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Research Article

Physical, Haematological and Biochemical Responses of Arabian Horses to Jereed (Javelin Swarm) Competition [1]

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Abstract

The aim of this study is to evaluate physical, haematological and biochemical changes in horses after acute exercise in a jereed game. Jereed is a traditional Turkish equestrian team sport. A total of 14 horses were included in this study. All horses were Arabian stallions aged between 4-14 years old. Respiration rate, heart rate, capillary refill time, mucous membrane and skin turgor (as an indicator for dehydration) were measured and blood samples were collected before the exercise (T0), immediately after first period (T1) and second period (T2), after 30 min (T3) and 60 min of recovery time (T4). Blood samples were analyzed for total cell counts and for determination of lactate, glucose, urea, total protein, albumin, globulin, calcium, magnesium, phosphorous, chloride, sodium, potassium, creatinine kinase, lactate dehydrogenase, aspartate aminotransferase and alkaline phospahatase. All parameters were analyzed using Linear Mixed effect model to evaluate changes of repeated measurements at 5 different time points and LSD was used as post-hoc test. Exercise caused significant increases in heart rate ($P<0.001$), respiratory rate ($P<0.001$), capillary refill time ($P<0.001$), mucous membrane colour ($P<0.001$), dehydration ($P<0.001$), red blood cells ($P<0.001$), hemoglobin ($P<0.001$), hematocrit ($P<0.001$), red cell dispersion width ($P<0.001$), total white blood cells ($P<0.001$), neutrophils ($P<0.001$), and basophils ($P<0.05$). It also caused significant increases in lactate ($P<0.01$), glucose ($P<0.001$), urea ($P<0.001$), total protein ($P<0.001$), albumin ($P<0.001$), globulin ($P<0.001$), sodium ($P<0.001$), potassium ($P<0.001$), creatinine kinase ($P<0.05$), lactate dehydrogenase ($P<0.001$), aspartate aminotransferase ($P<0.001$) and alkaline phosphatase ($P<0.001$) but a decrease in calcium ($P<0.01$), magnesium ($P<0.01$), phosphorous ($P<0.05$), and chloride ($P<0.001$). Although majority did not return to the baseline 30 or 60 minutes after competition ($P<0.05$) most were within or near to the reference range values.

Keywords: *Jeered, Horse, Exercise, Haematology, Biochemistry*

Cirit Müsabakalarının Arap Atlarında Oluşturduğu Fiziksel, Hematolojik ve Biyokimyasal Değişiklikler

Öz

Bu çalışmanın amacı cirit müsabakalarının atlarda oluşturduğu fiziksel, hematolojik ve biyokimyasal değişiklikleri incelemektir. Cirit, Türklerin oynadığı geleneksel bir atlı spor müsabakasıdır. Bu çalışmada, yaşları 4-14 arasında değişen, 14 adet Arap aygırı kullanıldı. İstirahat halinde (T0), müsabakanın hemen ilk (T1) ve ikinci devrelerinin sonunda (T2) ve müsabaka tamamlandıktan 30 dak. (T3) ve 60 dak. (T4) sonra atların kalp atım ve soluk sayıları, kapillar dolum zamanı, mukoz membran rengi, deri elastikiyeti ölçüldü ve intravenöz yolla kan örnekleri toplandı. Kan örneklerinden tam kan sayımı ve biyokimyasal olarak laktat, glikoz, üre, total protein, albumin, globulin, kalsiyum, magnezyum fosfor, klor, sodium, potasyum, kreatin kinaz, laktat dehidrogenaz, aspartat aminotransferaz, alkalin fosfataz analizleri yapıldı. 5 farklı zamanda tekrarlanan ölçümleri değerlendirmek için Linear Karışık etki modeli ve post-hoc test olarakta LSD kullanıldı. Atlı cirit müsabakası atların kalp atım ve soluk sayılarında ($P<0.001$), kapillar dolum zamanında ($P<0.001$), mukoz membran rengi ($P<0.001$), dehidrasyon ($P<0.001$), eritrosit sayısı ($P<0.001$), hemoglobin ($P<0.001$), hematokrit ($P<0.001$), toplam lökosit sayısı ($P<0.001$), nötrofil ($P<0.001$) ve bazofil ($P<0.05$) sayılarında artışa sebep olmuştur. Ayrıca, atların laktat ($P<0.01$), glikoz ($P<0.001$), üre ($P<0.001$), total protein ($P<0.001$), albumin ($P<0.001$), globulin ($P<0.001$), sodium ($P<0.001$), potasyum ($P<0.001$), kreatin kinaz ($P<0.05$), laktat dehidrogenaz ($P<0.001$), aspartate aminotransferaz ($P<0.001$), alkalin fosfataz ($P<0.001$) değerlerinde artış olmuştur. Kalsiyum ($P<0.01$), magnezyum ($P<0.01$), fosfor ($P<0.05$), klor ($P<0.001$) değerlerinde ise azalma oluşmuştur. Ölçülen parametrelerin büyük bir kısmı müsabaka bittikten 30 veya 60 dak. sonra başlangıç değerlerine geri dönemese de ($P<0.05$) referans aralığına çok yakın değerlere ulaştığı tespit edilmiştir.

