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Stress Responses of Stallions During Transport Period: The Variations of Complete Blood Count and Serum Biochemistry*

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Absract: In the present study the objective was to determine the effect of one transport journey on some stress indicators in horses. 11 adult stallion (all stallion) age 3-12 years including 7 Thoroughbred, 2 Arabian, 1 Dutch Warmblood, and 1 Friesian were enrolled. White blood cell counts and other relevant parameters were determined by an automated cell counter before and after transportation. Serum activities of relevant biomarkers were determined by use of an autoanalyzer. Serum cortisole level was detected by a microplate immunoassay technique. Sodium (Na), Potassium (K) and Clor (Cl) and Calsium (Ca) levels were also measured by use of autoanalyser. Mean Platelet (PLT), and Mean corpuscular hemoglobin concentration (MCHC) White Blood Cell (WBC) counts did not exhibit a variation for healthy horses during the study whereas RBC, Hb and Hct levels exhibited significant increases after transportation ($P<0.05$). Mean daily glucose concentration showed an increase after transportation ($P< 0.05$). Mean total protein, albumine, AST and CK levels presented increases on day 2, after transportation ($P< 0.05$). Mean blood urea nitrogen, creatinine, total bilirubine, ALT, ALP, LDH and GGT did not show significant changes throughout the study. Mean Sodium (Na), Potassium (K) and Clor (Cl) levels showed alterations after transportation ($P< 0.05$), whereas Calsium (Ca) levels showed significant decreases after transportation ($P< 0.05$).

Keywords: Biochemistry, Hematology, Stallion, Stress, Transport.

Uzun Transporta Bağlı Aygırların Strese Tepkileri: Tam Kan Sayımı ve Serum Biyokimya Varyasyonları

Öz: Bu çalışmada, transportun atlarda bazı stres göstergelerine etkisini belirlemek amaçlanmıştır. 7 Safkan, 2 Arap, 1 Hollanda Yarım Kan ve 1 Fresyan olmak üzere 3-12 yaş arası 11 olgun at (aygır) kayıt edildi. Transport öncesi ve sonrasında, otomatik kan sayımı ile beyaz kan hücresi sayımı ve diğer ilgili parametreler belirlendi. İlgili biyolojik belirteçlerin serum aktiviteleri bir otoanalizör kullanılarak belirlendi. Na, K, Cl ve Ca düzeyleri de otoanalizör kullanılarak ölçüldü. Ortalama Platelet (PLT) ve Oratalama Hemoglobin Konsantrasyonu (MCHC) Total Lökosit (WBC) sayıları çalışma süresince sağlıklı atlar için bir farklılık göstermezken, Alyuvarlar (RBC), Hemoglobin (Hb) ve Hematokrit (Hct) düzeyleri ulaşımdan sonra anlamlı artışlar sergiledi ($P<0.05$). Günlük ortalama glikoz konsantrasyonu transporttan sonra artış gösterdi ($P<0.05$). Ortalama total protein, albümin, Aspartat aminotransferaz (AST) ve Kreatinin kinaz (CK) düzeyleri, transportun ardından 2. günde artmaktadır ($P<0.05$). Ortalama kan üresi azotu, kreatinin, total bilirubin, Alanin aminotransferaz (ALT), Alkalen fosfataz (ALP), Laktat dehidrogenaz (LDH) ve Gama-gulutamil Transferaz (GGT), çalışma boyunca önemli değişiklikler göstermedi. Transporttan sonra ortalama Sodyum (Na), Potasyum (K) ve Klor (Cl) düzeyleri değişti ($P<0.05$), nakil sonrası Kalsiyum (Ca) düzeyleri anlamlı düşüş gösterdi ($P <0.05$).

Anahtar Kelimeler: Aygır, Biyokimya, Hematoloji, Stres, Transport.

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INTRODUCTION

Horses are transported for several reasons comprising sport (showing and racing) (1,2,3), breeding and slaughter for several times throughout their lives (4). It has been well recognized that in horses transport is a general stressor leading to salmonellosis (5), respiratory disease (6,7,8), elevated heart rates (9,10), and changes in plasma ascorbic acid (11), serum cortisol concentration (9,10,11,12,13) and muscle enzyme activities (13,14,15). Elevated sodium and blood glucose concentrations have also been detected in slaughtering horses (12,13,14,15,16).

Up to date the transportation of horses by road received attention, but limited scientific research has been (4) focused on both hematological and serum biochemical changes. Therefore the aim of this study was to determine the effect of one transport journey on some stress indicators in horses.

