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Effect of exercise on some minerals, metabolites and enzyme activities in the serum of trained Arabian horses

Roya POURMOHAMMAD, Mehrdad MOHRI*, Hesam SEIFI, Kamran SARDARI
Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

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Abstract: The exercise-induced variations of some biochemical parameters including calcium (Ca), phosphorous (P), iron (Fe), copper (Cu), manganese (Mn), selenium (Se), zinc (Zn), sodium (Na), potassium (K), chlorine (Cl), glucose, urea, creatinine, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK) were assessed in the serum of 25 Arabian horses. Blood samples were taken from the jugular vein 3 different times: before exercise, 5 h after exercise, and 18 h after exercise. Statistical analysis was performed using SAS (version 9.2; SAS Institute, Cary, NC, USA). Ca, Cl, glucose, and urea showed significant ($P < 0.05$) changes due to exercise. There was significant increase from before exercise to 5 h after exercise in urea concentrations while Ca and Cl concentrations significantly decreased. A significant ascending trend for Ca between 5 h and 18 h after exercise was also seen, while glucose and urea had a significantly descending trend. No significant time-based variations were observed for other variables. The results of the present study indicated that exercise could affect the value of some biochemical parameters, which could be used in future studies evaluating the health status of Arabian horses.

Key words: Arabian horse, trace minerals, electrolytes, biochemical parameters, exercise, dry climate

1. Introduction

Performance reduction in horses during exercise is mostly due to changes in biochemical parameters and electrolytes [1]. In order to optimize the training program of race horses, it is required to measure the biomedical and sport physiological parameters. It should be noted that biochemical alterations can be created by different types of exercises because they can show changes in the activity of different systems as well as the energy utilization [2,3].

Trace minerals are imperative parts of appropriate nutrition in horses. Selenium is one of the most important trace minerals which is responsible for the integrity of immune system and muscles. It was reported that horses with lower values of Se showed weaker performance. Copper (Cu) and zinc (Zn) are also necessary for superoxide dismutase (SOD) function, which is part of the antioxidant system. The role of iron (Fe), Zn, and Cu in intense exercise is a challenging topic. However, these trace elements regulate the physiological functions and they can potentially affect the level of performance in horses [4].

Glucose and electrolytes are other important factors to be considered in performance evaluation of horses. A balance is needed between production and consumption of energy to reach a high performance in horses. Shortage or abundance in the energy uptake from the diet can have negative effects on the performance of horses. The health of race horses depends on the analysis of energetic precursors such as glucose [5].

The function of electrolytes in horses is to maintain the osmotic pressure and nerve/muscle activities. It was seen that electrolytes were lost during endurance competitions depending on environmental parameters such as temperature, moisture, type of terrain, etc. [6,7].

During exercise, large amounts of fluid and electrolytes are lost due to sweating. If this condition prolongs, it can lead to imbalance in electrolyte concentrations in the serum. In the worst condition, it causes dehydration, which finally leads to weak performance of horses. Electrolytes such as sodium (Na), chlorine (Cl), potassium (K), phosphorous (P), and calcium (Ca) play important roles in physiological activities as well as muscle contraction. Although any kind of imbalance in these parameters along with the increase of blood lactate concentration can have negative effects on the performance of race horses, challenging and controversial results were reported in the literature in this regard [8,9]. Ca is needed for muscle contraction in endurance horses. It was observed that hypocalcemia led to metabolic failure during exercise and a

* Correspondence: mohri@um.ac.ir

reduction in ionized plasma Ca in show jumper horses was reported after exercise [10]. An increase in the frequency of synchronous diaphragmatic flutter was reported due to hypercalcemia during competition [11].

Biochemical variables which are associated with health status can act as performance indicators of race horses. Urea and creatinine are commonly used for the evaluation of kidney function. The concentrations of these two factors may be affected by dehydration and exercise [12].