Anahtar sözcükler: *Cirit, At, Egzersiz, Hematoloji, Biyokimya*

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INTRODUCTION

Physiological responses to exercise differ according to the type of exercise being performed. Equestrian sports vary in physiological demand depending on the speed and duration of the exercise bout. The physiological responses associated with exercise within equestrian sports also vary.

Exercise has many variable effects on physical, haematological and biochemical parameters of horses [1]. Some of these changes have been studied in equestrian sports such as flat racing [2-4], endurance [5-7], show jumping [8,9], eventing [10], reining [11], marcha [12], cavalcade [13], and polo [14,15]. However, to the best to our knowledge, there is no such a study focused on jereed horses.

Jereed is a traditional Turkish equestrian team sport played outdoors on horseback. It has been played by Turks for many centuries, dating back to the days of the Turkish states. The game were used to be played with native horse breeds of Anatolian Native, Kolu Kisa of Hinis, Turkish Arab, and East Anatolia which were raised for jereed game in old times [16,17]. Arabian horses, especially male ones which have not been involved in flat racing, have been preferred since the 1980s [18]. Apart from Turkey, jereed is also played in many Asian, Arabian, and Eastern European countries such as Iran, Afghanistan, Turkmenistan, *Kyrgyzstan*, Indonesia, and Hungary [19].

The game is held outdoors on a sandy pitch that is 140±10 m long by 40±5 m wide and played by two teams of 7 players. Each team has 3 reserve horses and players. The game lasts around one and half hours and is divided into 2 periods of 40 min with an interval of 10 min between them. All horses and players play in both periods if not injured or excluded. The jereed game requires an effort of constantly alternating intensities between high and low exercise. Horses do hard galloping with sudden pauses, high speed startups, and sudden changes of directions during the game. The speed and maneuver is determinative factor of game [20,21].

The aim of this study is to evaluate the response of physical, haematological and biochemical parameters after each period and during the recovery times. This study will be the first report of physiological and biochemical changes observed in horses during jereed competition. This study is noteworthy since it will serve as a basis of development of national training of jereed horses as well as jereed competition. Furthermore, these findings will make reference values for horses in jereed competition and help veterinarians to better judge the metabolic and electrolyte disturbances of horses during this competition.

