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Dynamic Hematological Responses in Endurance Horses: Unraveling Blood Physiological Markers of Exercise Stress and Recovery

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Abstract

This study aimed to investigate the effects of endurance exercise on erythrogram parameters and identify stress and inflammation markers that could serve as reliable indicators for assessing recovery in endurance horses. The study involved 26 Arabian endurance horses (4 stallions and 22 geldings) aged between 8 and 12 years, each completing a race (10 horses in 80 km, 10 horses in 120 km, and 6 horses in 160 km). Blood samples were collected at six different time points: at rest (T0), immediately after the race (T1), 3 hours after the race (T2), and 3, 7, and 14 days after the race (T3, T4, T5). The hemogram analyses included several hematological indices such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), eosinophil-to-lymphocyte ratio (ELR), red blood cell distribution width-to-platelet ratio (RDW/PLT), hemoglobin-to-red blood cell distribution width ratio (Hb/RDW), hemoglobin-to-platelet ratio (Hb/PLT), systemic inflammation index (SII), systemic inflammatory response index (SIRI), leukocyte shift index (LSI), and adaptation intensity index of L. Harkavy (AI). The findings revealed that some indices, such as NLR, PLR, SII, SIRI, MLR, RDW/PLT, and LSI, were sensitive to acute physiological changes related to the endurance race. These indices showed significant variations immediately after the race, indicating a stress and inflammatory response. In contrast, the ELR and AI indices displayed delayed and more prolonged responses, suggesting their utility in monitoring the post-exercise recovery phase. Overall, this study offers insights into applying hematological markers to assess endurance horses' stress, inflammation, and recovery. These findings could contribute to improved training and recovery strategies, promoting the health and welfare of equine athletes during and after intense physical exertion. Further research is recommended to explore these indices in larger samples and other equine sports.

Keywords

Cellular dynamics; endurance horses; hematological indices; immune responses; post-exercise monitoring

1. Introduction

Endurance racing, an increasingly challenging discipline within equestrian sport, has witnessed a surge in demands, particularly in terms of speed [1]. This escalation places metabolic, musculoskeletal, and cardiovascular stress on the participating athletes. A comprehensive understanding of these demands is imperative for optimizing performance, mitigating injury risks, and preventing overtraining.

Assessing fitness and recovery in endurance athletes often involves various methods, with serial blood count evaluation commonly utilized. However, interpreting hematological changes in the context of exercise presents distinctive challenges. Endurance races impose substantial physiological demands, resulting in transient alterations that can be challenging to differentiate from pathological shifts. Dehydration, splenic contraction, and exercise-induced changes in plasma volume further complicate the interpretation of hematological parameters [2].

Research has demonstrated that exercise triggers inflammation in response to local tissue damage in humans and horses [3–6]. The immune system orchestrates this response to restore tissue integrity and maintain homeostasis. In fit and healthy athletes, immune system imbalance and acute inflammation represent temporary states, considered the initial steps in the recovery process to restore tissue function [7]. However, unresolved acute inflammation poses a risk for chronic inflammation, a significant factor in various pathological conditions.

The innate immune system plays a pivotal role in the body and is crucial for combating infections and other stressors [8]. Intense exercise has been associated with a temporary weakening of the immune system, increasing the risk of respiratory disease in humans and horses, according to the "open window" hypothesis. Nevertheless, there is ongoing debate about the applicability of this theory to horses [9].

A study proposes [10] using established cellular inflammation markers in exercise immunology, advocating for data analysis solely from blood count in conjunction with classical blood count analyses and flow cytometry. The authors posit that blood-based inflammatory indices could serve as integrative, cost-effective, and time-efficient markers to detect cellular immune changes.

Our hypothesis posits that integrative cellular indices could serve as valuable parameters for evaluating effort and, particularly, recovery in horses subjected to endurance exercise. This study investigates the impact of endurance exercise on erythrogram parameters and platelet changes over 14 days following a competition. Additionally, we seek to identify potential stress and inflammation markers that can serve as reliable parameters for assessing recovery from inflammation after a race in endurance horses.

2. Materials and Methods

Twenty-six Arabian and cross Arabian horses that had completed an FEI (Fédération Equestre and Internationale) endurance race and had cleared the last veterinary inspection (hydration status, rectal temperature, gut sounds, and lameness exam) were included in this study. Six horses completed a 160 km race, ten completed a 120 km race, and ten completed an 80 km race. As the FEI categories require a minimum of two years of training experience, the horses included in the study had at least two years of training.

Due to the previously reported differences in hematological parameters between male and female athletic horses [11,12], we have opted to exclusively include males in this study, as they constitute the majority among endurance horses. The study enrolled 26 horses, consisting of 4 stallions and 22 geldings, aged between 8 and 12 years and weighing 400-440 kg.

These horses were owned by endurance riders and housed in their farms and private training centers in São Paulo. A day before the race, all the horses were transported to the competition venue and kept under similar stall conditions.