Other biochemical parameters such as cytoplasmic enzymes are also utilized in the assessment of muscle injury during exercise. Among these enzymes, creatine kinase (CK) is the most specific muscle enzyme. An increase in the serum activity of CK and lactate dehydrogenase (LDH) after severe exercise was observed in healthy horses, which might be due to an increase in skeletal muscle membrane leakage. An increase in the plasma activity of CK and aspartate aminotransferase (AST) after exercise was also observed even without any clinical sign or change in the structure of the muscle cells. The values of enzyme activities can increase due to a change in the membrane permeability, increase in enzyme synthesis, and poor clearance of enzyme [13].

Since all of the studied horses are kept in Yazd with a hot and dry climate, the environmental conditions may affect the thermoregulation and heat loss [14]. Therefore, the impact of climate was also taken into consideration in the current study.

The aim of this study was to evaluate the alteration of some serum biochemical parameters, trace minerals, and electrolytes after the training of horses in a hot and dry climate for different durations and to analyze the impact of these alterations on the performance of the horses. It is assumed that there is a correlation between the change in serum biochemical parameters and exercise that will help trainers to assess the health status of their horses as well as help them set better training schedules.

2. Materials and methods

2.1. Horses and training

In the current research, a total of 25 Arabian race horses (eight mares and seventeen stallions) from the same training center located in the Iranian city of Yazd were studied. Yazd is located at the center of Iran with a hot and dry climate. The mean and median ages of the horses were 6.8 and 5 years, respectively. The mean, median, minimum, and maximum ages for mares were 6.1, 4, 2, and 12 and for stallions they were 7.1, 6.5, 2, and 20, respectively. The healthiness of the horses was previously confirmed by physical examinations by a local vet. All horses were dewormed and vaccinated in a similar program. The mares were not pregnant during the study. All animals were housed in the same stable in individual boxes under natural summer photoperiod (sunrise at 05:00 hours, sunset at 19:00 hours) and 22–25 °C indoor temperature. Diets were formulated using the 1989 National Research Council (NRC) tables of nutrient requirements and recommendations for horses. The ingredient composition of the diet consisted of dry alfalfa hay (about 1.5% of body weight) and concentrates (about 1% of body weight). The concentrate included a mixture of barley, corn, wheat, molasses, and vitamin, mineral, and amino acid supplements. The chemical composition of the concentrate was designed as follows: crude protein, 13%; energy, 1300 kcal/kg; Zn, 0.4%; Cu, 0.15%; Fe, 0.35%; manganese (Mn), 0.42%; Se, 0.0015%; cobalt, 0.027%; chrome, 0.0008%; antioxidants, 0.013%; Ca, 1.22%; lysine, 0.88%; methionine, 1.35%; L-carnitine, 0.33%; vitamin A, 1,000,000 IU/kg; vitamin D3, 250,000 IU/kg; vitamin E, 20,000 mg/kg; vitamin K, 6500 mg/kg; vitamin B1, 6500 mg/kg; vitamin B2, 5000 mg/kg; vitamin B3, 20,000 mg/kg; vitamin B5, 25,000 mg/kg; vitamin B6, 10,000 mg/kg; vitamin B9, 2000 mg/kg; vitamin B12, 6 mg/kg; and vitamin C, 65,000 mg/kg. Salt and water were available ad libitum.

All horses underwent training 6 days per week with a rest day on Saturday. Training started at 06:00 hours every day and lasted for 1.5 h. All horses participated in the same daily training program, which included warming up (20 min), walking (1 h), trotting, and galloping (10 min). According to the performance, individual abilities, and the age of each horse, the trainer may have slightly changed the standard procedure for some horses. The intensity and duration of training were approximately similar for all of the horses.

2.2. Blood samples

All of the procedures and the experiments were done with respect to the ethical standards in the 1975 Declaration of Helsinki, which was revised in 2000 and 2008, and the study was approved by national law as well (ethical permission no: 3/41419).

Blood was taken from the jugular vein of each horse 3 times: immediately before exercise, 5 h after exercise, and 18 h after exercise. Sampling was done within 3 days and all the measurements were obtained in less than 1 month. The blood was taken into tubes without anticoagulant and centrifuged at 1800 × *g* for 15 min; the serum was separated within 30 min after collection. All samples were refrigerated at –80 °C within 2 h after being collected. The serum was used to measure Ca, P, Fe, Cu, Mn, Se, Zn, Na, K, Cl, glucose, urea, and creatinine concentrations along with LDH, AST, alanine aminotransferase (ALT), and CK activities.

