Original Article

Effect of Moderate and Intense Exercise on Hematological Parameters and Oxidative Stress Biomarkers in Young Crossbred Horses

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ABSTRACT

Background: Exercise is vital for horse health because it improves fitness and strength. However, it can also stress the body by producing harmful molecules that may damage cells. Young horses with mixed breeding may respond differently to exercise compared to purebred horses, yet little is known about how their blood and stress responses adapt to different exercise levels.

Objectives: This study explored how moderate and intense exercise affects blood health and stress markers in young horses with mixed breeding. It aimed to determine whether moderate exercise protects their health while intense exercise poses challenges.

Methods: Ten young horses (aged 3–4 years; four females and six males) completed a six-week moderate exercise program, ending with one intense exercise session. Blood samples were collected one hour after exercise on days 0, 14, 42, and 43 to measure blood cell counts (red and white cells and platelets) and stress markers (indicators of cell protection and damage). Data were analyzed to detect changes over time.

Results: Blood cell counts remained stable, showing the horses' ability to handle exercise stress. Moderate exercise improved protective markers and reduced damage indicators, suggesting better cell health. However, the intense session increased stress markers, indicating temporary cell strain.

Conclusion: Moderate exercise benefits young mixed-breed horses by enhancing cell protection, while intense exercise may cause stress that requires recovery. Tailored exercise plans can optimize horse health and performance.

Keywords: Exercise physiology, Oxidative stress biomarkers, Hematological parameters, Antioxidant defense mechanisms, Equine health and performance

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Introduction

Exercise is integral to equine health, enhancing cardiovascular fitness, musculoskeletal development, and metabolic efficiency. However, it also induces physiological challenges, notably oxidative stress, which is critical for performance horses, including crossbred athletes in disciplines, such as racing, eventing, and endurance (de Meeûs d'Argenteuil et al., 2021). Oxidative stress occurs when reactive oxygen species (ROS), generated during increased oxygen metabolism, exceed antioxidant defenses, potentially causing lipid, protein, and DNA damage (Ott et al., 2022). Young crossbred horses, such as Thoroughbred-Turkmen crosses, may exhibit different physiological responses compared to purebreds due to their genetic diversity; however, their oxidative adaptations to varying workloads remain underexplored (Bażanów et al., 2020).

Previous studies indicate that endurance exercise alters hematological indices, like hematocrit (HCT) and hemoglobin (HGB), which reflect oxygen transport efficiency, and oxidative stress biomarkers, such as superoxide dismutase (SOD) and catalase (CAT), which neutralize ROS (Williams, 2016). Moderate exercise enhances total antioxidant capacity (TAC) and reduces malondialdehyde (MDA) levels, a lipid peroxidation marker, suggesting adaptive responses to training stress (Kacprzyk et al., 2026). In contrast, high-intensity exercise increases ROS production, often overwhelming antioxidant defenses and elevating carbonyl (CO) levels, which indicate protein oxidation (Tkaczenko et al., 2023). These responses vary by genetic makeup, exercise intensity, duration, nutrition, and age. Moderate exercise boosts antioxidant enzyme activity (e.g. CAT, glutathione peroxidase [GPx]) and intense exercise depletes reserves (Ohmura et al., 2013).

Young crossbred horses that are still undergoing physiological maturation may respond differently to exercise than mature or purebred horses. Although research on purebreds has outlined general oxidative trends, data on crossbreds are limited, leaving gaps in understanding how genetic diversity influences hematological stability and oxidative modulation (Mukai et al., 2026). This study addressed these gaps by examining the effects of moderate and intense exercise on hematological parameters and oxidative stress biomarkers in young crossbred horses over a six-week period, followed by a high-intensity bout. We hypothesized that moderate exercise enhances antioxidant defenses and promotes resilience, while intense exercise induces oxidative strain and necessitates greater recovery support.

Materials and Methods

Study population

Ten young crossbred horses (four mares and six stallions), aged 3–4 years, were selected from a single stable in a temperate region. These Thoroughbred-Turkmen crosses underwent veterinary assessments, including physical examinations, hematological analysis, and biochemical profiling, to confirm health status (Mukai et al., 2026). Routine care, including deworming and vaccinations, was maintained. The horses were housed in individual stalls under natural spring photoperiods (12–14 hours daylight). The mares were confirmed to be neither pregnant nor in estrus.