Blood samples were collected via jugular venipuncture in vacuum tubes with EDTA (ethylenediaminetetraacetic acid) to conduct a hemogram. Leukocyte differential slides were prepared on-site and stained with the May-Griinwald Giemsa method. The blood count was immediately processed using an automated hematology analyzer (BC 2800Vet, Mindray, USA) in the laboratory at the event's premises. Figure 1 shows the time samples, which consisted of three collections at the race venue (T0: at rest, before tacking; T1: immediately after the final vet check, and T2: 3 hours after T1) and three collections at the horse's origin farm (T3: 3 days after the race; T4: 7 days after the race, and T5: 14 days after the race).

Using absolute blood cell counts, we calculated several integrated hematological indices, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), eosinophil-to-lymphocyte ratio (ELR), red blood cell distribution width-to-platelet ratio (RDW/PLT), hemoglobin-to-red blood cell distribution width ratio (Hb/RDW), and hemoglobin-to-platelet ratio (Hb/PLT). Additionally, we calculated two indices that consider three cellular types:

The systemic inflammation index (SII):

$$SII = \frac{(Neutrophlis\ x\ Platelets)}{Lymphocytes}$$

The systemic inflammation response index (SIRI):

$$SIRI = \frac{(Neutrophils\ x\ Monocytes)}{Lymphocytes}$$

The Leukocyte Shift Index (LSI) evaluates changes in the count of neutrophils and lymphocytes following a stress event such as exercise:

$$LSI = \frac{(Post-exercise\ neutrophil\ count - Post-exercise\ lymphocyte\ count)}{(Pre-exercise\ neutrophil\ count - pre-exercise\ lymphocite\ count)}$$

The adaptation intensity index of L. Harkavy (AI) was calculated using the differential cells according to the following formula:

$$AI = \frac{Lymphocytes}{Segmented\ neutrophils}$$

The open-source Jamovi statistical platform (version 1.2.1.1) was used for data analysis. It was retrieved from https://www.jamovi.org. The Shapiro-Wilk test was used to evaluate the distributions of the traits before conducting further statistical analyses. Non-parametric repeated measures were analyzed using the Friedman test and compared using the Durbin-Conover pairwise test. The significance level was set to $p < 0.05$ for all statistical analyses.

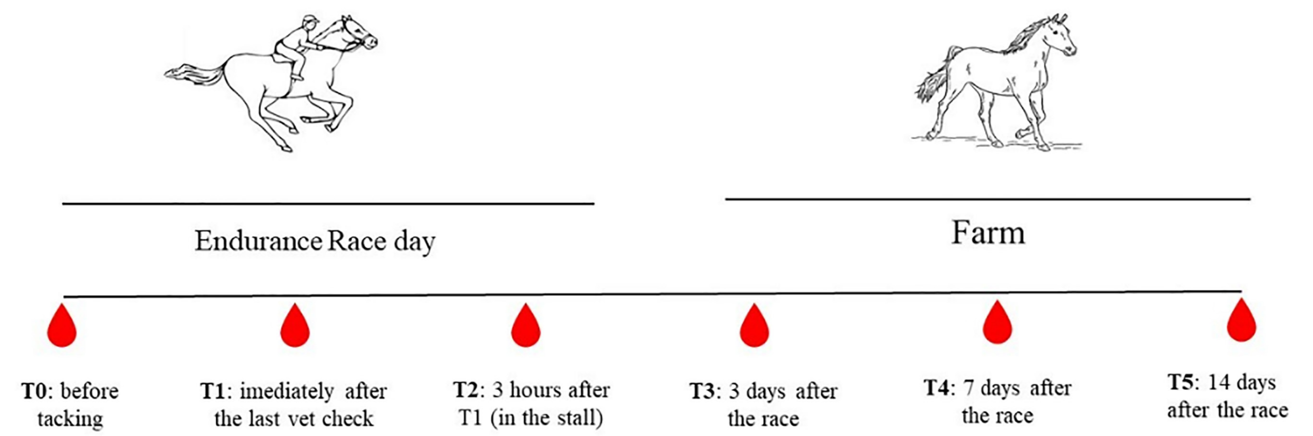

Figure 1: Blood sampling times in endurance horses that completed 80 km, 120 km, and 160 km events. Blood samples were collected before the event (pre-exercise), immediately after the event (post-exercise immediate), 3 hours post-event at the same location, and on subsequent days (3 days, 7 days, and 14 days post-event) at their respective stables.

3. Results

All the horses in the study passed the veterinary inspection before and after the race and completed the required distance. The average speed of the horses was 17.12 (±1.06) km/h for 160 km, 17.88 (±1.20) km/h for 120 km, and 19.20 (±1.88) km/h for 80 km. However, due to the small number of horses in each class, their ranking positions could not be analyzed or reported.

The current study has found alterations in various blood parameters such as red blood cell count, hemoglobin, mean cell hemoglobin concentration, hematocrit, neutrophils, monocytes, platelets, and plateletcrit on the day of the competition (T1 and T2). However, these parameters returned to normal or lower levels after three days (T3). Lymphocytes and eosinophils showed alterations on the day of the competition but showed higher levels after T3, as demonstrated in **Table 1**.