Serum Se, Mn, Fe, Zn, and Cu were measured by ICP-OES (SPECTRO ARCOS, Germany). Extra pure water (18.2 MΩ cm^{-1} water) was used as a blank. Prior to

digestion, the frozen samples were allowed to melt and then were vortexed to become a homogeneous matrix. Each sample was immediately pipetted thoroughly to avoid settling and then 500 µL of sample was transmitted into an acid-washed glass tube. Then 600 µL of Suprapur nitric acid (65%, HNO_3, Merck), 400 µL of concentrated hydrochloric acid (HCl, Merck), and 200 µL of hydrogen peroxide (30%, H_2O_2, Merck) were added to each sample and 18.2 MΩ cm^{-1} water was added to provide a final volume of 6 mL. Tubes were placed in a water bath at 70 °C for 5 min and centrifuged at 9000 rpm for 10 min. Supernatant was harvested and analyzed immediately. The detection limit for all measured trace elements was 1 µg/L and the average accuracy was 95%.

Ca, P, urea, creatinine, glucose, LDH, AST, ALT, and CK values were measured using Pars Azmoon commercial kits (Tehran, Iran) with an autoanalyzer (Mindry, BS-200E, Shenzhen, China). Na, K, and Cl concentrations were measured by ion-selective electrode (STARLYTE III, Alfa Wassermann, the Netherlands)

2.3. Statistical analysis

Statistical analysis was performed using SAS (version 9.2; SAS Institute, Cary, NC, USA). Because serum metabolites were measured over time, a repeated measure of ANOVA using PROC MIXED was used. All outcome variables were screened for normality by visual assessment of the distributions and calculation of kurtosis and skewness. The distributions of Ca, P, Se, Mn, Zn, K, glucose, and LDH were normal. Serum concentrations of Cu, Fe, Na, Cl, urea, creatinine, ALT, AST, and CK were skewed to the right. For Fe distribution, a logarithmic transformation was done to achieve a normal distribution. The distributions of urea, creatinine, and CK were transformed to square root to get a normal distribution. Inverse transformation was performed for concentrations and activities of Cu, Na, Cl, ALT, and AST to achieve normal distribution. Variables considered in the model include time of sampling (immediately before exercise, 5 h and 18 h after exercise), sex (stallion and mare), and age (younger than 5 years old as age group 1 and older than 5 years old as age group 2). All variables were offered to the model and then removed in a backward stepwise elimination approach. Interactions between intervention and the significant covariates were tested and included in the general model if significant. The horses were considered as a random effect and error term. The mathematical model of the statistical analysis is defined by the following equation:

$$Y_{ijkm} = \mu + T_i + A_j + G_k + TA_{ij} + TG_{ik} + H_m + e_{ijkm}$$

Here I, j and k subscripts are the time of sampling, age, and sex as independent variables, respectively, and subscript m refers to individual horses. Y_{ijkm} is the output variable depending on µ (overall mean), T_i (time of sampling effect: i = 1 for immediately before exercise, i = 2 for 5 h after exercise and i = 3 for 18 h after exercise), A_j (age effect: j = 1 for horses younger than 5 years old and j = 2 for horses older than 5 years old), G_k (effect of sex: k = 1 for stallion and k = 2 for mare), TA_{ij} (interaction between time and age), TG_{ik} (interaction between time and sex), H_m (random horse effect), and e_{ijkm} (random error term).

All values were reported as least squares means and standard errors (SEs). In all analysis the value of $P \leq 0.05$ was considered as significant.

3. Results

All results are reported in Tables 1–4, respectively.

It can be inferred from these tables that urea, glucose, Ca, and Cl have shown significant changes over time due to exercise. The variations of least square mean ± standard error (LSM ± SE) of these parameters with respect to time are shown in Figures 1–4, respectively.