MATERIALS and METHODS

Horses and Study Design

Eleven adult stallion aged 3-12 years including 7 Thoroughbred, 2 Arabian, 1 Dutch Warmblood, and 1 Friesian were enrolled. All horses have had prior experience of transport; indeed 2 weeks prior to the study was performed, all horses were loaded and unloaded form the van in an attempt to ensure that all horses loaded without hesitation. This study was conducted in accordance with the ethical guidelines of animal experiments.

A study design consisted of one 19 h transport period from Nevsehir to Antalya, with a total of 867 km travelled. On day 0, prior to study horses were housed individually in their natural boxes and physiological data were collected to obtain baseline values. On day 1 horses were loaded to a commercial van. Travel commenced for 19 hour period. Horses were unloaded on day 2 and housed individually in boxes for post-transport period. Water was always available to the horses in their boxes.

A commercial van was designed to carry out a total of 11 stallions and was pulled by a tractor. Horses were randomly enrolled in groups paralel to the length of the van, as described previously (21). Five horses faced the direction of the journey, and six other faced the opposite direction. Bedding (straw) was placed on the floor for secure footing. Windows were allowed to open fully for fresh air. Travel was iniated at early morning and continued for 19 h.

Laboratory Analyses

White blood cell counts and were determined by an automated cell counter (Abacus Junior Vet 5, Hungary). Serum activities of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Creatinine kinase (CK), Lactate dehydrogenase (LDH), Gama-glutamil transferase (GGT) and glucose, Blood Urea Nitrogen (BUN), creatinine, total protein, albumine, total bilirubine levels were determined by use of an autoanalyzer (Spotchem ez sp-4430, Arkray, Japan). Serum cortisole levels and electrolyte analysis [Na, K, Cl and Ca], were detected by a microplate immunoassay technique using autoanalyzer in a private commefical lab.

Statistical Analyses

The effects of transportation and measurements taken after transport were evaluated using means for day 1 and 2, respectively, using Paired Samples t-test. Significance was declared whenever $P<0.05$.

RESULTS

Blood Measurements

The WBC counts did not exhibit a variation and remained within the reference range (5.5 to 14.3 × 10^3/_L) for healthy horses during the study. Mean PLT and MCHC did not show significant levels (Table 1). RBC, Hb and Hct levels exhibited variations with increases after transportation ($P<0.05$). MCHC exhibited variations with slight decrease after transportation ($P<0.05$) (Table 1).

Table 1. Mean haematological values (± SD) of measurements and P-values of comparisons between day 0 (d 0) and following a 19-h posttransport period (d 2).
Tablo 1. 0. Hematolojik ölçüm değerlerinin ortalaması (± SD) ve 0.(d 0) gün ile 19 saatlik bir nakliye sonrası dönem (d 2) arasındaki karşılaştırmaların P-değerleri.

	N	O. hour	19. hour	SD	P	
WBC ($x10^3/\mu L$)	11	7.67	7.73	0.815	0.809	
RBC ($x10^6/\mu L$)	11	7.77	12.82	2.005	0.000	***
HB (g/dL)	11	11.91	16.93	1.415	0.000	***
HCT (%)	11	40.54	50.79	1.768	0.000	***
MCV (fL)	11	46.40	40.88	5.605	0.006	***
MCHC (g/dL)	11	33.39	33.50	1.877	0.845	
PLT ($x10^6/\mu L$)	11	150.25	147.92	6.945	0.269	

Serum Metabolites and Enzymes

Mean daily glucose concentration showed an increase after transportation (P<0.05*).* (Table 2). Mean total protein, albumine, AST and CK levels showed increases on day 2, after transportation (P<0.05). Neither serum concentrations of blood urea nitrogen nor of creatinin exhibited variation or difference (P>0.05) between days 1 and 2. Besides mean values of total bilirubine, ALT, ALP, LDH and GGT did not show significant changes throughout the study (Table 2).

Mean Na, K and Cl levels showed variations with increases after transportation (P< 0.05), whereas Ca levels showed significant decreases after transportation (P<0.05). Mean serum cortisol levels showed significant increases after transportation (P<0.05) (Table 2).

Table 2. Mean serum biochemical and metabolite values (± SD) of measurements and P-values of comparisons between day 0 (d 0) and following a 19-h posttransport period (d 2). Daily means (SD) and P values.
Tablo 2. Ortalama serum biyokimyasal ve metabolit değerleri (± SD) ve 0. gün (d 0) ile 19 saat sonrası nakliye sonrası dönem (d 2) arasındaki karşılaştırmaların P değerleri. Günlük ortalama (SD) ve P değerleri.