Following the ride, there was an increase in hematocrit levels during T1 and T2 compared to before the ride (T0). This shows that there was a concentration of red blood cells during the exercise. The levels of red blood cells and hemoglobin increased after the ride, which is a normal response to the increased need for oxygen during exercise. Immediately after the ride, there was a significant increase in neutrophils and a decrease in lymphocytes, indicating a stress response. There was an increase in lymphocytes from T3 to T5. Monocytes showed a noticeable increase after the race (T1 and T2) and decreased from T3 to T5. On the race day, there was a distinct decrease in eosinophils, followed by higher levels after T3. The levels of platelets increased significantly after the ride, which may be related to the increased demand during exercise. The values for mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) were generally stable across the time points with no significant changes, indicating no major alterations in red blood cell size, hemoglobin content, or distribution width. PCT increased at T1 and T2, then returned to basal levels from T3 to T5.

After the competition, there was a significant increase in NLR, suggesting potential inflammation, as shown in **Table 2**. However, there was a significant decrease in NLR at T3 and T4. Similarly, PLR increased post-ride but decreased over the subsequent days. SII and SIRI indicated the systemic inflammatory response, significantly increasing post-ride. A decrease followed these indices in successive days. Furthermore, MLR increased post-ride, which reflects potential stress or inflammation responses, and there was a decrease in ELR at T1 and T2 and an increase at T3.

After a horse has undergone endurance exercise, it enters an immediate post-ride period where signs of acute stress and inflammation are evident. This is reflected in increased NLR, PLR, SII, and SIRI. However, subsequent days (T3 and T4) indicate a resolution of inflammation, as evidenced by decreasing levels of NLR and PLR.

Monitoring the LSI and AI indices over time provides insights into the balance between exercise-induced stress and adaptive responses. There was an increase in LSI and a decrease in the AI values at T1 and T2, which corresponded to the conclusion of the competition, indicating stress. However, at T3, T4, and T5, the values were within the range considered "quite an adaptation," returning to the adaptive threshold only 14 days post-competition.

Table 1: Median values (Friedman test) of hematological parameters measured in endurance horses at different times: before the race (T0), immediately after the race (T1), three hours after the race (T2), three days after the race (T3), seven days after the race (T4), and fourteen days after the race (T5).

	Sample time						
	T0	T1	T2	T3	T4	T5	*p*
Eritrogram							
PCV (%)	36.0 (35.5)^{B}	43.5 (44.0)^{A}	41.0 (41.8)^{A}	34.3 (33.8)^{B}	32.2 (32.0)^{B}	33.8 (34.0)^{B}	<.001
RBC (10^3/mm^3)	13.5 (7.59)^{B}	18.6 (8.87)^{A}	17.3 (8.25)^{A}	14.0 (7.39)^{B}	14.7 (8.00)^{B}	14.4 (8.20)^{B}	<.001
Hemoglobin (g/dL)	13.6 (13.3)^{B}	16.0 (16.1)^{A}	20.1 (14.8)^{A}	13.6 (12.9)^{B}	12.5 (12.4)^{B}	13.0 (12.4)^{B}	<.001
MCV (fL)	51.7 (51.4)	52.2 (51.2)	51.2 (50.5)	51.2 (51.0)	51.2 (51.0)	51.9 (52.0)	0.785
MCH (pg)	18.6 (18.7)	18.4 (18.7)	18.7 (18.2)	18.1 (17.7)	18.9 (17.8)	18.7 (18.2)	0.830
MCHC (g/dL)	35.9 (35.8)^{B}	37.9 (36.0)^{A}	37.8 (36.1)^{A}	35.5 (35.5)^{B}	35.3 (35.0)^{B}	36.4 (34.7)^{B}	0.002
RDW (%)	17.5 (17.3)	17.4 (17.5)	17.7 (17.6)	16.7 (17.0)	16.7 (17.0)	16.5 (16.7)	0.332
Leukogram							
Neutrophils (10^3/mm^3)	5900 (5650)^{B}	11632 (11400)^{A}	12691 (12850)^{A}	4514 (4350)^{C}	5200 (5000)^{B}	4823 (4800)^{B}	<.001
Lymphocytes (10^3/mm^3)	2077 (1650)^{C}	2350 (1600)^{C}	1591 (1500)^{D}	2832 (2400)^{B}	3536 (2800)^{A}	2573 (2950)^{B}	<.001
Monocytes (10^3/mm^3)	443 (418)^{C}	833 (709)^{A}	586 (600)^{B}	486 (401)^{C}	469 (400)^{C}	354 (320)^{D}	<.001
Eosinophils (10^3/mm^3)	183.2 (90.0)^{C}	49.1 (0.0)^{D}	63.2 (0.0)^{D}	735.5 (675.0)^{A}	749.5 (450.0)^{A}	230.0 (190.0)^{B}	<.001
Platelets							
Platelets (10^3/mm^3)	153 (156)^{C}	305 (321)^{A}	214 (201)^{B}	162 (168)^{C}	171 (158)^{C}	144 (134)^{D}	<.001
MPV (fL)	6.73 (6.70)	6.70 (6.60)	6.57 (6.55)	6.76 (6.75)	6.66 (6.70)	6.58 (6.50)	0.736
PDW (%)	15.8 (15.8)	15.9 (15.9)	15.9 (15.9)	16.1 (16.0)	16.0 (16.1)	16.1 (16.0)	0.170
PCT (%)	0.103 (0.104)^{C}	0.231 (0.147)^{A}	0.174 (0.160)^{B}	0.097 (0.101)^{C}	0.125 (0.107)^{C}	0.098 (0.105)^{C}	<.001

Different letters in the same line mean statistical difference ($p < 0.05$). PCV = packed cell volume; RBC = red blood cells; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; RDW = red cell distribution width; MPV = mean platelet volume; PDW = platelet distribution width; PCT = plateletcrit.