There was a significant increase from before exercise to 5 h after exercise in urea values, while Ca and Cl concentrations significantly decreased in the same time intervals. A meaningful ascending trend was also observed for Ca between 5 h after exercise and 18 h after exercise, whereas the trends for glucose and urea meaningfully descended in the same time intervals. No significant changes were observed for other variables.

Considering the effects of age and sex (as reported in Tables 2 and 3), it can be inferred that only age has a significant effect on Cl concentration. The means and standard errors of Cl concentrations were calculated as 97.18 ± 1.12 and 102.11 ± 0.95 in horses aged below and above 5 years, respectively ($P = 0.0008$). Since both time and age have significant effects on Cl concentrations, their interacting effects are analyzed and reported in Table 4. Based on these results, it can be deduced that younger horses have much stronger effects on the significant variations of Cl concentrations during exercise.

4. Discussion

The aim of this study was to investigate whether training of Arabian horses could change some serum biochemical variables, trace elements, and electrolytes before and after exercise or not. Researchers suggested that exercise in horses could change some biochemical parameters of serum that were related to different factors such as individual differences, breed, and the level of exercise [12,15,16].

A significant decrease in Ca concentration has been observed in the current research ($P \leq 0.05$). Binding of ionized Ca^{2+} to lactate, ionized phosphate, or plasma albumin may be due to such a decrease. In addition, the ionized Ca tends to flow into working muscle in order to restore Ca within the sarcoplasmic reticulum, which could be another reason

Table 1. The concentrations of measured variables (LSM ± SE) in the serum of Arabian horses before and after exercise.

Parameter	Unit	Before exercise	5 h after exercise	18 h after exercise	P-value
Ca	mg/dL	13.53 ± 0.17[a]	12.15 ± 0.18[b]	13.54 ± 0.17[a]	0.007
P	mg/dL	4.00 ± 0.17	3.85 ± 0.17	3.97 ± 0.17	0.776
Cu	mg/L	0.90 ± 0.09	0.97 ± 0.10	0.90 ± 0.09	0.745
Fe	mg/L	2.29 ± 0.30	2.34 ± 0.29	2.08 ± 0.26	0.971
Mn	ppb	15.05 ± 1.38	13.43 ± 1.38	13.36 ± 1.38	0.453
Se	ppb	73.32 ± 6.42	71.15 ± 6.39	63.25 ± 6.39	0.111
Zn	mg/L	0.95 ± 0.05	0.99 ± 0.05	0.97 ± 0.05	0.356
Na	mEq/L	140.7 ± 12.1	140.1 ± 12.3	139.1 ± 12.1	0.463
K	mEq/L	3.69 ± 0.15	3.84 ± 0.15	3.83 ± 0.15	0.580
Cl	mEq/L	99.90 ± 0.96[a]	97.65 ± 0.87[b]	98.04 ± 0.93[ab]	0.031
Glucose	mg/dL	76.30 ± 4.9[ab]	85.14 ± 4.9[a]	70.22 ± 4.9[b]	0.031
Urea	mg/dL	32.50 ± 1.43[a]	35.81 ± 1.29[b]	32.82 ± 1.42[a]	0.016
Creatinine	mg/dL	1.58 ± 0.07	1.66 ± 0.07	1.60 ± 0.07	0.361
LDH	U/L	649.4 ± 36.8	693.1 ± 36.8	637.9 ± 36.8	0.305
ALT	U/L	10.25 ± 0.82	11.38 ± 1.02	11.22 ± 0.99	0.432
AST	U/L	267.4 ± 16.2	291.4 ± 19.4	279.8 ± 17.8	0.524
CK	U/L	467.8 ± 21.4	508.9 ± 33.2	479.8 ± 19.7	0.752

In each row, values with different superscripts are significantly different (P ≤ 0.05).

Table 2. The concentrations of measured variables (LSM ± SE, min and max) in the serum of Arabian horses for different age groups.

