to a lesser extent, from the bone marrow and lymph nodes (Larson *et al*., 2013). A second phase of leukocytosis characterized by mild lymphopenia and marked neutrophilia was observed 30-minutes after racing, suggesting a possible release of the pool of wall granulocytes and increased

mobilization from the bone marrow (Pedersen and Hoffman-Goetz, 2000). This dramatic change in leukocytic kinetics was supported by an observable increase in the N/L ratio. Cortisol, which stimulates the production and release of neutrophils from the bone marrow and suppresses the number of circulating lymphocytes (Soroko *et al*., 2021), could be said to be responsible for these findings.

The total protein concentration, which determines the total serum protein fractions, is a useful screening test in equine medicine used as a sensitive indicator to assess changes or pathological states in liver function and the immune system (Aulbach *et al*., 2023). Albumin constitutes the primary (48–76 % of TP) and most osmotically active protein component in equine serum (Wannicka, 2011), whereas a globulin fraction is a heterogeneous group of blood proteins including carrier proteins, enzymes, immunoglobulins, and other inflammatory molecules (Robert *et al*., 2010). Serum urea and creatinine concentrations are common indices reflecting protein metabolism and the efficiency of renal functions. Moreover, creatinine is a breakdown product of muscular creatine phosphate, and its level depends directly on muscle mass and its activity. Significantly increased albumin, total serum protein, urea, and creatinine concentrations observed during racing are most likely due to dehydration, and this result is consistent with previous findings (Robert *et al*., 2010; O'Reilly *et al*., 2017).

Both the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis are activated to improve oxygen delivery to the working muscles and this activation is demonstrated by rapid increases in the circulating levels of adrenocorticotropin (ACTH), cortisol, adrenaline, and noradrenaline, allowing these hormones to be utilized in the evaluation of activity-induced stress (Soroko *et al*., 2021). In the present study, the concentration of cortisol was significantly elevated immediately after the race, with a peak elevation observed 30 minutes after the race, which corresponds with the second peak of leukocytosis (neutrophilic). Recent studies also reported increased concentrations of blood cortisol in racing horses (Zobba *et al*., 2011; Soroko *et al*., 2021).

The concentration of blood lactate increases in response to exercise or training and is generally regarded as an indicator of fitness or intensity of physical activity because it reflects the dependence on anaerobic metabolic pathways (Miazga *et al*., 2023). In the present study, plasma lactate rose significantly immediately after and 30 minutes after racing as compared to control and other sampling times. Significantly increased concentrations of plasma lactate have been reported post-exercise in sport horses (Handerson, 2013). Glucose and lipid profiles reflect the energy economy and its fluctuations in a living organism. Racing did not significantly affect the pattern of plasma glucose levels and lipid metabolism in this study. This result is consistent with the findings of Trilk *et al*. (2002); however, other studies have reported elevated blood glucose concentrations in racing horses (McKenzie *et al*., 2014; Souza *et al*., 2018). The nature of feeding and the intensity of racing might be responsible for this observable difference.

Creatine kinase, lactate dehydrogenase, and, to a lesser extent, aspartate aminotransferase are the most muscle-specific enzymes. Under normal conditions, the activities of muscle enzymes in the blood plasma are maintained at a certain level, and their increase is considered a symptom of

muscle damage (Marichal *et al*., 2023). Significantly higher levels of serum AST, LDH, and CK found in investigated horses immediately after racing are probably the result of leakage from intact muscle fibers resulting from muscular activity (Kingston, 2008), rather than a consequence of oxidative stress-induced muscle or liver damage. The latter is also supported by the decline to the pre-race value 30 minutes after racing and the comparable activities of GGT reported throughout the experiment.

Growing evidence indicates that the antioxidant defense systems of mammalian tissues are capable of adaptation in response to exercise or racing (Kinnunen, 2011). The present study demonstrated no significant changes in the activities of SOD, GPx, CAT or MDA. This lack of significant changes in antioxidant enzymes might be ascribed to the acclimatization of investigated horses to this kind of racing activity. The activities of antioxidant enzymes determined in the investigated horses were in general agreement with previous data in horses (Siqueira *et al*., 2014), but disagreed with others (Bollinger *et al*., 2023; Bujuk *et al*., 2024). The inconsistency in this finding could be a reflection of the differences in intensity and duration of racing.

Conclusion

The hemato-biochemical effects observed in racing horses can be attributed to the physiological stress imposed by either dehydration and/or the demands of racing. The reported "pseudoleukocytosis" reflects the horses' adaptations to racing. The elevation of liver and muscle enzymes could be linked to increased enzyme leakages and, as a result, increased muscular activity. Notably, the observation that the horses did not develop racing-induced oxidative stress indicates a balance between pro-oxidants and antioxidants and could suggest that racing evokes specific adaptations, which could increase the body's resistance to oxidative stress and lower the level of oxidative damage. The fact that all parameters returned to their pre-race values by 24 hours post-racing is an indication that the horses were in good physical health condition and were fit to compete in subsequent racing activity.

Approval of Ethical Comission

The study protocol was approved by University of Ilorin Animal Care and Use Committee (Approval number, URE/FVM/2022/039; 15th, March 2022).

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Authors Contribution

JAA - Conceptualization, methodology, and writing (original draft). AAA, MA and AOO - Analysis, Investigation, methodology, and writing (review and editing). BS and SA - Conceptualization and supervision. All authors reviewed and approved the final version of this manuscript for publication.

Conflict of Interest

The authors declare that they have no conflict of interest that could influence the work reported in this manuscript.

Data Availibility Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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