Exercise protocol

The six-week training regimen included six sessions per week, with rest on Saturdays. Sessions began at 9 A.M. with a 5-minute walking warm-up (1.5 m/s), followed by 4000–5000 meters of trotting (4–5 m/s, adjusted to each horse's capacity), and a 10-minute walking cool-down (1.5 m/s). On day 43, the horses completed an intense exercise bout consisting of a 15-minute warm-up (5 minutes of walking and 10 minutes of trotting), followed by a 1000-meter race on a dirt track at ~17 m/s, which was completed in under one minute. Environmental conditions were consistent (18–22 ˚C, 50–60% humidity).

Diet and nutrition

The horses received a standardized diet to minimize nutritional variability: 6 kg of dry alfalfa hay (18% crude protein and 90% dry matter) and 5 kg concentrates (92% dry matter and 13% crude protein) containing minerals (zinc, copper, iron, manganese, selenium, and calcium) and amino acids (lysine and methionine). The concentrate included an energy blend of barley, corn, wheat, and molasses (3.2 Mcal/kg digestible energy). Salt blocks and fresh water were available ad libitum, and the horses were fed at 7 A.M. and 5 P.M. Stable conditions were maintained at 18–20 ˚C.

Sample collection and analysis

Blood samples were collected one hour post-exercise via jugular venipuncture on days 0 (baseline), 14, 42 (end of the moderate phase), and 43 (post-intense). Hematological samples were collected in K-EDTA tubes. The

samples were stored on ice and processed within two hours. Hematological parameters (HCT, HGB, white blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width, and platelet distribution width (PDW)) were analyzed using a NIHON KOHDEN Celltac G MEK-9100K Analyzer calibrated for equine standards (Williams, 2016). Oxidative stress biomarkers (TAC, MDA, CAT, SOD, glutathione (GSH), GPx, and CO) were quantified using Biorex Fars ELISA Kits (Biorex Fars Co., Tehran, Iran) via spectrophotometric and enzymatic assays, with intra-assay coefficients of variation below 5% (Tkaczenko et al., 2023). Heart rate and respiratory rate were recorded to assess physiological stability.

Statistical analysis

Data normality was assessed using Shapiro-Wilk tests, confirming non-normal distribution. Friedman tests were used to compare measurements across sampling days, with Wilcoxon signed-rank tests were used for pairwise evaluations. Analyses were performed using SPSS software, version 27 (IBM Corp., Armonk, USA), with significance set at $P<0.05$ (Ott et al., 2022).

Results

Vital signs

Vital signs recorded one hour after exercise remained within equine physiological ranges (Table 1). Heart rate ranged from 49.3 to 51.7 bpm ($P>0.05$), respiratory rate ranged from from 14.1 to 15.6 bpm ($P>0.05$), and body temperature ranged from 37.07 to 37.29 ˚C ($P=0.052$), indicating cardiovascular and thermoregulatory stability.

Hematological parameters

Hematological indices showed no significant changes ($P>0.05$) and remained within equine reference ranges (Table 2). MCV, MCHC, WBC, PLT, and platelet metrics—PDW), mean platelet volume (MPV), and plateletcrit (PCT)—were stable, with no sex-specific differences ($P>0.05$).

Oxidative stress biomarkers

Oxidative stress biomarkers exhibited significant changes ($P<0.05$, Table 3). TAC increased from 0.37 mmol/L (day 0) to 0.92 mmol/L (day 42, $P<0.05$) and slightly declined to 0.90 mmol/L post-intense exercise (day 43, $P<0.05$ vs baseline). MDA levels decreased from 0.84 nmol/mL (day 14) to 0.41 nmol/mL (day 42, $P<0.05$), then reduced to 0.34 nmol/mL post-intense exercise ($P<0.05$). SOD activity rose from 311.65 U/mL (day 14) to 496.5 U/mL (day 42, $P<0.05$), then dropped to 374.95 U/mL post-intense exercise ($P>0.05$). CAT increased from 11.68 U/mL (day 0) to 24.7 U/mL (day 42) and remained elevated at 28.05 U/mL post-intense exercise ($P<0.05$). GSH levels rose from 1.05 U/mL (day 0) to 7.58 U/mL (day 43, $P<0.05$). GPx activity peaked at 37.85 U/mL (day 14) and stabilized at 32.9 U/mL post-intense exercise ($P=0.033$). CO levels decreased from 66.55 U/mL (day 0) to 16.93 U/mL (day 42), then rose slightly to 19.1 U/mL after intense exercise ($P<0.05$).