Parameter	Unit	≥5 years old			>5 years old			P-value
		LSM ± SE	min	max	LSM ± SE	min	max	
Ca	mg/dL	12.91 ± 0.17	11.1	13.9	13.23 ± 0.17	11.8	14.1	0.1
P	mg/dL	3.95 ± 0.17	2.9	4.7	3.93 ± 0.17	3.0	4.9	0.6
Cu	mg/L	0.95 ± 0.1	0.57	1.43	0.87 ± 0.08	0.41	1.53	0.4
Fe	mg/L	2.26 ± 0.3	1.04	5.12	2.21 ± 0.29	1.23	4.8	0.83
Mn	ppb	13.4 ± 1.34	8.3	19.2	14.4 ± 1.41	9.4	20.8	0.25
Se	ppb	62.42 ± 6.21	41.2	102.5	72.40 ± 7.1	41.4	104.7	0.13
Zn	mg/L	1.00 ± 0.06	0.67	1.21	0.94 ± 0.04	0.73	1.14	0.37
Na	mEq/L	139.26 ± 9.2	111	167	140.67 ± 8.9	116	168	0.58
K	mEq/L	3.80 ± 0.14	2.9	4.7	3.77 ± 0.16	3.1	5.1	0.94
Cl	mEq/L	97.18 ± 1.1[a]	92	103	102.1 ± 0.95[b]	97	106	0.0008
Glucose	mg/dL	79.18 ± 5	59	122	75.26 ± 5	48	93	0.25
Urea	mg/dL	33.00 ± 1.71	25	42	34.42 ± 1.1	27	42	0.95
Creatinine	mg/dL	1.59 ± 0.06	1.3	2	1.63 ± 0.08	1.2	2.2	0.75
LDH	U/L	644.8 ± 36	389	920	675.5 ± 37	490	972	0.80
ALT	U/L	10.72 ± 1.03	6	18	11.18 ± 0.9	6	18	0.47
AST	U/L	274.2 ± 16.2	182	382	284.9 ± 19.4	178	415	0.86
CK	U/L	500.9 ± 29.2	375	757	500.9 ± 29.2	398	613	0.31

In each row, values with different superscripts are significantly different (P ≤ 0.05).

Table 3. The concentrations of measured variables (LSM ± SE) in the serum of Arabian horses for different sexes.

Parameter	Unit	Stallion	Mare	P-value
Ca	mg/dL	12.99 ± 0.18	13.15 ± 0.16	0.85
P	mg/dL	3.72 ± 0.17	4.16 ± 0.17	0.2
Cu	mg/L	0.97 ± 0.09	0.88 ± 0.11	0.15
Fe	mg/L	2.31 ± 0.29	2.16 ± 0.29	0.4
Mn	ppb	13.91 ± 1.26	13.98 ± 1.5	0.46
Se	ppb	68.69 ± 6.67	69.79 ± 6.11	0.75
Zn	mg/L	0.96 ± 0.05	0.98 ± 0.05	0.82
Na	mEq/L	140.6 ± 11.8	139.3 ± 13.2	0.54
K	mEq/L	3.85 ± 0.13	3.72 ± 0.16	0.63
Cl	mEq/L	99.3 ± 1.1	97.76 ± 0.95	0.73
Glucose	mg/dL	76.22 ± 4.8	78.22 ± 5.1	0.98
Urea	mg/dL	32.78 ± 1.40	34.64 ± 1.32	0.29
Creatinine	mg/dL	1.6 ± 0.07	1.63 ± 0.07	0.58
LDH	U/L	611.7 ± 28.7	708.6 ± 45.3	0.1
ALT	U/L	10.61 ± 0.75	11.3 ± 1.32	0.13
AST	U/L	254.1 ± 16.2	304.9 ± 19.4	0.2
CK	U/L	440.5 ± 22.2	529.5 ± 31.4	0.09

Table 4. Time × age interaction analysis for Cl concentrations (LSM ± SE values).

