P-ISSN: 2304-3075; E-ISSN: 2305-4360

International Journal of Veterinary Science

www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2023.014

# The Influence of Various Physical Activity on Hematological and Biochemical Parameters in Draft and Sport Horses

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**Article History:** 23-104 Received: 09-Jan-23 Revised: 09-Feb-23 Accepted: 10-Feb-23

## ABSTRACT

Good horse performance depends on a genetic predisposition, training, health status, and optimal values of hematological and biochemical blood parameters. This research aimed to monitor 20 hematological and 15 biochemical blood parameters during different types of physical activity in clinically healthy 20 sports and 20 working horses with an average age of 8.33 years. Sport male horses had higher MCV, MCH, number, and percentage of neutrophils, eosinophils, creatinine, and bilirubin, while mares had higher values of RDW, lymphocytes, monocytes, basophils, MPV, glucose, BUN-to-creatinine ratio. Physical activity in draft horses led to a statistically significant increase in the number of leukocytes and neutrophils and significant hypocalcemia, while in sport horses, there was a significant increase in RBC, HCT, and HGB and significant hypoglycemia after rest. Significantly higher values of neutrophils and creatinine were recorded in male draft horses, while in mares, there were higher values of lymphocytes and amylase. This study proved that sport horses have better aerobic capacity and performance, are better adapted to the type and intensity of physical activity, and are kept in better conditions than draft horses. The different types of physical activity and other physiological factors can significantly change hematological and biochemical parameters in the draft and sports horses. Monitoring of hematological and biochemical blood parameters during different types of exercise is essential to assessing horses' performance, welfare status, and health status.

**Key words:** Physical Activity, Hematological Parameters, Biochemical Parameters, Draft Horses, Sport Horses.

## INTRODUCTION

Physical activity affects changes in the reference ranges of hematological and biochemical parameters in horses' blood. The efficiency of the body's metabolic processes during different types of physical activity depends on the value of hematological and biochemical parameters in the horse's blood (Witkowska-Piłaszewicz et al. 2020; Massányi et al. 2022). Exercise-induced horses respond in a coordinated manner to the intensity of physical effort where adaptations are made to various body systems. The horses compensatory physiological response during exercise changes in hematological and blood biochemical parameters. This compensatory physiological response involves the integration of multiple organ systems which communicate via neural and endocrine pathways (Trailović 2008; Hinchcliff et al. 2008; Mc Gowan and Hodgson 2014; Cabrera et al. 2022).

The neuro-endocrine response of the body during exercise is induced by the release of adrenaline, which leads to the contraction of the spleen, where by accumulated blood rich in erythrocytes and leukocytes reaches the circulation (Kurosawa et al. 1998; Snow et al. 1983). Hematocrit increases linearly with enhance training intensity until the intensity reaches ¾ speed (Persson 1982; Muñoz 1999). Neutrophilia is induced by an increase in cortisol, which follows energy changes in the body during physical exertion. An elevated concentration of cortisol in the plasma stimulates bone marrow with consequent neutrophilia, lymphopenia, and eosinopenia (Rose 1982). Exercise induced leukocytosis depends on the intensity and duration of physical activity. The influence of workload on the WBC number depending on the intensity and duration of exercise. Hypercortisolemia at the same time can lead to eosinopenia, which definitely does not affect the value of the neutrophil/lymphocyte

**Cite This Article as:** Isović J, Čamo D, Ćutuk R and Zahirović A, 2023. The influence of various physical activity on hematological and biochemical parameters in draft and sport horses. International Journal of Veterinary Science 12(5): 708-714. https://doi.org/10.47278/journal.ijvs/2023.014

ratio due to the predominant increase in the number of neutrophils (Daud et al. 2019; Djoković et al. 2021). Physical exercise causes thrombocytosis, resulting from increased platelet mobilization from the lungs and spleen (Hammer 1991). The values of glucose concentration in horses' blood vary depending on the intensity and type of exercise, but they move within the reference intervals (Hodgson and McKeever 2014). Physical activity induces dehydration which leads to changes in the concentration of total proteins (Trailović 2008), creatinine, urea, bilirubin, electrolytes, and calcium, which negatively affects sports performance in horses (McCutcheon and Geor 1996; Allaam et al. 2014; Gomes et al. 2020).