Discussion

The stability of hematological parameters ($P>0.05$) contrasts with findings in purebred horses, where endurance training often increases HCT and RBC counts due to hemoconcentration from fluid shifts and splenic contraction (Navas de Solis, 2019; Vlaeva et al., 2023). This resilience in young crossbred horses may reflect genetic diversity or hybrid vigor, enabling hematologi-

Table 1. Vital signs of the studied crossbred horses

Parameter	Median±SE				P
	Day 0	Day 14	Day 42	Day 43	
Weight (kg)	386.5±19.98	386.5±14.98	386.5±14.98	386.5±15.78	0.392
Body condition score	6.9±0.18	6.9±0.18	6.9±0.18	6.9±0.18	1.000
Heart rate (bpm)	50.6±1.49	49.3±1.48	51.4±1.3	51.7±0.65	0.104
Respiratory rate (bpm)	15±0.72	14.1±0.57	15.1±0.32	15.6±0.34	0.163
Temperature (°C)	37.29±0.08	37.18±0.03	37.08±0.02	37.07±0.03	0.052

Table 2. Hematological parameters of the studied crossbred horses

Parameter	Median±SE				P
	Day 0	Day 14	Day 42	Day 43	
PCV (%)	37.49±1.15	39.69±1.2	37.79±1.15	46.44±4.07	0.132
Hemoglobin (g/dL)	12.87±0.48	13.3±0.42	12.69±0.38	15.36±1.26	0.213
RBC (10^6/µL)	8.36±0.32	8.97±0.22	8.29±0.33	9.76±0.69	0.086
MCV (fL)	45.67±1.12	44.33±1.12	45.07±1.24	47.24±1.63	0.112
MCH (pg)	15.31±0.54	14.8±0.37	14.89±0.36	15.96±0.77	0.300
MCHC (g/dL)	33.42±0.44	33.57±0.11	33.6±0.32	33.49±0.47	0.448
WBC (10^3/µL)	10.59±1.62	10.9±0.63	12.18±1.05	10.39±0.59	0.169
Platelets (10^5/µL)	109.6±8.75	113.4±6.66	203.5±60.4	317.5±73.52	0.175
PCT (%)	0.06±0.01	0.08±0.01	0.13±0.05	0.25±0.06	0.112
MPV (fL)	7.28±0.1	7.02±0.09	7.36±0.14	7.61±0.13	0.056
PDW (%)	13.48±0.33	13.73±0.19	13.05±0.40	12.74±0.31	0.051
Neutrophils (%)	35.4±2.5	40.9±4.34	38.4±2.71	32.8±2.27	0.378
Lymphocytes (%)	48.3±3.74	49.5±4.1	54.8±2.67	59.6±2.13	0.243
Monocytes (%)	12.7±1.66	7.6±1.39	5.2±0.68	6±0.94	0.055
Eosinophils (%)	3±1	2.2±0.81	1.6±0.50	1.6±0.65	0.648
Basophils (%)	0±0	0±0	0±0	0±0	1.000
Bands (%)	0.6±0.43	0.2±0.2	0±0	0±0	0.300

cal stability under controlled exercise intensities. The consistent vital signs (heart rate and respiratory rate) further support cardiovascular adaptability, aligning with observations that training enhances equine homeostasis (Ebisuda et al., 2024).

Significant increases in TAC and CAT by day 42 ($P<0.05$) corroborate the results of Brkljača Bottegaro et al. (2018), who reported enhanced antioxidant defenses with moderate exercise. The reduction in MDA levels suggests decreased lipid peroxidation, likely driven by CAT-mediated neutralization of hydrogen peroxide (Ott et al., 2022). This adaptation may reflect hormesis, where moderate ROS levels stimulate antioxidant enzyme production (Radak et al., 2013). Conversely, the increases in MDA and slight decline in TAC after intense exercise ($P<0.05$) align with Latham et al. (2021), indicating oxidative stress caused by rapid ROS accumulation that exceeds antioxidant capacity. The sustained elevation in CAT after intense exercise suggests ongoing enzymatic activity, although it is insufficient to fully counter oxidative stress.