4. Discussion

The endurance race causes notable alterations in several blood parameters, reflecting the significant physiological demands placed on the horses [13]. Immediately after the race, we observed a sharp increase in Packed Cell Volume (PCV), Red Blood Cell Count (RBC), and Hemoglobin (Hb), indicating hemoconcentration likely due to dehydration and splenic contraction. This increase in red blood cell parameters reflects the body's adaptation to maintain oxygen delivery during intense physical exertion. During exercise, a horse's spleen contracts to expel reserve amounts of blood, increasing the number of red blood cells in circulation and ultimately enhancing oxygen delivery. This is possible due to the spleen's large storage capacity, per the previous findings [14]. This study observed an increase in RBC, PCV, hemoglobin concentration, and MCHC in T1 and T2, and these levels returned to normal beyond T3.

Horses may exhibit an increase in MCV following high-intensity exercise, attributed to the release of larger erythrocytes from the spleen. The RDW index measures RBC distribution generated by a hematological automated machine. It reflects the heterogeneity in erythrocyte size and is the most sensitive indicator for determining the degree of anisocytosis. There was no significant alteration in these parameters in the horses of this study at any collection time, aligning with the findings reported in the literature [15–18].

Platelets exhibit significant variations during exercise, crucial in hemostasis and immune modulation. A previous study [2] highlighted the importance of MPV, PDW, and PCT. The increase in platelet count and PCT during T1 and T2, followed by normalization beyond T3, suggests a dynamic response. MPV and PDW remained unaltered, emphasizing the nuanced platelet dynamics during exercise.

Plateletcrit (PCT) changes during and after the endurance race suggest significant physiological adaptations to exercise-induced stress. Immediately following the race, PCT increased dramatically, indicating a surge in platelet activity likely driven by splenic contraction and exercise-induced stress. This initial rise is consistent with the body's response to potential microtraumas and preparation for hemostasis and tissue repair [19]. As the recovery progressed, PCT levels gradually decreased, reflecting a return to normal hemodynamics and a resolution of the acute stress response. This decline could be attributed to rehydration and hemodilution as the horses regained lost fluids and decreased inflammation as the body's immune response stabilized. The decrease in PCT also suggests that the horses recovered efficiently, with their physiological systems returning to baseline. These findings highlight the importance of monitoring PCT in endurance horses to assess their recovery status. A rapid return to baseline levels of PCT can indicate successful recovery, while prolonged elevation may signal ongoing stress or a slower recovery process. Understanding these patterns can guide training adjustments and inform recovery strategies, contributing to endurance horses' overall health and performance. To develop comprehensive recovery protocols, further research could explore the relationships between PCT, other hematological markers, and endurance performance.

Assessing inflammation and immune status due to exercise remains challenging but pivotal. Studies delve into cytokine level changes, activating monocytes and cells to produce proinflammatory cytokines [5,6,20]. This study aligns with such findings, observing neutrophilia and lymphocytopenia in early recovery, akin to responses observed in pathological conditions. Leukocytes in the peripheral blood system can be a universal indicator for assessing the body's overall homeostasis. Prolonged periods of intense physical activity can lead to significant leukocytosis in endurance horses, which may last up to 48 hours. This happens due to an increase in the release of cortisol, which leads to neutrophilia. At the same time, hypercortisolemia can cause eosinopenia, but this does not affect the value of the Ne/Ly ratio because of the predominant increase in the number of neutrophils [20]. The observed decrease in neutrophils and the increase in lymphocytes during the recovery period, specifically from T3 (three days after the race) to T5 (14 days after the race), indicate a shift from the acute stress response to a more stable, recovery-focused immune state. This pattern reflects the body's efforts to return to homeostasis following the intense physiological demands of endurance racing. This pattern can be an essential marker for trainers and veterinarians, suggesting that horses move from the acute stress phase to a more adaptive, healing state.

Monocytes are critical components of the immune system, acting as phagocytes that help clear pathogens and cellular debris. They also play a role in signaling to other immune cells [21]. In this study, monocytes showed significant changes in response to the endurance race, indicating their involvement in the immune and inflammatory processes following intense exercise. Immediately after the race, monocyte counts increased significantly, suggesting an early immune response. This monocyte surge could be linked to the body's need to address tissue damage and initiate repair mechanisms following the physical exertion of the endurance race. Given their role in the immune system, the elevated monocyte levels might also indicate a transition from acute stress to recovery and repair. However, as the recovery phase progressed, monocyte counts gradually decreased. Monocyte levels began to normalize three days post-race, aligning with the overall reduction in other stress markers, such as neutrophils. This trend suggests that monocytes are involved in the early stages of inflammation and recovery, then taper off as the body's immune response stabilizes.