Time	Before exercise		5 h after exercise		18 h after exercise	
Age	5 years old≥	>5 years old	5 years old≥	>5 years old	5 years old≥	>5 years old
Mean Cl concentration	95.85 ± 1.13	101.75 ± 0.92	99.78 ± 1.06	102.91 ± 0.86	96.32 ± 1.11	102.06 ± 0.89

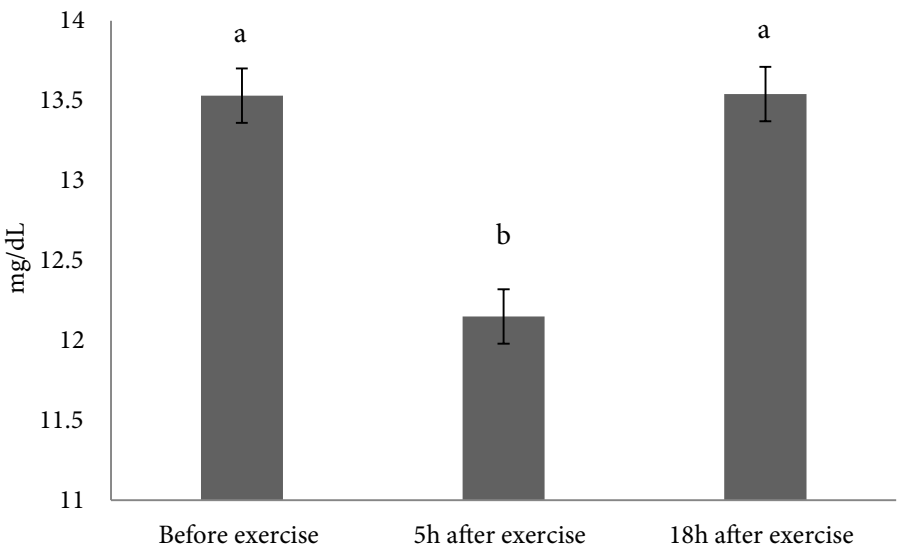

Figure 1. Ca values (LSM ± SE) in the serum of Arabian horses before and after exercise.

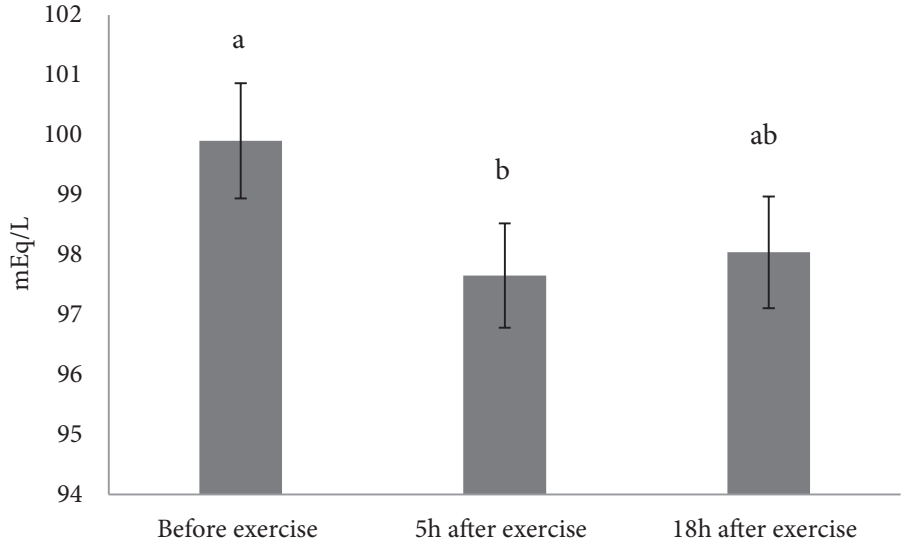

Figure 2. Cl values (LSM ± SE) in the serum of Arabian horses before and after exercise