MATERIAL and METHODS

The study received ethical approval from the local ethic committee of animal experiments in Kocaeli University (No: 2017-22). The study was performed in Turkey (Çayırova, Kocaeli, N40° 47′ 59′, E 29° 25′ 0′) in July 2017 during official national jeered competition for Kocaeli provience held by Turkish Federation of Traditional Sports. Games were played according to the national jereed rules. The mean temperature, humidity and wind were 29°C, 31%, 9 km/sn (from North West), respectively (Data supplied from Turkish Regional Meterological Service website). All seven horses on both teams were studied. A total of 14 horses were included in this study. All horses were Arabian stallions, aged between 4-14 years and weighed approximately 330 kg. Horses were clinically examined in the morning before the game (T0). Thereafter, they were examined immediately after first period (T1), immediately after second period (T2) and after 30 min (T3) and 60 min of recovery period (T4). Heart rate, respiratory rate, capillary refill time, mucous membane colour, and signs of deydration were examined. Mucose membrane were described as normal (1), hyperemic (2) and congested (3). Capillay refill time was tested by pressing one's thumb firmly into the gum, blanching colour out, removing quickly, and counting seconds for gum to turn to normal, pink color. Skin turgor test (dehydration) was measured by pinching the skin on the neck into a tent, then counting seconds until it returned to normal contour.

In all time points, blood samples were collected by jugular venipuncture into 2 mL ethylenediaminetetraacetic acid vacutainer tubes (K3EDTA, Greiner Bio-one GmbH, USA) for haematological evaluation. Blood samples were collected into 2 mL sodium floride-ethylenediaminetetraacetic acid vacutaner tubes (FE Sodium Floride/K3EDTA, Greiner Bio-one GmbH, USA) for lactate measurement and 8.5 mL serum separator tube (Vacutainer SST Plus; BD) for all other biochemical analyses. Serum was separated by centrifugation at 4000 rpm for 5 min (Eppendorf Centrifuge 5702 R, Germany) and refrigerated until the next day. All analysis were carried out in the Equine Hospital of the Turkish Jockey Club. Red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell dispersion width (RDW), white blood cells (WBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS), basophils (BASO) and platelets (PLT) were analysed using Abott Cell-Dyn 3500 Hematology analyser (Abbott Diagnostics, Santa Clara, CA). Lactate, glucose, urea, total protein, albumin, globulin, calcium (Ca), magnesium (Mg), phosphorous (P), sodium (Na), potassium (K), chloride (Cl), creatinine kinase (CK), lactate dehydrogenase (LDH) aspartate aminotransferase (AST), alkaline phosphatase (ALP) were measured using Dimension Xpand biochemistry analyser (Dimension Xpand plus, Siemens, USA).

Statistical Analyses

Collected variables were analyzed by using Linear Mixed effect model to evaluate changes of repeated measure-

ments at 5 different time points and LSD was used as post-hoc test. Due to the fact that variances at each time point were different and correlations between measurements were different for each time pairing, unstructured covariance type was selected for modeling. All statistical analyses were performed with SPSS for Windows version 24.0 and a P value <0.05 was accepted as statistically significant.

RESULTS

All results are shown as means±standard error of mean (SEM) in *Table 1, Table 2* and *Table 3*. All animals were examined clinically before game at T0. At T0, mean values of respiratory rate, heart rate, capillary refill time and mucous membrane colour were near to reference range. Mean value for dehydration were mildly high *(Table 1)*. At T1, respiratory rate (P<0.001), heart rate (P<0.001), capillary refill time (P<0.001), skin turgor time (P<0.001), and mucous membrane colour (P<0.01) were significantly increased. There was no significant difference in these parameters between T1 and T2 whereas all values at T2 were significantly higher than T0 (P<0.001). At T3, respiratory rate (P<0.01), heart rate (P<0.001), capillary refill time (P<0.05) and mucous membrane (P<0.001) were significantly higher compared to T0. Respiratory rate, heart rate and mucous membrane colour were decreased significantly from T2 to T4 (P<0.05, P<0.001, P<0.05; respectively). At T4, heart rate (P<0.01), capillary refill time (P<0.001), mucous membrane (P<0.05) and skin turgor (P<0.01) were significantly higher than T0 while there was no significant difference in respiratory rate between T4 and T0.

RBC, HGB and HCT were significantly higher at T1 and T2 (each, P<0.001) than T0. Their values at T3 (each, P<0.05) and T4 (each, P<0.001) were significantly lower than T2 but significantly higher than T0 (each, P<0.001) *(Table 2)*.