Applying different horse training programs requires a mandatory assessment of the animal's physiological response to the physical activity. Breed affiliation, sex, age, and the difference in geographical, climatic, and environmental conditions, diet, and purposes, can affect hematological and biochemical parameters in draft and sport horses (Shawaf et al. 2018; Czech et al. 2018). In order to better understand the physiology of sport and draft horses, and to identify horses welfare, it is necessary to conduct more detailed studies of the influence of other factors on the reference values of hematological and biochemical blood parameters (Lesimple 2020). Therefore, the aim of the present study was to investigate the influence of different type of physical activity on hematological and biochemical parameters in draft and sport horses.

## MATERIALS AND METHODS

The Ethics Committee Veterinary Faculty, University of Sarajevo, Bosnia, and Herzegovina approved the study. The protocol number is 07-03-1000-2/21 from 10.01.2022 at the Ethics Committee Veterinary Faculty, University of Sarajevo, Sarajevo, Bosnia, and Herzegovina.

The study included 40 clinically healthy horses divided on purpose into two groups draft and sport horses. A group of 20 draft horses consisted of ten males and ten females, with an average age of 9 years. A group of 20 sport horses comprised ten males and ten females with an average age of 8.33 years. Draft horses were used to pull carriages on the Vrelo Bosne Promenade in Sarajevo and to haul wood in the wider area of Sarajevo Canton. Sports horses were used for endurance competitions throughout Bosnia and Herzegovina, dressage, and recreational riding. Based on medical history and physical examination, the horses were clinically healthy. The first blood sampling was performed by puncturing the jugular vein, after which the horses were subjected to a physical activity test.

Draft horses were tested by pulling a double-harnessed carriage with a coachman (weighing 400-500kg) along a 500m long avenue, while pack horses hauled wood in the wider area of the Sarajevo Canton. The horses carried 0.3$m^3$ of raw pine wood (the specific weight of raw pine is 750–850 kg/$m^3$) on the pack saddles secured on their backs along a 300m road measured in a straight line, with an altitude difference of 80m.

The sport horses were subjected to lunge training. The horse training on a lunge lasted a total of 15 minutes. First, the horse moved for 5 minutes at a walk and then at a trot to the left and right side for two and a half minutes. The horse went from a trot to a gallop to the left and right side for two minutes. During the last minute of the lunge training, the horse moved at a walk again.

Sport horses training on the lunge lasted a total of 15min. First, the horse moved for 5min, at a walk, and then at a trot to each side for two and a half minutes. He went from trot to gallop to each side for two minutes. During the last minute of lunging, the horse moved at a walk. Blood samples were collected immediately after the physical activity and 5-6 hours after rest.

For hematological analysis, blood was collected in an EDTA microtube. A complete blood count (CBC), including a count of erythrocytes (RBC), the value of hematocrit (HCT), hemoglobin (HGB), mean volume of erythrocytes (MCV), mean value of corpuscular hemoglobin (MCH), mean concentration of samples hemoglobin in erythrocytes (MCHC), the width of the distribution of erythrocytes (RDW), the total number of leukocytes (WBC), number of neutrophils (NEU), number lymphocytes (LYM), number of monocytes (MONO), eosinophils (EOS), basophils (BASO), percentage representation %NEU, %LYM; % MONO, %EOS, %BASO, number of platelets PLT, mean volume of platelets MPV, was performed by an automatic hematology laser reader "IDEXX Laser Cyte."

For biochemical analysis, blood was taken in heparin microtubes and included the concentration of glucose, creatinine, urea, BUN/urea ratio, phosphorus (P), calcium (Ca), total proteins (TP), albumin, globulin, total bilirubin (TBL), cholesterol, then the activity of enzymes alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and amylase. Biochemical analysis was performed on an IDEXX Catalyst One Chemistry Analyzer.

In the statistical processing of the results, a one-way ANOVA was applied using the General Linear Model (GLM) procedure in the statistical program Minitab 17 (10) with a post hoc Tukey test, and a two-way ANOVA which were used for examining the significance of individual differences, when the load phase showed a significant effect. The two-way ANOVA was used to examine the interactions between the phases of workload and the sexes of the horse. Differences between the two examined groups of horses (working versus sports) were tested with the T-test for independent samples. The correlation between the examined parameters was calculated by Pearson's correlation coefficients (r). P values less than 0.05 was statistically significant.