Inflammation markers are commonly used in human performance settings to measure the impact of exercise and the recovery process. The response of leukocytes to physical exertion is essential in objectively assessing the body's degree of adaptation. By analyzing individual recovery kinetics using these markers, exercise programs can be adjusted to better suit the individual's needs [22–24]. The current study aimed to introduce the established cellular immune inflammation markers in equine exercise physiology.

A prior study [25] explored various inflammatory markers across athletic disciplines, including those derived from red blood cells (such as RDW/PLR, Hb/RDW, and Hb/PLT) and the eosinophil-to-lymphocyte ratio, among other established indicators.

The stability of MCHC, Hb/RDW, and Hb/PLT during and after the endurance race indicates that intense exercise did not affect red blood cell morphology and oxygen-carrying capacity. The decrease in RDW/PCT suggests a temporary shift towards platelet activation, possibly as a physiological response to stress and hemostatic needs during the race. These findings underscore the complex adaptive mechanisms that endurance horses use to maintain physiological balance during and after intense exercise.

The NLR has long been used as a stress marker in horses, reflecting the relationship between specific and non-specific immunity. According to various studies, it effectively predicts exercise stress and fitness [20,23,24]. The hormone cortisol mediates the increase in neutrophils after long-duration exercise, and its effects can last for several hours or even days after the race. On the day of the competition (T0-T2), there was an increase in NLR, PLR, SII, SIRI, and MLR. These indices are calculated using cells that participate in and orchestrate the acute inflammatory response, such as neutrophils, lymphocytes, monocytes, and platelets. Therefore, they provide a reliable recovery scenario as they returned to baseline values from T3 onwards.

The Monocyte-to-Lymphocyte Ratio (MLR) index describes the balance between the effector and afferent chains of the immune response. It has been found that an imbalance between the different subpopulations of leukocytes, especially between neutrophils and monocytes and between lymphocytes and monocytes, can indicate overtraining in men [23]. Monocytosis was observed at T1 in the horses of the present study, with values decreasing at T2 and normalizing by T3. The monocyte-to-lymphocyte ratio (MLR) mirrored the dynamics of monocytes, exhibiting a similar pattern across the observed time points.

During a competition day, the PLR, SII, and SIRI indexes were also found to be sensitive. When horses exercise, their platelet count increases as the spleen, bone marrow, and lungs release fresh platelets to aid in the healing of microtrauma caused by exercise [26]. Platelets are crucial in primary hemostasis and possess various proinflammatory properties, making them useful as an inflammation marker. However, limited information is available regarding the hemostatic changes that occur after exercise in horses. Some studies have suggested that exercise-induced thrombocytosis in horses leads to changes in the clotting mechanisms, a physiological adaptation of the hemostatic system to exercise [27–29]. All horses in the study showed a significant increase in platelet count, SII, SIRI, and PLR at T1 ($p < 0.05$) and a decrease at T2, albeit different from the baseline levels, with normalization after three days.

Table 2: Median values (Friedman test) for key hematological indices in endurance horses measured at various time points: before the ride (T0), immediately after the ride (T1), three hours after the ride (T2), three days post-race (T3), seven days post-race (T4), and fourteen days post-race (T5).

	Sample time						
Indices	T0	T1	T2	T3	T4	T5	*p*
NLR	3.85 (3.92)^{B}	8.20 (8.42)^{A}	9.81 (8.98)^{A}	1.90 (1.89)^{C}	2.20 (2.03)^{C}	2.34 (1.60)^{C}	<.001
PLR	0.097 (0.090)^{C}	0.219 (0.207)^{A}	0.169 (0.135)^{B}	0.078 (0.072)^{C}	0.080 (0.055)^{C}	0.076 (0.056)^{C}	<.001
SII	478 (503)^{B}	2548 (2286)^{A}	2156 (1815)^{A}	307 (334)^{B}	414 (292)^{B}	329 (236)^{B}	<.001
SIRI	945 (858)^{B}	6226 (5835)^{A}	5509 (5664)^{A}	855 (814)^{B}	824 (766)^{B}	840 (710)^{B}	<.001
MLR	0.26 (0.23)^{B}	0.54 (0.42)^{A}	0.43 (0.42)^{A}	0.26 (0.17)^{B}	0.18 (0.16)^{B}	0.18 (0.12)^{B}	<.001
ELR	0.103 (0.073)^{B}	0.041 (0.000)^{C}	0.024 (0.000)^{C}	0.355 (0.274)^{A}	0.327 (0.172)^{A}	0.113 (0.072)^{B}	<.001
LSI	0.18 (0.00)^{C}	2.43 (2.30)^{A}	2.90 (2.60)^{A}	0.44 (0.00)^{B}	0.43 (0.00)^{B}	0.59 (0.12)^{B}	<.001
AI	0.35 (0.00)^{B}	0.22 (0.00)^{B}	- 0.12 (- 0.08)^{C}	0.62 (0.40)^{A}	0.68 (0.52)^{A}	0.54 (0.42)^{A}	<.001
RDW/PLT	0.19 (0.12)^{A}	0.06 (0.05)^{B}	0.06 (0.09)^{B}	0.12 (0.10)^{A}	0.12 (0.10)^{A}	0.14 (0.12)^{A}	<.001
Hb/RDW	0.78 (0.75)	0.82 (0.82)	0.89 (0.83)	0.81 (0.78)	0.75 (0.75)	0.79 (0.76)	0.788
Hb/PLT	0.09 (0.08)	0.07 (0.05)	0.09 (0.07)	0.09 (0.08)	0.08 (0.07)	0.09 (0.08)	0.891