RDW was significantly higher both at T1 (P<0.01) and T2 (P<0.001) than T0. Their values both at T3 and T4 were significantly higher than T0 (each, P<0.001). MCV did not show any significant difference at T1 but decreased significantly at T2 (P<0.05) and its value at T2 significantly lower than T0 (P<0.001). MCV value at T4 was significantly lower than T0 (P<0.05).

WBC was increased significantly at T1 (P<0.01) because of increases in the numbers of neutrophils and monocytes (P<0.001 and P<0.01, respectively). At T2, the numbers of WBC, NEU and BASO were higher than T0 (P<0.001, P<0.001, P<0.05; respectively). The number of WBC was significantly higher at T4 than T0 (P<0.001) because of significant increases in NEU (P<0.01), MONO (P<0.01) and BASO (P<0.05) numbers. There was no significant difference in the number of PLT among all periods (P>0.05).

Ca, Mg and Cl concentrations decreased significantly from T0 to T1 (P<0.001, P<0.01, P<0.001; respectively) while Na was increased significantly (P<0.001*) (Table 3)*. Ca, Mg, Cl and P were significantly lower at T2 than T0 (P<0.01, P<0.01, P<0.001, P<0.05; respectively) while Na and K were higher (each, P<0.001). Ca, Mg, Cl increased significantly from T2 to T4 (P<0.001, P<0.05, P<0.001; respectively) and there was no significant difference in these parameters between T4 and T0 (each, P>0.05). Na and K were significantly higher (both, P<0.05) and P were significantly lower (P<0.001) at T4 than T0.

Total protein, albumin and globulin increased significantly from T0 to T2 (each, P<0.001) and were significantly higher at T3 (each, P<0.001) and T4 (each, P<0.001) than T0.

Lactate, glucose and urea were increased significantly at T1 (P<0.001, P<0.001, P<0.01; recpectively) and were significantly higher at T2 (P<0.01, P<0.001, P<0.001; respectively) than T0. Lactate and glucose significantly decreased from T2 to T4 (for each, P<0.01) while urea increased (P<0.05). Lactate, glucose and urea were significantly higher at T4 than T0 (P<0.01, P<0.01, P<0.001; respectively).

Table 1. *Physical parameters at rest (T0), immediately after first (T1) and second period (T2), after 30 min (T3) and 60 min of recovery time (T4). Comparisons between eight time points are shown (T1 vs. T0, T1 vs. T2, T2 vs. T0, T2 vs. T3, T3 vs. T0, T3 vs. T4, T2 vs. T4, and T4 vs. T0)*

Parameters (with reference range)[Δ]	T0 X_0±SEM	T1 X_1±SEM	T2 X_2±SEM	T3 X_3±SEM	T4 X_4±SEM	Statistical Difference (P)							
						T1 vs T0	T1 vs T2	T2 vs T0	T2 vs T3	T3 vs T0	T3 vs T4	T2vsT4	T4vsT0
Respiratory rate (10-16 respiration/min)	24±2	63±6	71±7	94±6	39±4	***	Ns	***	***	**	Ns	*	Ns
Heart rate (28-40 beats/min)	40±2	83±4	79±6	62±5	54±3	***	Ns	***	**	***	*	***	**
Capillary refill time (1-2 sec)	2.0±0.2	3.3±0.4	3.1±0.3	2.7±0.2	3.1±0.2	***	Ns	***	Ns	*	Ns	Ns	***
Mucous membrane	1±0.00	1.5±0.2	1.7±0.2	1.7±0.2	1.4±0.1	**	Ns	***	Ns	***	*	*	*
Skin turgor test/ dehydration (1-2 sec)	2.6±0.4	4.1±0.4	4.4±0.3	3.1±0.2	3.7±0.3	***	Ns	***	***	Ns	Ns	Ns	**

** P<0.05, ** P<0.01, *** P<0.001,* ***Ns:*** *Not significant,* ***Δ:*** *Reference ranges are referred* [22]

Table 2. *Haematological parameters at rest (T0), immediately after first (T1) and second period (T2), after 30 minutes (T3) and 60 minutes of recovery time (T4). Comparisons between eight time points are shown (T1 vs. T0, T1 vs. T2, T2 vs. T0, T2 vs. T3, T3 vs. T0, T3 vs. T4, T2 vs. T4 and T4 vs. T0)*