## RESULTS

### Hematological Tests Reuslts

In working horses, physical activity had a statistically significant effect on the values of WBC (P=0.0034) and neutrophils (P=0.026), whereby the highest values were recorded after rest, and they differed significantly from the values before the activity (Table 1). The average values of the differential blood count between the genders in draft horses were statistically significantly different in

**Table 1:** Hematological parameters in draft horses by gender, before, after exercise, and after rest.

| Draft Horses | Before Physical Activity | | After Physical Activity | | After Rest | | Pool. SD | Physcial activity | Gender | Physical activityxGennder |
|---|---|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | | | | |
| RBC ($\times10^{12}$/L) | 9.92 | 8.88 | 9.28 | 9.16 | 8.38 | 9.01 | 1.89 | ns | ns | ns |
| HCT (%) | 45.51 | 39.79 | 42.21 | 41.51 | 37.73 | 40.63 | 9.33 | ns | ns | ns |
| HGB (g/dL) | 16.23 | 14.38 | 15.22 | 14.87 | 13.66 | 14.65 | 3.03 | ns | ns | ns |
| MCV (fL) | 45.70 | 44.82 | 45.54 | 45.30 | 45.03 | 44.91 | 2.35 | ns | ns | ns |
| MCH (pg) | 17.39 | 16.23 | 18.38 | 16.25 | 18.35 | 16.22 | 3.89 | ns | ns | ns |
| MCHC (g/dL) | 36.03 | 36.23 | 36.06 | 35.87 | 36.25 | 36.16 | 0.91 | ns | ns | ns |
| RDW (%) | 28.89 | 28.12 | 28.29 | 28.30 | 27.58 | 28.21 | 2.5 | ns | ns | ns |
| WBC ($\times10^{9}$/L) | 6.91$^{b}$ | 7.39$^{ab}$ | 7.86$^{ab}$ | 7.13$^{b}$ | 8.41$^{a}$ | 7.94$^{ab}$ | 1.23 | 0.034 | ns | ns |
| NEU (%) | 57.06$^{ab}$ | 47.63$^{cd}$ | 54.65$^{abc}$ | 46.40$^{d}$ | 59.34$^{a}$ | 50.77$^{bcd}$ | 7.96 | ns | <0.001 | ns |
| LYM (%) | 32.48$^{c}$ | 42.49$^{ab}$ | 35.58$^{bc}$ | 44.89$^{a}$ | 31.51$^{c}$ | 39.92$^{ab}$ | 7.77 | ns | <0.001 | ns |
| MONO (%) | 6.1 | 5.18 | 6.04 | 4.82 | 5.74 | 5.10 | 2.49 | ns | ns | ns |
| EOS (%) | 4.01 | 4.27 | 3.44 | 3.63 | 3.00 | 3.81 | 2.3 | ns | ns | ns |
| BASO (% ) | 0.33 | 0.43 | 0.31 | 0.26 | 0.41 | 0.40 | 0.29 | ns | ns | ns |
| NEU ($\times10^{9}$/L) | 3.97$^{bc}$ | 3.50$^{bc}$ | 4.32$^{ab}$ | 3.44$^{c}$ | 4.98$^{a}$ | 4.05$^{bc}$ | 0.94 | 0.026 | 0.003 | ns |
| LYM ($\times10^{9}$/L) | 2.24$^{c}$ | 3.15$^{ab}$ | 2.78$^{abc}$ | 3.39$^{a}$ | 2.64$^{bc}$ | 3.14$^{ab}$ | 0.76 | ns | 0.001 | ns |
| MON ($\times10^{9}$/L) | 0.43 | 0.39 | 0.69 | 0.36 | 0.49 | 0.40 | 0.35 | ns | ns | ns |
| EOS ($\times10^{9}$/L) | 0.26 | 0.32 | 0.27 | 0.27 | 0.25 | 0.29 | 0.16 | ns | ns | ns |
| BASO ($\times10^{9}$/L) | 0.02 | 0.03 | 0.03 | 0.02 | 0.03 | 0.03 | 0.02 | ns | ns | ns |
| PLT ($\times10^{9}$/L) | 117.40 | 111.30 | 113.70 | 115.90 | 125.20 | 114.60 | 32.64 | ns | ns | ns |
| MPV(fL) | 11.56 | 14.23 | 11.30 | 12.25 | 11.54 | 11.98 | 2.73 | ns | ns | ns |

Results are expressed as mean, Pool.SD – pooled standard deviation, $^{abcd}$ values in the same row that do not contain the same letter differ significantly (P<0.05), ns- not significant.

the number of neutrophils (P=0.003) and lymphocytes (P=0.001), as well as their percentage representation P <0.001).