Different letters in the same line mean statistical difference ($p < 0.05$). NLR = neutrophil-to-lymphocyte ratio; PLR = platelet-to-lymphocyte ratio; SII = systemic inflammation index; SIRI = systemic inflammation response; MLR = monocyte-to-lymphocyte ratio; ELR = eosinophil-to-lymphocyte ratio; LSI = leukocyte shift index; AI = adaptive response index; RDW/PLT = red cell distribution width-to-platelet ratio; Hb/RDW = hemoglobin-to-red cell distribution ratio; Hb/PLT = hemoglobin-to-platelet ratio.

After the competition, the horses exhibited a significant decrease in eosinophils. However, all horses in this study showed an increase in eosinophils on the third day, which persisted until the seventh day after the competition. These findings are consistent with other research conducted on endurance horses [30]. Eosinophils are cells that generate various cytokines, some of which help maintain a healthy balance in the body, while others contribute to inflammation and release proinflammatory mediators that can damage tissues. Both endurance and strength exercises in humans can activate eosinophils through non-allergic means, as these cells also play a role in the regeneration of injured muscles by modifying the production of glycosaminoglycans by fibroblasts [31].

The complement system (C3, C4, and C5) may be activated in injured muscles, which can then activate eosinophils. A study conducted on human ultramarathon runners found elevated serum concentrations of eosinophil cationic protein (ECP). Previous research [31,32], observed that ECP (eosinophil cationic protein) levels and the number of eosinophils remained abnormal for 72 hours after an event. This is consistent with the findings of the current study. However, in horses, eosinophilia (an increase in eosinophil count) was observed for up to seven days, possibly due to their larger muscle mass compared to humans. The eosinophil-to-lymphocyte ratio (ELR) reflected the changes in eosinophil levels and remained high for a week. Since eosinophils have non-allergic functions and the ELR remained elevated for several days following the competition, this indicator is considered appropriate for evaluating the recovery of horses.

The Leukocyte Shift Index (LSI) is a measure that helps assess changes in the distribution of different types of white blood cells, such as neutrophils and lymphocytes. The movement of these cells depends on the intensity and duration of exercise. Studies have investigated the relationship between leukocyte counts and physical fitness, revealing an inverse relationship between the total leukocyte count and physical fitness [33]. However, the available search results do not specifically discuss the adaptation of the Leukocyte Shift Index to exercise.

According to research conducted on human athletes [34], Adaptation Intensity (AI) values are utilized to identify different types of Adaptive Reactions (AR). These AR types include stress (0.3 and below), orientation (0.31 - 0.5), quiet adaptation (0.51 - 0.7), re-activation (0.71 - 0.9), and increased activation (0.9 and above). The values of the Adaptation Intensity Index (AI) declined at T1 and T2, corresponding to the conclusion of the competition, indicating stress. However, at T3 and T4, there was an increase, with the values falling within the range considered "quite an adaptation," and returning to the adaptive threshold only 14 days post-competition. The most significant discrepancy between neutrophil and lymphocyte values, as observed by the Leukocyte Shift Index (LSI), also occurred post-competition (T1 and T2) and stabilized from the third day post-competition (T3). This observed stabilization in T3 indicates a more balanced state between neutrophils and lymphocytes, suggesting that the initial stress response was subsiding, and the horses were entering a recovery phase.

Based on the obtained results, it can be concluded that specific hematological indices, such as NLR, PLR, SII, SIRI, MLR, RDW/PLT, and LSI, are sensitive to acute changes associated with physical exertion during equine competition. These changes were observed on the day of the event, indicating the ability of these indices to reflect the immediate physiological response to exertion. On the other hand, the ELR and AI indices exhibited delayed and persistent responses, making them more specific indicators of the post-exercise recovery phase. The persistence of these changes beyond the third day suggests that these indices could provide valuable insights into prolonged adaptation and recovery in equine athletes.

An integrated approach that considers indices sensitive to the acute phase of exertion and those indicative of prolonged recovery can provide a more comprehensive and accurate evaluation of equine physiological performance in sporting environments. A thorough understanding of these blood parameter changes and indices offers a robust framework for assessing endurance horses' stress, inflammation, and recovery, which can help trainers and veterinarians develop optimal training and recovery protocols to enhance equine athletes' overall health and performance. However, further research is necessary, including animals in training across multiple sports, to determine precise cutoff points for horses.