Parameters (with reference range)[Δ]	T0 Xo±SEM	T1 X_1±SEM	T2 X_2±SEM	T3 X_3±SEM	T4 X_4±SEM	Statistical Difference (P)							
						T1 vs T0	T1 vs T2	T2 vs T0	T2 vs T3	T3 vs T0	T3 vs T4	T2vsT4	T4vsT0
RBC (6.0-10.4x10^6/µL)	8.09±0.40	10.13±0.45	10.89±0.46	10.14±0.56	10.01±0.57	***	*	***	*	***	Ns	**	***
HGB (10.1-16.1 g/dL)	13±0.6	17±0.7	18±0.7	17±0.9	16±0.9	***	Ns	***	*	***	Ns	**	***
HCT (32%-43%)	39±2	50±2	52±2	48±2	48±2	***	Ns	***	*	***	Ns	**	***
MCV (37-49 fL)	48.4±0.8	48.3±0.8	47.7±0.8	48.1±0.8	47.8±0.8	Ns	*	***	Ns	Ns	Ns	Ns	*
MCH (13.7-18.2 pg)	16.6±0.2	16.4±0.2	16.4±0.2	16.5±0.2	16.4±0.2	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
MCHC (35.3-39.3 g/dL)	34.3±0.2	34.3±0.3	34.6±0.2	34.4±0.2	34.4±0.3	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
RDW (17%-27%)	24.7±0.5	26.4±0.6	27.5±0.6	26.9±0.6	26.7±0.8	**	*	***	Ns	***	Ns	Ns	***
WBC(5.6-12.1x10^3/µL)	7.8±0.4	9.1±0.3	11.6±1.0	11.5±1.2	12.1±1.0	**	*	***	Ns	**	Ns	Ns	***
NEU(2.9-8.5 x10^3/µL)	5.9±0.4	7.2±0.4	9.6±0.9	7.9±0.9	8.6±0.9	***	*	***	*	*	Ns	Ns	**
LYM(1.2-5.1 x10^3/µL))	1.7±0.1	1.7±0.2	1.6±0.2	2.6±0.5	2.05±0.2	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
MONO (0.0-0.7 x10^3/µL)	0.15±0,02	0.09±0.01	0.24±0.06	0.80±0.23	0.90±0.21	**	*	Ns	*	*	Ns	*	**
EOS (0.0-0.8 x10^3/µL)	0.17±0.03	0.13±0.02	0.18±0.04	0.18±0.06	0.34±0.10	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
BASO (0.0-0.3 x10^3/µL)	0.02±0.00	0.02±0.00	0.02±0.00	0.03±0.01	0.10±0.03	Ns	Ns	*	Ns	Ns	*	**	*
PLT (117-256x10^3/µL)	133.8±11.2	149.8±25.9	162.8±22.6	182.3±28.4	161.1±27.1	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns

** P<0.05, ** P<0.01, *** P<0.001,* ***Ns:*** *Not significant,* ***Δ:*** *Reference ranges are referred* [22]

CK, LDH, AST and ALP increased significantly from T0 to T1 (P<0.001, P<0.001, P<0.01, P<0.001; respectively), and to T2 (P<0.05, P<0.001, P<0.001, P<0.001; respectively). CK, LDH, AST and ALP were significantly higher at T3 (P<0.05, P<0.001, P<0.001, P<0.001; respectively) and at T4 (P<0.05, P<0.05, P<0.001, P<0.001; respectively) than T0.

DISCUSSION

To the knowledge of literature review, the evaluation of the physical, hematological and biochemical responses of horses to jeered competition is the first.