The number of neutrophils was higher in the blood of male draft horses compared to females, while the number of lymphocytes was higher in mares (Table 1).

The effect of gender and activity were completely independent, which is reflected in the fact that no interactions were statistically established between them (physical activity x gender).

After physical activity, hematological parameters in sports horses had significantly higher values of RBC (P=0.041), HCT (P=0.019), and HGB (P=0.021) compared to the values before activity and after rest (Table 2).

Within the group of sports horses, statistically significant differences between male and female individuals (Table 2) were recorded in the values of MCV (P=0.026), MCH (P=0.021), and RDW (P=0.012). MCV and MCH values were higher in males compared to females, while RDW values were higher in females compared to males. Also, statistically significant differences between the genders were recorded in the values of WBC (P<0.001), monocytes (P=0.009) and basophils (P=0.013), as well as the percentage of neutrophils (P<0.001), lymphocytes (P<0.001), monocytes (P=0.003) and eosinophils (P=0.003). The number of neutrophils and eosinophils and percentage representation in males' blood was higher than in females, while the opposite was found for lymphocytes and monocytes. A higher number of basophils was recorded in mares' blood, statistically significantly different from the number of basophils in males.

A statistically significant difference between the genders, regardless of work activity, was also observed in MPV (P=0.018). Mares had a higher average MPV compared to males (Table 2).

**Impact of Physical Activity on Biochemical Parameters**

The average values of biochemical parameters in the blood of draft horses indicate that the concentration of calcium (Ca) in the phases before and after exercise was statistically significantly higher than that of the same parameter after rest (Table 3).

In draft horses, a statistically significant difference between the genders was observed with the values of amylase (P<0.001) and creatinine (P=0.041). The higher amylase concentration was recorded in mares, while males had higher creatinine values (Table 3). No statistically significant interaction between the gender effect and the physical activity effect was confirmed.

Physical activity had a statistically significant effect (P=0.031) on blood glucose levels in sports horses, where the lowest values were recorded after activity (Table 4). The average values of blood biochemical parameters in sport horses showed statistically significant differences between the genders in the concentration of glucose (P=0.001), creatinine (P<0.001), bilirubin (P<0.001), and the ratio of BUN to creatinine (P=0.002). Among the mentioned parameters, no statistically significant interaction between the effect of activity and gender was confirmed. Sport mares had higher blood glucose values than males, while the opposite was found in the case of creatinine and bilirubin. Also, a higher ratio of BUN to creatinine was recorded in the blood of female individuals (Table 4).

## DISCUSSION

The results of hematological and biochemical blood parameters of draft and sport horses were within the reference values. The results of this research determined significant differences between individual hematological and biochemical parameters concerning the type of activity, breed, and gender of draft and sport horses.