The stability of SOD and GSH levels differs from findings in endurance studies, which reported GSH depletion and increased SOD turnover in Thoroughbreds reporting (White et al., 2001). The brief duration of the intense bout (<1 minute) may explain this difference, as Marlin & Nankervis (2002) note that SOD responses vary by exercise type and breed. The lack of sex-specific antioxidant differences contrasts with Andriichuk et al. (2014), who reported elevated SOD activity in purebred mares. This suggests that genetic diversity or young age may mitigate such disparities in crossbreds.

Moderate exercise likely fosters mitochondrial efficiency and antioxidant gene expression (Powers & Jackson, 2008), and genetic diversity in crossbreds may enhance resilience compared with purebreds (Bos et al., 2018). The oxidative stress observed after intense exer-

Table 3. Oxidative stress biomarkers in the studied crossbred horses

Biomarker	Median±SE				P
	Day 0	Day 14	Day 42	Day 43	
TAC (mmol/L)	0.37±0.09	0.6±0.05	0.92±0.1	0.9±0.15	0.001*
MDA (nmol/mL)	0.48±0.04	0.84±0.23	0.41±0.08	0.34±0.09	0.010*
SOD (U/mL)	298.75±11.39	311.65±12.04	496.5±166.7	374.95±51.54	0.040*
CAT (U/mL)	11.68±1.48	38.4±24.66	24.70±2.84	28.05±2.52	0.001*
GSH (U/mL)	1.05±0.35	2.33±0.29	5.5±1.85	7.58±2.85	0.001*
GPx (U/mL)	33.4±6.24	37.85±7.02	34.85±10.1	32.9±12.59	0.033*
Protein carbonyl (U/mL)	66.55±28.12	43.55±16.74	16.93±6.73	19.1±7.9	0.001*

*Significantly different from baseline (day 0), P<0.05.

cise indicates a mismatch between ROS production and antioxidant mobilization, particularly in young horses with developing physiological systems (Arfuso et al., 2022). The stability of GPx activity and GSH levels suggests that, although the intense bout was stressful, it did not deplete deeper antioxidant reserves, unlike endurance efforts.

Compared to purebreds, crossbreds exhibit better oxidative balance during moderate exercise but remain vulnerable to intense efforts (Nocera et al., 2020). The one-hour post-exercise sampling may underestimate peak ROS levels or delayed antioxidant responses (Zhang et al., 2025). Future studies should extend sampling to 24–48 hours, incorporate muscle biopsies, and explore diverse crossbred lineages.

These findings support structured training that prioritizes moderate exercise to build antioxidant defenses and reserves intense efforts for later developmental stages, with extended recovery or antioxidant supplementation (e.g. vitamin E, selenium) to mitigate lipid peroxidation (Garcia et al., 2022). This approach optimizes performance and welfare in young crossbred horses by leveraging their genetic resilience.

Conclusion

Moderate exercise enhances antioxidant defenses in young crossbred horses by increasing TAC and CAT while reducing MDA levels, indicating reduced oxidative stress. Intense exercise, however, elevates MDA and partially depletes TAC, signaling oxidative risk. However, intense exercise elevates MDA and partially depletes TAC, signaling oxidative risk. These findings support tailored training regimens that emphasize moderate exercise to build resilience, with cautious integration of intense bouts to prevent oxidative overload. Further research should explore long-term recovery kinetics, nutritional interventions, and breed-specific adaptations to optimize equine health and performance.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Ferdowsi University of Mashhad, Mashhad, Iran (Code: IR.UM.REC.1403.126).

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Authors' contributions

Conceptualization, methodology, statistical analysis, review and editing: Gholamreza Mohammadi; Data collection: Abdolhamid Bakhshizadeh; Formal analysis and data interpretation: Gholamreza Mohammadi and Mehrdad Mohri; Writing the original draft: Gholamreza Mohammadi and Abdolhamid Bakhshizadeh; Administrative, technical, and material support: All authors.

Conflict of interest

The authors declared no conflict of interest.

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