In this study, respiratory rate at T4 (39±4) were significantly lower than T2 (71±7) and reached the baseline (T0). Heart rate at T4 (54±3) were significantly lower than T2 (79±6) but did not reach the baseline (40±2). Heart rate recovery might be a good indicator for assessing fitness level of warm blood sport horses [23]. However, ambient temperature and humidity also affect the heart rate. Higher heart rate elevation in the hot conditions increases cardiac output to provide skin blood for heat dissipation [24]. The present study was performed in July at 29°C temperature and 31% humidity. This might explain that heart rate at 60 min after exercise did not reach the resting value at T0. Actually, in endurance competition, horses having 64 beats/min heat rate within 30 min at the final inspection are considered as fit [25]. In polo, horses with 48 beats/min of heart rate at 30 min after exercise were considered as fit although not reached the resting value [14]. The mean capillary refill time and skin turgor time at T0 (2.0±0.2, 2.6±0.4; respectively) observed in the present study shows that horses might be mildly dehydrated. According to personel communications with horse owners, the last time of horses to consume water was 10 h before the competition. This might explain the mild dehydration observed in the horses before competition. Thereafter, capillary refill time was increased and skin turgor was decreased (as time increased for skin to return to normal contour) at T1 and T2. These might be attributed to the fact that more dehydration occured after exercise. When the exercise is performed in hot conditions, body temperature rises excessively, the demands of skin blood flow for heat dissipation arise resulting in dehydration [26]. Furthermore, the condition did not improve after 60 min of rest. This might be explained by the fact that horses were

Table 3. *Biochemical parameters at rest (T0), immediately after first (T1) and second period (T2), after 30 minutes (T3) and 60 minutes of recovery time (T4). Comparisons between eight time points are shown (T1 vs. T0, T1vs. T2, T2 vs. T0, T2 vs. T3, T3 vs. T0, T3 vs. T4, T2 vs. T4 and T4 vs. T0)*

Parameters (with reference range)Δ	**T0 Xo±SEM**	**T1 X_1±SEM**	**T2 X_2±SEM**	**T3 X_3±SEM**	**T4 X_4±SEM**	**Statistical Difference (P)**							
						T1 vs T0	**T1 vs T2**	**T2 vs T0**	**T2 vs T3**	**T3 vs T0**	**T3 vs T4**	**T2 vs T4**	**T4 vs T0**
Lactate (0-2 mmol/L)	0.69±0.08	14.67±1.98	9.23±2.54	4.79±1.41	2.79±0.69	***	Ns	**	**	*	*	**	**
Glucose (62-134 mg/dL)	92.07±2.76	158.08±8.68	180.86±17.56	158.29±19.49	143.50±17.32	***	Ns	***	Ns	**	Ns	**	**
Urea (24-48 mg/dL)	27.86±1.61	30.17±1.79	36.89±2.06	37.81±1.95	39.18±2.09	**	***	***	Ns	***	*	*	***
TotalProtein (5.6-7.6 g/dL)	6.71±0.13	7.31±0.22	7.74±0.20	7.63±0.23	7.73±023	***	**	***	Ns	***	Ns	Ns	***
Albumin (2.6-4.1 g/dL)	3.49±011	3.92±0.16	4.03±0.19	4.01±0.19	4.09±0.19	***	Ns	***	Ns	***	*	Ns	***
Globulin (2.6-4.0 g/dL)	3.21±0.14	3.39±0.15	3.71±0.14	3.62±0.14	3.64±0.14	Ns	**	***	Ns	***	Ns	Ns	***
Calcium (10.2-13.4 mg/dL)	11.80±0.15	11.18±0.21	11.09±0.27	11.40±0.29	11.69±0.24	***	Ns	**	***	Ns	*	***	Ns
Magnesium (1.4-2.3 mg/dL)	2.06±0.06	1.86±0.06	1.84±0.08	1.86±0.09	1.97±0.10	**	Ns	**	Ns	*	**	*	Ns
Phosphorous (1.5-4.7 mg/dL)	3.09±0.15	2.94±0.20	2.48±0.25	2.24±0.21	2.34±0.22	Ns	Ns	*	Ns	***	Ns	Ns	***
Sodium (128-142 mmol/L)	136.07±1.13	139.00±1.07	138.79±1.04	138.93±1.32	138.57±1.44	***	Ns	***	Ns	**	Ns	Ns	*
Potassium (2.9-4.6 mmol/L)	3.99±0.14	4.52±0.28	5.66±0.45	5.35±0.67	5.62±0.72	Ns	**	***	Ns	*	Ns	Ns	*
Chloride (98-109 mmol/L)	98.29±0.95	93.92±1.34	94.36±1.36	95.93±1.39	97.00±1.49	***	Ns	***	***	*	**	***	Ns
CK (60-330 U/L)	220.00±23.05	342.38±32.27	520.86±108.08	602.57±139.23	638.14±162.27	***	Ns	*	*	*	Ns	Ns	*
ALP (138-251 U/L)	136.21±7.01	164.08±8.85	174.50±11.12	173.64±10.32	175.36±11.54	***	Ns	***	Ns	***	Ns	Ns	***
LDH (112-456 U/L)	335.43±11.49	440.00±20.15	479.86±28.83	478.00±33.66	444.50±38.45	***	Ns	***	Ns	***	*	*	*
AST (160-412 U/L)	381.500±63.549	424.846±57.133	460.357±60.498	464.929±64.433	474.500±65.508	**	*	***	Ns	***	**	Ns	***