**Table 2:** Hematological parameters in sport horses by gender, before, after exercise, and after rest.

| Sport Horses | Before physical activity | | After physical activity | | After rest | | Pool. SD | Physcial activity | Gender | Physical activityx Gennder |
|---|---|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | | | | |
| RBC ($\times10^{12}$/L) | 8.75$^{b}$ | 9.02$^{ab}$ | 10.35$^{a}$ | 9.98$^{ab}$ | 8.76$^{b}$ | 9.53$^{ab}$ | 1.64 | 0.041 | ns | ns |
| HCT (%) | 38.69$^{b}$ | 38.08$^{b}$ | 46.80$^{a}$ | 42.73$^{ab}$ | 38.80$^{b}$ | 40.56$^{ab}$ | 7.31 | 0.019 | ns | ns |
| HGB (g/dL) | 14.00$^{b}$ | 13.84$^{b}$ | 16.68$^{a}$ | 15.38$^{ab}$ | 14.08$^{b}$ | 14.68$^{ab}$ | 2.43 | 0.021 | ns | ns |
| MCV (fL) | 44.29$^{a}$ | 42.60$^{b}$ | 44.90$^{a}$ | 43.05$^{b}$ | 44.30$^{a}$ | 42.82$^{b}$ | 2.86 | ns | 0.028 | ns |
| MCH (pg) | 16.06$^{a}$ | 15.49$^{b}$ | 16.09$^{a}$ | 15.50$^{ab}$ | 16.12$^{a}$ | 15.49$^{b}$ | 0.97 | ns | 0.021 | ns |
| MCHC (g/dL) | 36.27 | 36.38 | 34.82 | 36.04 | 36.38 | 36.21 | 1.68 | ns | ns | ns |
| RDW (%) | 27.50$^{b}$ | 29.19$^{ab}$ | 28.831$^{ab}$ | 30.15$^{a}$ | 27.56$^{b}$ | 29.58$^{ab}$ | 2.51 | ns | 0.012 | ns |
| WBC ($\times10^{9}$/L) | 7.12 | 7.90 | 7.39 | 8.29 | 7.99 | 8.22 | 2.09 | ns | ns | ns |
| NEU (%) | 57.37$^{a}$ | 49.04$^{bc}$ | 55.57$^{ab}$ | 47.42$^{c}$ | 59.45$^{a}$ | 49.89$^{bc}$ | 7.83 | ns | <0.001 | ns |
| LYM (%) | 33.50$^{c}$ | 44.18$^{a}$ | 35.95$^{bc}$ | 44.10$^{a}$ | 31.40$^{c}$ | 41.07$^{ab}$ | 7.94 | ns | <0.001 | ns |
| MONO (%) | 5.030$^{bc}$ | 5.78$^{ab}$ | 4.68$^{c}$ | 5.74$^{ab}$ | 5.45$^{abc}$ | 5.88$^{a}$ | 0.93 | ns | 0.003 | ns |
| EOS (%) | 3.59$^{a}$ | 2.82$^{b}$ | 3.42$^{a}$ | 2.60$^{b}$ | 3.30$^{a}$ | 2.58$^{b}$ | 1.34 | ns | 0.003 | ns |
| BASO (% ) | 0.47 | 0.53 | 0.38 | 0.49 | 0.35 | 0.58 | 0.3 | ns | ns | ns |
| NEU ($\times10^{9}$/L) | 4.15 | 3.89 | 4.16 | 3.91 | 4.83 | 4.11 | 1.48 | ns | ns | ns |
| LYM ($\times10^{9}$/L) | 2.32$^{c}$ | 3.31$^{ab}$ | 2.61$^{bc}$ | 3.67$^{a}$ | 2.44$^{c}$ | 3.38$^{ab}$ | 0.88 | ns | <0.001 | ns |
| MON ($\times10^{9}$/L) | 0.36$^{b}$ | 0.43$^{ab}$ | 0.35$^{b}$ | 0.47$^{a}$ | 0.43$^{ab}$ | 0.48$^{a}$ | 0.12 | ns | 0.009 | ns |
| EOS ($\times10^{9}$/L) | 0.26 | 0.22 | 0.25 | 0.21 | 0.26 | 0.21 | 0.11 | ns | ns | ns |
| BASO ($\times10^{9}$/L) | 0.03$^{ab}$ | 0.04$^{ab}$ | 0.03$^{b}$ | 0.04$^{ab}$ | 0.03$^{b}$ | 0.05$^{a}$ | 0.02 | ns | 0.013 | ns |
| PLT ($\times10^{9}$/L) | 124.60 | 117.20 | 106.40 | 119.50 | 115.90 | 116.70 | 30.97 | ns | ns | ns |
| MPV(fL) | 11.80$^{ab}$ | 12.52$^{a}$ | 11.95$^{ab}$ | 12.44$^{a}$ | 11.54$^{b}$ | 12.0$^{ab}$ | 0.9 | ns | 0.018 | ns |

Results are expressed as mean, Pool.SD – pooled standard deviation. $^{abcd}$ values in the same row that do not contain the same letter differ significantly (P<0.05). ns - not significant.