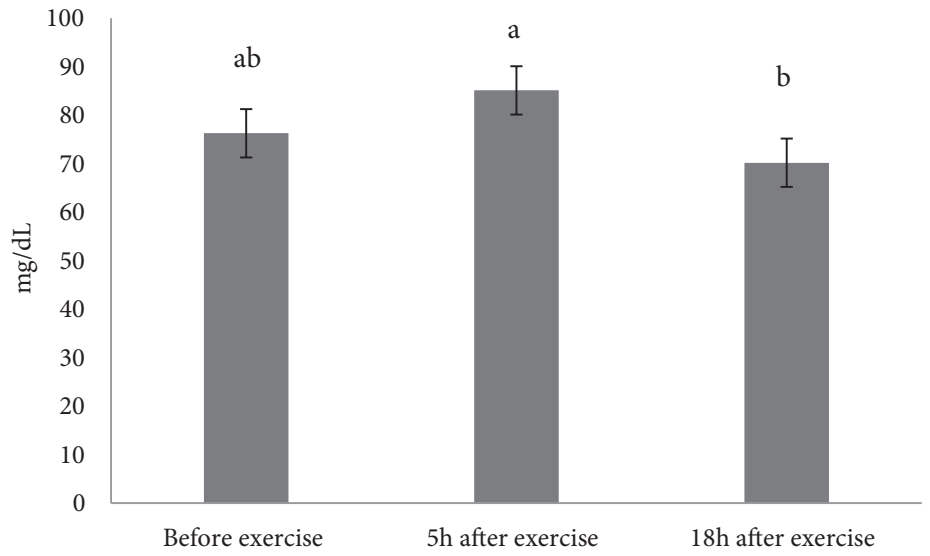

Figure 3. Glucose values (LSM ± SE) in the serum of Arabian horses before and after exercise.

for hypocalcemia [17]. Other researchers claimed that hypocalcemia might occur due to the persistent activity of calcitonin and/or Ca loss due to sweating [18]. The study performed by Grimston et al. in humans showed that exercise-induced elevation of calcitonin was not due to hemoconcentration; rather, it occurred as a result of secretion stimulation by hypercalcemia [19]. Additionally, Alosia et al. [20] reported that, in humans, by increase in the concentration of calcitonin, hemoconcentration-induced hypercalcemia was somewhat suppressed. Inoue et al. [21] stated that loss of Ca as a result of sweating in horses during exercise was significant and could neutralize hemoconcentration-induced increase in Ca. In a study performed by Kanungo et al. [22], no meaningful exercise-induced changes in Ca concentration were observed.

The values of Cu, Fe, Mn, Se, and Zn did not show any significant variations in the current study. It was suggested that the amount of Zn in the diet could affect the value of plasma Zn [21]. In the present study, the mean value of Zn insignificantly increased. Hemoconcentration can cause a temporary elevation in serum Cu during exercise [21], but in the current study, the serum Cu did not show any significant increase. In the current study, an increase in Fe after exercise was observed, but it was not significant. Hemolysis and increase in the hepatic blood flow can elevate Fe concentration during exercise [23].

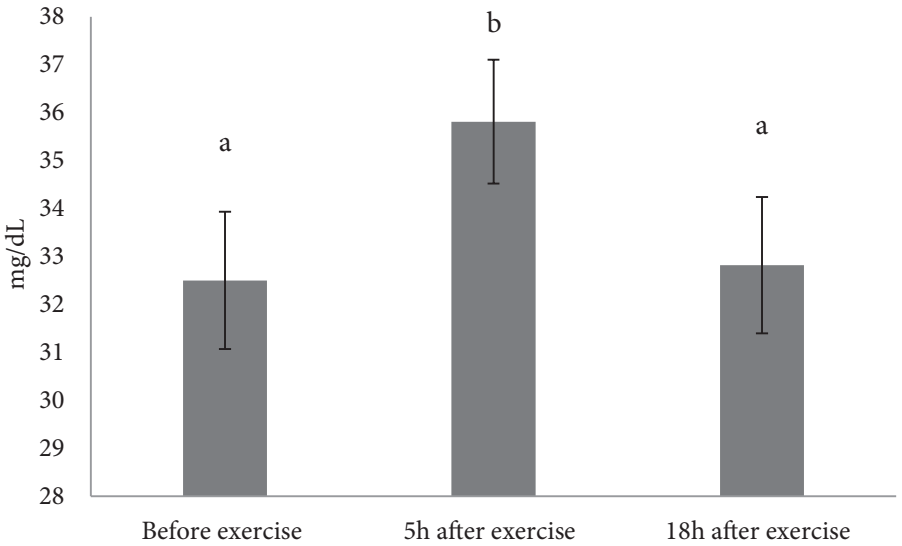

Figure 4. Urea values (LSM ± SE) in the serum of Arabian horses before and after exercise.