** P<0.05, ** P<0.01, *** P<0.001,* ***Ns:*** *Not significant, Δ: Reference ranges are referred* [27]

not given water within two hours after competition in jeered competition since most horse owners traditionally believe that it could be dangerous for horses.

Total protein, albumin and globulin concentrations were increased at T2. These parameters did not reach the resting values after 30 and 60 min of recovery but were within the reference range. Increases in plasma proteins with an increased capillary refill time and a decreased skin turgor might be attributed to the acute dehydration. The increase in serum protein concentrations after exercise has been observed in other equestrian competitions such as polo [14,15], flat racing [26], cross country [10] and endurance [24]. It has been reported that hyperproteinemia occurs in athletic horse as a result of dehydration [1]. It has been also reported that there is an increase in total protein and albumin concentration as a result of intercompartmental fluid shifts or real fluid loss [24]. Total protein concentration returns to baseline by 30 min after high intensity and short duration of exercises while total protein recovery requires more time in prolonged exercises or exercises with excessive sweating [1]. The fact that jeered competition lasts approximately for 90 min and horses usually are not given water within two hours after exercise might explain the reason for total protein not to be recovered 30 or 60 min after competition in the present study.

Urea concentration was increased after exercise and did not reach the baseline after 30 or 60 min of recovery but were within reference range. Transient increase in urea concentration with exercise has been found normal responses to high and low intensity exercises as a result of reduction in renal blood flow or real fluid loss [1].

The RBC, HGB and HCT were increased after exercise. It has been reported that erythrocytes from spleen reservoir are realeased to the circulation under the influence of catecholamine during exercise [1]. Apart from spleen contraction, the increase in HCT might be attributed to intercompartmental fluid shifts or real fluid loss by sweating. RBC, HGB and HCT at T4 (10.01±0.57, 16±0.9, 48±2; respectively) were significantly lower than T2 (10.89±0.46, 18±0.7, 52±2; respectively). These parameters at T4 did not reach the baseline but were near to the reference range. Circulating erythrocytes return to spleen over a period of 1-2 h to reach preexercise values [1].

MCV were decreased but RDW were increased at T2. Both of the parameters did not return to the baseline 60 min after competition but were within reference range. Both conditions observed in the present study may be attributed to the fluid shifts from erythrocytes to the extracellular compartment because of dehydration. Actually an increase in RDW has been observed in human athletes after acute exhaustive exercise [28].