5. Conclusion

Our study on endurance horses investigated hematological and inflammatory responses following prolonged exercise, providing valuable insights into blood cell dynamics. The utilization of NLR, PLR, SII, SIRI, RDW/PLR, MLR, and LSI as markers on the day of competition, along with ELR and AI as delayed recovery indicators offers a promising approach. These integrated cell indices can guide training and medical care for endurance horses, emphasizing the significance of monitoring strategies for performance improvement and equine well-being.

Authors' Contributions

Conceptualization: RFS and WRF; methodology: RFS and WRF; data collection: RFS; data analysis: RFS; writing—original draft preparation: RFS; writing—review and editing: RFS and WRF. All authors have read and agreed to the published version of the manuscript.

Data Availability

The data supporting the findings of this study are available on request from the corresponding author.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval

The Ethics Committee approved this study for the Use of Animals of FMVZ-USP, protocol n. 2606/2012.

References

[1] Nagy A, Murray JK, Dyson S. Elimination from elite endurance rides in nine countries: A preliminary study. Equine Veterinary Journal 2010;42:637–43. https://doi.org/10.1111/j.2042-3306.2010.00220.x.

[2] Satué K, Gardón JC, Muñoz A. Interpretation of platelets in the horse. Journal of Hematology Research 2017;4:19–25. https://doi.org/10.12974/2312-5411.2017.04.3.

[3] Cywinska A, Witkowski L, Szarska E, Schollenberger A, Winnicka A. Serum amyloid A (SAA) concentration after training sessions in Arabian race and endurance horses. Bmc Veterinary Research 2013;9:91–91. https://doi.org/10.1186/1746-6148-9-91.

[4] Cywinska A, Turło A, Witkowski L, Szarska E, Winnicka A. Changes in blood cytokine concentrations in horses after long-distance endurance rides. Medycyna Weterynaryjna-Veterinary Medicine-Science and Practice 2014;70:568–71.

[5] de Siqueira RF, Fernandes WR. Post-ride inflammatory markers in endurance horses. Ciência Rural 2016;46:1256–61. https://doi.org/10.1590/0103-8478cr20151070.

[6] Page AE, Stewart JC, Holland RE, Horohov DW. The impact of training regimen on the inflammatory response to exercise in 2-year-old thoroughbreds. Journal of Equine Veterinary Science 2017;58:78–83. https://doi.org/10.1016/j.jevs.2017.08.011.

[7] Eming SA, Wynn TA, Martin P. Inflammation and metabolism in tissue repair and regeneration. Science 2017;356:1026–30. https://doi.org/10.1126/science.aam7928.

[8] Cappelli K, Amadori M, Mecocci S, Miglio A, Antognoni MT, Razzuoli E. Immune response in young thoroughbred racehorses under training. Animals (Basel) 2020;10:1809. https://doi.org/10.3390/ani10101809.

[9] Witkowska-Piłaszewicz O, Pingwara R, Winnicka A. The effect of physical training on peripheral blood mononuclear cell ex vivo proliferation, differentiation, activity, and reactive oxygen species production in racehorses. Antioxidants (Basel) 2020;9:1155. https://doi.org/10.3390/antiox9111155.

[10] Joisten N, Walzik D, Schenk A, Bloch W, Zimmer P, Wahl P. Aqua cycling for immunological recovery after intensive, eccentric exercise. European Journal of Applied Physiology 2019;119:1369–75. https://doi.org/10.1007/s00421-019-04127-4.

[11] Muñoz A, Riber C, Trigo P, Castejón F. Age- and gender-related variations in hematology, clinical biochemistry, and hormones in Spanish fillies and colts. Research in Veterinary Science 2012;93:943–9. https://doi.org/10.1016/j.rvsc.2011.11.009.

[12] Silvestre-Ferreira AC, Cotovio M, Maia M, Queiroga F, Pires MJ, Colaço A. Reference intervals for haematological parameters in the Lusitano horse breed. Acta Veterinaria Hungarica 2018;66:530–41. https://doi.org/10.1556/004.2018.047.

[13] Poškienė I, Juozaitienė V, Antanaitis R. A comparison of haematological and biochemical blood indices between the Žemaitukai and Arabian horses participating in endurance competitions. Acta Veterinaria Brno 2021;90:159–69. https://doi.org/10.2754/avb202190020159.

[14] Pellegrini Masini A, Baragli P, Tedeschi D, Lubas G, Martelli F, Gavazza A, *et al*. Behaviour of mean erythrocyte volume during submaximal treadmill exercise in the horse. Comparative Haematology International 2000;10:38–42. https://doi.org/10.1007/s005800070025.

[15] Zobba R, Ardu M, Niccolini S, Cubeddu F, Dimauro C, Bonelli P, *et al*. Physical, hematological, and biochemical responses to acute intense exercise in polo horses. Journal of Equine Veterinary Science 2011;31:542–8. https://doi.org/10.1016/j.jevs.2011.03.010.