**Table 3:** Biochemical parameters in draft horses by gender before, after exercise, and after rest.

| Draft Horses | Before physical activity | | After physical activity | | After rest | | Pool. SD | Physcial activity | Gender | Physical activityx Gennder |
|---|---|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | | | | |
| Glucose (mmol/L) | 4.54 | 4.94 | 4.75 | 4.45 | 4.76 | 5.07 | 0.84 | ns | ns | ns |
| Creatinine (µmol/L) | 109.00$^{a}$ | 95.90$^{b}$ | 113.30$^{a}$ | 96.60$^{b}$ | 111.00$^{a}$ | 94.10$^{b}$ | 28.47 | ns | 0.041 | ns |
| Urea (mmol/L) | 4.59 | 4.23 | 4.61 | 4.41 | 4.52 | 4.57 | 1.02 | ns | ns | ns |
| BUN/Urea | 11.30 | 11.30 | 11.22 | 11.60 | 11.00 | 12.50 | 4.03 | ns | ns | ns |
| P (mmol/L) | 0.98 | 1.16 | 0.99 | 1.16 | 0.95 | 0.93 | 0.22 | ns | ns | ns |
| Ca (mmol/L) | 2.99$^{a}$ | 2.91$^{ab}$ | 2.98$^{a}$ | 2.85$^{ab}$ | 2.53$^{b}$ | 2.66$^{ab}$ | 0.45 | 0.003 | ns | ns |
| TP (g/L) | 73.80 | 72.60 | 74.10 | 73.60 | 75.20 | 74.60 | 4.74 | ns | ns | ns |
| Albumin (g/L) | 28.80 | 28.20 | 29.20 | 29.50 | 29.20 | 29.20 | 1.96 | ns | ns | ns |
| Globulin (g/L) | 45.00 | 44.20 | 45.00 | 44.00 | 46.10 | 45.30 | 4.2 | ns | ns | ns |
| Album/Glob | 0.65 | 0.63 | 0.6 | 0.68 | 0.64 | 0.65 | 0.08 | ns | ns | ns |
| ALT (U/L) | 10.00 | 38.50 | 10.00 | 33.40 | 10.00 | 30.10 | 49.78 | ns | ns | ns |
| ALP (U/L) | 188.10 | 172.70 | 185.90 | 207.90 | 182.70 | 183.50 | 50.65 | ns | ns | ns |
| GGT (U/L) | 30.30 | 28.40 | 33.30 | 28.40 | 38.40 | 22.00 | 19.91 | ns | ns | ns |
| TBIL (µmol/L) | 17.60 | 16.70 | 19.10 | 18.00 | 19.80 | 17.90 | 6.55 | ns | ns | ns |
| Cholesterol (mmol/L) | 2.08 | 2.22 | 2.23 | 2.22 | 2.17 | 2.22 | 0.39 | ns | ns | ns |
| Amylase (U/L) | 25.30$^{b}$ | 32.90$^{a}$ | 32.56$^{a}$ | 35.00$^{a}$ | 27.40$^{b}$ | 34.60$^{a}$ | 5.3 | 0.028 | <0.001 | ns |
| Glucose (mmol/L) | 4.54 | 4.94 | 4.75 | 4.45 | 4.76 | 5.07 | 0.84 | ns | ns | ns |
| Creatinine (µmol/L) | 109.00$^{a}$ | 95.90$^{b}$ | 113.30$^{a}$ | 96.60$^{b}$ | 111.00$^{a}$ | 94.10$^{b}$ | 28.47 | ns | 0.041 | ns |
| Urea (mmol/L) | 4.59 | 4.23 | 4.61 | 4.41 | 4.52 | 4.57 | 1.02 | ns | ns | ns |
| BUN/Urea | 11.30 | 11.30 | 11.22 | 11.60 | 11.00 | 12.50 | 4.03 | ns | ns | ns |

Results are expressed as mean, Pool.SD – pooled standard deviation. $^{abcd}$ values in the same row that do not contain the same letter differ significantly (P<0.05). ns - not significant.

According to Rose (1982), a significant increase in WBC and neutrophils was observed after an intensive endurance test. Fear, stress, exhaustion, and high-intensity exercise, increase the release of cortisol, which causes leukocytosis with an increase in the number of mature segmented neutrophils, lymphopenia, and eosinopenia (Welles 2000; Bhutta et al. 2022). The influence of cortisol on the redistribution of leukocytes depending on the type and intensity of the activity has not been sufficiently investigated and requires further investigations (Maśko et al. 2021). Insignificantly higher values of WBC in males were published for Lipizzan horses (Čebulj-Kadunc et al. 2002). Satué et al. (2020) found statistically higher values of leukocytes and granulocytes in females of older age groups, probably because Spanish female thoroughbreds have slower maturation of leukocytes . The influence of age was not recorded in our study. Higher percentages of eosinophils recorded in males were the opposite of the results of Pađen et al. (2014), where higher values of eosinophils in mares are caused by staying on pasture and continuous infestations by different types of parasites. Compared to draft horses, sports horses have better aerobic capacity and performance. Exercise-induced aerobic capacity and increased RBC, HCT, and HGB were reported for Spanish thoroughbred horses (Muñoz et al. 1997). The different breed has influenced the hemogram values; Arabina horses have the significantly better aerobic capacity,