The means of serum Na and K concentrations did not show any significant changes. However, the mean value of Cl significantly decreased after exercise. A considerable loss of Cl ions was detected as a result of sweating [24]. In a previous study by Kanungu et al. [22], Cl values were observed to be low up to 4 h after exercise, which was similar to the results of this research. However, there was no explanation for this change.

The variation of Na and K in Kanungo's work was reported to be nonsignificant, which was similar to the observations of the this study. Soliman and Nadim [24] suggested a mild decrease in Na and a significant reduction in K values after severe exercise, which can be related to sampling time. The Na values may increase, decrease, or remain unchanged based on environmental conditions and the duration of endurance competition [17]. The results of this study concerning the changes in K after exercise were in accordance with those of previous studies [25]. The elevation of serum K is related to acid/base status; however, the status of acid/base was not assessed in this study. In contrast to the present work, no significant change of Cl concentration was reported by Robert et al. [26] after two months of low-intensity exercise.

Considering the effect of a hot climate (similar to this study), Backhouse [16] suggested that horses competing in hot environments might be subjected to biochemical disturbances that can lead to weaker performance of the horses. The same study [16] suggested that electrolyte loss without replacement from the diet is the main cause of poor performance in the horses. Based on that study, chloride is deeply concentrated in sweat and prolonged sweating in hot climates causes hypochloremia, which is similar to the findings of the current study.

In this study, the glucose values increased up to 5 h after exercise and then decreased from 5 h to 18 h after exercise. Controversial results are reported for the exercise-induced variations of glucose concentration, but the general trend is that there is no considerable variation in glucose amounts 3–6 days after exercise [27]. One reason for these controversial results in humans is the reduction in body fat, which causes long-term and indirect effects of exercise on glucose metabolism. Another justification for these differences is based on the residual effect of the last training session. It has also been shown that an acute and considerable effect of exercise is the elevation of glucose metabolism up to several hours after exercise [28,29]. The change in glucose values in horses during exercise strongly depends on the intensity and duration of the exercise. The increase in the outlet of glucose after exercise is caused by the decrease in the insulin/glucagon ratio. High-performing horses are more capable of utilizing glucose than poor-performing ones [30]. The increase in glucose may be correlated with hyperactivity of the sympathetic system and activation of hepatic glycogenolysis [16]. In the work done by Kanungo et al. [22], a significant reduction in glucose values was reported immediately after exercise, which was due to glucose utilization. Those researchers mentioned that the glucose values increased between 4 and 8 h after exercise because of reduced stress and enough resting time.

In this study, the concentration of urea was significantly increased up to 5 h after exercise and then decreased from 5 h to 18 h after exercise. No significant change was seen in serum creatinine values. Increases in urea may be related to prerenal factors as well as hemoconcentration.

Therefore, the increase of urea concentration may result from increased protein catabolism for energy production that is essential for muscle contraction. Rose et al. [23] observed no exercise-induced change in urea levels of horses after 3-day events. The work published by Fillipo et al. [31] also showed significant increases in urea and creatinine in mares after a marcha gait competition.

No significant changes were observed in serum enzyme activities including CK, LDH, AST, and ALT in the present study. The change in enzyme activities is related to parameters such as duration of exercise, intensity of exercise, and the physical condition of each animal. When horses are adapted to the exercise, the level of serum enzymes remains stabilized [31].

In conclusion, in order to assess the fitness of sport horses, it is necessary to take equine exercise physiology science into consideration. Since physiological and biochemical parameters depend on the performance, it is required to evaluate the changes in values of these parameters before and after training programs. The presented data can be used to evaluate the response of some biochemical parameters, electrolytes, and trace minerals caused by exercise to further study the health and performance of Arabian horses.

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