[16] Adamu L, Adzahan NM, Abdullah R, Ahmad B. Effects of race distance on physical, hematological and biochemical parameters of endurance horses. American Journal of Animal and Veterinary Sciences 2010;5:244–8. https://doi.org/10.3844/ajavsp.2010.244.248.

[17] Wanderley EK, Manso Filho HC, Manso HECCC, Santiago TA, McKeever KH. Metabolic changes in four beat gaited horses after field marcha simulation. Equine Veterinary Journal 2010;42:105–9. https://doi.org/10.1111/j.2042-3306.2010.00288.x.

[18] Horohov DW, Sinatra ST, Chopra RK, Jankowitz S, Betancourt A, Bloomer RJ. The effect of exercise and nutritional supplementation on proinflammatory cytokine expression in young racehorses during training. Journal of Equine Veterinary Science 2012;32:805–15. https://doi.org/10.1016/j.jevs.2012.03.017.

[19] Ehrmann C, Engel J, Moritz A, Roscher K. Assessment of platelet biology in equine patients with systemic inflammatory response syndrome. Journal of Veterinary Diagnostic Investigation 2021;33:300–7. https://doi.org/10.1177/1040638720983791.

[20] Cywińska A, Wyszyńska Z, Górecka R, Szarska E, Witkowski L, Dziekan P, *et al*. The effect of the 162 km endurance ride on equine peripheral blood neutrophil and lymphocyte functions. Polish Journal of Veterinary Sciences 2010;13:279–85.

[21] Dimitrov S, Hulteng E, Hong S. Inflammation and exercise: Inhibition of monocytic intracellular TNF production by acute exercise via β(2)-adrenergic activation. Brain, Behavior, and Immunity 2017;61:60–8. https://doi.org/10.1016/j.bbi.2016.12.017.

[22] Walzik D, Joisten N, Zacher J, Zimmer P. Transferring clinically established immune inflammation markers into exercise physiology: focus on neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and systemic immune-inflammation index. European Journal of Applied Physiology 2021;121:1803–14. https://doi.org/10.1007/s00421-021-04668-7.

[23] Zacher J, Wesemann F, Joisten N, Walzik D, Bloch W, Predel G. Cellular Integrative immune markers in elite athletes. International Journal of Sports Medicine 2023;44:298–308. https://doi.org/10.1055/a-1976-6069.

[24] Wong CW, Smith SE, Thong YH, Opdebeeck JP, Thornton JR. Effects of exercise stress on various immune functions in horses. American Journal of Veterinary Research 1992;53:1414–7. https://doi.org/10.2460/ajvr.1992.53.08.1414.

[25] Holcomb KE, Tucker CB, Stull CL. Physiological, behavioral, and serological responses of horses to shaded or unshaded pens in a hot, sunny environment1. Journal of Animal Science 2013;91:5926–36. https://doi.org/10.2527/jas.2013-6497.

[26] Arfuso F, Giannetto C, Interlandi C, Giudice E, Bruschetta A, Panzera MF, *et al*. Dynamic metabolic response, clotting times and peripheral indices of central fatigue in horse competing in a 44 km endurance race. Journal of Equine Veterinary Science 2021;106:103753. https://doi.org/10.1016/j.jevs.2021.103753.

[27] Korytko Z, Kulitka E, Chornenka H, Zachidnyy V. Use of integral hematological indices for diagnostics of athletes' adaptive processes. Journal of Physical Education and Sport 2019;19:214–8.

[28] Assenza A, Tosto F, Casella S, Fazio F, Giannetto C, Piccione G. Changes in blood coagulation induced by exercise training in young athletic horses. Research in Veterinary Science 2013;95:1151–4. https://doi.org/10.1016/j.rvsc.2013.07.005.

[29] Miglio A, Falcinelli E, Mezzasoma AM, Cappelli K, Mecocci S, Gresele P, *et al*. Effect of first long-term training on whole blood count and blood clotting parameters in thoroughbreds. Animals (Basel) 2021;11:447. https://doi.org/10.3390/ani11020447.

[30] Siqueira RF, Fernandes WR. [Hematologic response of endurance horses that ran different distances in the post-race period]. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2017;69:543–50. https://doi.org/10.1590/1678-4162-9296.

[31] McKune A, Smith L, Semple S, Wadee A. Non-allergic activation of eosinophils after strenuous endurance exercise. South African Journal of Sports Medicine 2004;16:12. https://doi.org/10.17159/2078-516x/2004/v16i2a182.

[32] Dufaux B, Heine O, Prinz U, Soll C, Rost R. Effect of a short maximal physical exercise on the eosinophil cationic protein. International Journal of Sports Medicine 1993;14:468–70. https://doi.org/10.1055/s-2007-1021213.

[33] Chung P-S, Tsai K-Z, Lin Y-P, Lin Y-K, Lin G-M. Association between Leukocyte Counts and Physical Fitness in Male Military Members: The CHIEF study. Scientific Reports 2020;10:6082–6082. https://doi.org/10.1038/s41598-020-63147-9.

[34] Marakushyn DI, Isaieva IM, Karmazina IS, Hloba NS, Oluwasegun EA, Makarova KM. Female vs. male: difference in immune response, 2019.

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