**Table 4:** Biochemical parameters in sports horses by gender, before, after exercise, after rest.

| Sport Horses | Before physical activity | | After Physical activity | | After rest | | Pool. SD | Physcial activity | Gender | Physical activityx Gennder |
|---|---|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | | | | |
| Glucose (mmol/L) | 4.79$^{b}$ | 5.24$^{ab}$ | 4.03$^{c}$ | 4.96$^{ab}$ | 4.72$^{bc}$ | 5.56$^{a}$ | 0.8 | 0.031 | 0.001 | ns |
| Creatinine (µmol/L) | 95.40$^{a}$ | 72.70$^{b}$ | 99.20$^{a}$ | 77.90$^{b}$ | 100.50$^{a}$ | 74.20$^{b}$ | 17.54 | ns | <0.001 | ns |
| Urea (mmol/L) | 4.94 | 4.83 | 5.10 | 4.87 | 5.0 | 4.49 | 0.8 | ns | ns | ns |
| BUN/Urea | 13.30$^{bc}$ | 16.90$^{a}$ | 13.30$^{bc}$ | 16.10$^{ab}$ | 12.90$^{c}$ | 15.10$^{abc}$ | 3.42 | ns | 0.002 | ns |
| P (mmol/L) | 1.01 | 1.08 | 1.07 | 1.09 | 0.99 | 1.11 | 0.19 | ns | ns | ns |
| Ca (mmol/L) | 3.04 | 3.01 | 2.96 | 3.02 | 2.97 | 2.94 | 0.32 | ns | ns | ns |
| TP (g/L) | 72.10 | 73.80 | 73.40 | 74.60 | 72.40 | 74.90 | 4.58 | ns | ns | ns |
| Albumin (g/L) | 29.60 | 29.60 | 30.30 | 30.60 | 29.70 | 30.60 | 1.49 | ns | ns | ns |
| Globulin (g/L) | 42.40 | 43.70 | 43.20 | 44.10 | 42.80 | 44.50 | 4.03 | ns | ns | ns |
| ALT (U/L) | 10.00 | 11.50 | 10.00 | 11.10 | 10.00 | 12.60 | 4.13 | ns | ns | ns |
| ALP (U/L) | 170.08 | 193.60 | 177.30 | 198.00 | 171.50 | 189.80 | 46.14 | ns | ns | ns |
| GGT(U/L) | 22.60 | 19.10 | 23.40 | 19.80 | 22.30 | 19.30 | 6.94 | ns | ns | ns |
| TBIL (µmol/L) | 21.30$^{a}$ | 14.80$^{b}$ | 23.70$^{a}$ | 15.30$^{b}$ | 22.60$^{a}$ | 16.10$^{b}$ | 5.66 | ns | <0.001 | ns |
| Cholesterol (mmol/L) | 1.97 | 1.92 | 2.03 | 1.97 | 1.98 | 1.960 | 0.3 | ns | ns | ns |
| Amylase (U/L) | 28.80 | 33.80 | 34.80 | 35.80 | 31.40 | 33.70 | 5.86 | ns | ns | ns |
| Glucose (mmol/L) | 4.79$^{b}$ | 5.24$^{ab}$ | 4.03$^{c}$ | 4.96$^{ab}$ | 4.72$^{bc}$ | 5.56$^{a}$ | 0.8 | 0.031 | 0.001 | ns |
| Creatinine (µmol/L) | 95.40$^{a}$ | 72.70$^{b}$ | 99.20$^{a}$ | 77.90$^{b}$ | 100.50$^{a}$ | 74.20$^{b}$ | 17.54 | ns | <0.001 | ns |
| Urea (mmol/L) | 4.94 | 4.83 | 5.10 | 4.87 | 5.0 | 4.49 | 0.8 | ns | ns | ns |
| BUN/Urea | 13.30$^{bc}$ | 16.90$^{a}$ | 13.30$^{bc}$ | 16.10$^{ab}$ | 12.90$^{c}$ | 15.10$^{abc}$ | 3.42 | ns | 0.002 | ns |
| P (mmol/L) | 1.01 | 1.08 | 1.07 | 1.09 | 0.99 | 1.11 | 0.19 | ns | ns | ns |