Increased leukocytosis was observed because of a significant rise in the neutrophil count at T1 and T2, with rises in monocytes at T1 and basophils at T2. Lymphocyte count was not affected but the increase in neutrophil: lymphocyte ratio (NEU/LYM T0 = 3.47, NEU/LYM T1 = 4.24 and NEU/LYM T2 = 6.00) was observed in the present study. It has been suggested that exercise causes the mobilization of marginated leucocytes sequestered in the spleen and capillary beds to the circulation [1,2,29]. It has been reported that neutrophil : lymphocyte ratio has been decreased after polo competition [14] while neutrophil: lymphocyte has been increased after endurance [1]. In high intensity and shorter duration of exercises, relatively more lymphocytes are realesed from spleen to the circulation under the effect of catecholamines [2,14]. Low to modeate intensity but longer duration exercises produces a marked leukocytosis due to neutrophilia and lymphopenia under the effect of cortisol [1,30]. The values at T3 and T4 did not reach to the baseline but were in the reference range 30 and 60 min after exercise. The increased leucocyte count after exercise returns to baseline values witin 6 h [31].

Lactate and glucose levels were significantly increased at T1 (14.67±1.98, 158.08±8.68; respectively) and T2 (9.23±2.79, 180.86±17.56; respectively). It has been reported that lactate concentrations immediately after exercise are less than 2 mmol/L in endurance race, 10.24 mmol/L in polo competition, 20-25 mmol/L in throughbred racing [14,26,32]. Lactate increases have been found to be associated with anaerobic metabolism of pyruvate and the stimulaton of glycogenolysis [1,33]. It has been reported that glucose level increases in all form of exercise because of hepatic glycogenolysis [26]. In high intensity with short duration exercises, glucose concentrations have peak value of 180-206 [1]. Lactate and glucose levels at T4 (2.79±0.69, 143.50±17.32; respectively) were significantly lower than T2 (9.23±2.79, 180.86±17.56; respectively). These parameters did not reach the baseline at T4 but were near to the reference range.

Ca, Mg, P and Cl concentrations were decreased at T2. The decrease observed in Ca, Mg, P concentrations could have been caused by intracellular movement, which is necessary for their use in muscle function and/or their loss with sweating [14]. Ca and Mg concentration reached the baseline after 30 and 60 min of recovery; respectively. P concentration did not return to the baseline at T4 but were in the reference range. The decrease in chloride concentration after exercise could have been the result of sweating. Chloride has been considered as the principle anion lost in sweat [1,13]. However, chloride concentration reached the baseline at T4. It has been reported that when plasma chloride concentration falls through sweating, renal reabsorption of Cl occurs for compensation [1].

The increase in Na concentration after exercise was observed in this study. Na concentration did not return to the baseline 60 min after exercise but were in reference values. It has been reported that the increase in Na concentration occurs following high intensity exercise

such as flat racing [1]. Na concentrations have been reported to be unchanged, decreased, increased, depending on the environmental conditions and duration of endurance competition [1,34]. It has been suggested that the increase in sodium concentration could have been the result of fluid movement out of extracellular space [35]. It has been also reported that the increase in Na concentration might be because of renal reabsorption of Na to compensate dehydration [36,37]. The decrease in Na concentration after calcavede competition might be associated with the loss of Na from sweating [38]. The intensity and duration of exercise as well as environmental conditions might all affect the amount of fluid lost by sweating and hence, Na concentration in blood.

The increase in K concentration was observed at T2. It has been reported that the increase in potassium concentration after high intensity exercise might be due to the impairment of Na-K pump by increased hydrogen ions in muscle cell [1]. On the contrary, the decrease of K concentration has been detected in endurance competition in which horses have slower speed but longer durations [13]. The decrease of K in that study has been indicated to be associated with the loss of this element in sweat [13].

LDH, CK, AST and ALP concentrations were increased at T1 and T2 and did not reach to the baseline at T4 but were near to reference range except CK. These enzyme activities have been shown to be increased following racing in galloping but still remain within normal reference ranges [1]. Similiary, moderate increases in CK and AST have been observed following endurance exercises [1]. The reason for CK concentration to be high and not to reach near reference range values at T4 might be due to small muscle damage since potassium concentration was also high at T4 compared to reference values.

Physical, haematological and biochemical changes observed in this study were the physiological responses of horses to jereed competition which has a characteristic of mixed aerobic/anaerobic pathway metabolism. Most of the parameters changed significantly after exercise. Although majority did not return to the baseline 30 or 60 min after competition most were within or near to the reference range values.

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