Results are expressed as mean, Pool.SD – pooled standard deviation. $^{abcd}$ values in the same row that do not contain the same letter differ significantly (P<0.05). ns - not significant.

enabling a better body supply of oxygen (Poškienė et al. 2021). In our study, sports horses belong to the Arabian breed, while draft horses belong to a mix of Bosnian mountain horses crossed with different cold-blooded breeds. The higher values of the erythrogram in stallion were confirmed (Andriichuk and Tkachenko 2017).

The slightly higher values of erythrogram in stallions are probably caused by the influence of androgenic hormones on erythropoiesis (Andriichuk and Tkachenko 2017). The significantly higher MPV in sports mares compared to males correlates with the statistically insignificant finding of Andriichuk and Tkachenko (2017). Intensive exercise activates blood coagulation and enhances blood fibrinolysis in horses (El-Sayed et al. 2005). Platelet activation is a physiological response of the hemostatic system to physical activity.The intensive horse training leads to increased platelet aggregation and that hypercoagulability in horses with pulmonary hemorrhages is associated with significant thrombocytosis after the race (Giordano et al. 2010). The significant hypocalcemia found in draft horses after rest was confirmed by Pourmohammad et al. (2019). Hypocalcemia after exercise can probably be caused in the following ways: binding calcium to the troponin molecule, lactates, phosphates, and plasma albumins, and restoring stored calcium ions within the sarcoplasmic reticulum (Vervuert et al. 2002; Dummont et al. 2012).

Physical activity has a variable influence on glucose concentration in horses' blood. In contrast to our survey, which showed a significantly lower glucose concentration in sport horses after physical activity, but according to Gonçalves et al. (2022), the same results were reported in sport horses at the beginning of the exercise. However, a mild increase in glycemia was recorded with higher exercise intensity and stress. The serum glucose concentration in horses is an indicator of the balance between the process of glycolysis in the activated muscles, and the process of hepatic glycogenolysis, the determination of the value of the serum concentration gives little information about the metabolism of carbohydrates during different types of physical exertion (Hodgson and McGowan 2014). Statistically significant differences between the genders in a concentration of amylase, creatinine, and bilirubin were reported in Croatian Posavina cold-blooded horses (Pađen et al. 2014) and Bosnian mountain horses (Rukavina et al. 2018). Adaptation of the Posavina horses, cold-blooded Croatian horses, to the severe and harsh conditions of the external environment and the management can affect the reference values of the examined hematological and biochemical parameters (Pađen et al. 2014). Breed, gender, age, differences in geographical and climatic management conditions, diet, purpose, various diagnostic reagents, methods, and instruments can affect the reference values of hematological and biochemical parameters in the Bosnian mountain horse. The mentioned factors led to the obtained values being significantly different from the reference values for other warm-blooded horse breeds (Rukavina et al. 2018). Our research results confirmed the findings of Pađen et al. (2014) and Rukavina et al. (2018) that hematological and biochemical parameters can be influenced by other factors such as gender and breed.

## Conclusion

Further research is needed to examine the influence of different factors to better understand the dynamics of the horse's homeostatic system during different types of physical activity. As long as there is an adequate evaluation, hematological and biochemical parameters can be helpful indicators of the general health status and performance of draft and sports horses.

## Acknowledgment

This research was funded by the Ministry Science, Higher Education, and Youth of Canton Sarajevo Bosnia and Herzegovina.

The authors acknowledge the support of Ćazim Crnkić, DVM Ph.D., Department of Animal Production and Biotechnology, Veterinary Faculty, University of Sarajevo Bosnia and Herzegovina for statistical analysis.

**Conflict of Interests**

The authors declare that they have no conflict of interest.

**Authors' Contribution**

Jasminka Isović: Conceived and designed the experiments, performed the experiments, analyzed, collected and interpreted the data. Wrote the manuscript. Denis Čamo: Contributed to the concept and design of the study, drafting the paper and critical revision of the manuscript. Ramiz Ćutuk: Planning and coordination of the whole study, analyzed and interpreted the data. Amir Zahirović: Planning and coordination of the whole study, drafting the manuscript and critical revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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