	N	O. hour	19. hour	SD	P	
Glucose (mg/dL)	11	89.83	111.83	5.846	0.000	***
Blood Urea Nitrogen (mg/dL)	11	14.33	14.50	1.800	0.754	
Creatinin (mg/dL)	11	1.11	1.14	0.143	0.438	
Total Protein (g/dL)	11	6.25	7.53	0.407	0.000	***
Albumine (g/dL)	11	3.20	3.82	0.332	0.000	***
Total Bilirubine (mg/dL)	11	1.34	1.33	0.144	0.845	
ALT (IU/L)	11	13.75	14	2.800	0.763	
AST (IU/L)	11	311.17	396.67	40.218	0.000	***
ALP (IU/L)	11	188.08	187	6.815	0.593	
CK (IU/L)	11	12.33	28.84	3.091	0.000	***
LDH (IU/L)	11	271.17	270.25	9.209	0.737	
GGT (IU/L)	11	13	13.33	1.614	0.489	
Na^{++} (mmol/L)	11	138.17	149.50	1.442	0.000	***
K^+ (mmol/L)	11	3.07	4.49	0.362	0.000	***
Cl^- (mmol/L)	11	102.75	119.17	4.420	0.000	***
Ca^{++} (mmol/L)	11	3.09	2.69	0.175	0.000	***
Cortisole (µg/dL)	11	1.65	2.90	0.215	0.000	***

DISCUSSION and CONCLUSION

One of the most important problem is that the response to road transport equine practice worldwide (24). As a consequence several immunological, physiological, and metabolic alterations might prone horses to elevated susceptibility to disorders (diarrhea, colics, pneumonia laminitis and trauma) (24,25). Transportation is composed of several psychological stimuli, resulting with stress disrupting metabolism and homeostasis of affected animals (26,27,28).

Transportation includes various stressors comprising loading, unloading and penning in a novel and unfamiliar environment and confinement along with motion, vibrations, changes in climatic conditions, inadequate ventilation and food and water deprivation. Animals react and addict respond to challenges in their environment by various interacting mechanisms including behavioural, haematological, physiological and neuro-hormonal parameters. Age, sex and physiological conditions may also have infleunce on the behaviour of animals during handling and transport (17,18,19).

In a prior study analyzing relationships between transportation of 24 cold-blooded female horses forwarded to both long and short distances and an inflammatory condition [acute phase proteins, oxidative stress and muscle injury. Plasma and serum were withdrawn prior to transportation (T0), immediately after transportation (T1) and finally during slaughter (T2). Fibrinogen was elevated in all studied groups especially in fillies after long distance transportation, (205±7.07 mg/dl at TO; 625±35.35 mg/dl at T1, and 790±14.14 mg/dl at T2). Malondialdehyde levels rose at T1 and reached the maximal level during T2. CK activity was also increased at T1 in younger horses, According to the latter study an intensified acute phase response along withoxidative stress and muscle trauma indicated an inflammatory condition (24). In the present study altough the present authors did not evaluate direct biomarkers of stres or oxidative status, cortisole was markedly increased in horses following transportation indicating relative stress as was also determined (25,29).

Significantly higher levels of GOT, GPT, LDH and ALT in contrast to the basal values were reported for sedentary horses following transportation (20). In addition, haematological and serum biochemical changes occur in horses confined during air transport (13). In the present study significantly higher levels in glucose, total protein, albumin, AST and CK levels ($P< .05$) were detected after transportation.

In horses in general CPK and AST are clinically important with muscular diseases because of their high activity in skeletal muscle (21,22,23). Moderate increases in CPK may be associated with moderate exercise or a training program, indeed more severe exercise and competitions may induce increases over 1.000 IU/L. In this study, the CK elevated following transport, and the activity of AST rose in response to transport. The increase in those two serum enzymes indicates minimal muscular insult due to 19 h of transport.

During stressful conditions as exercise or transportation, the hypothalamic-pituitary-adrenal axis activates and may result with an elevated plasma cortisol. Plasma cortisol concentration exhibited variation, with increase during 19h of transport. After the stressor (i.e., transportation) ceased, cortisol dramatically declined. This may be explained with cortisol's relatively short half-life of 1 to 1.5 h (23). The peak in cortisol levels was greater than post-transit cortisol levels evaluated in slaughter horses traveling for up to 30 h (20) but similar to concentrations in moderately exercised horses (20).

In a previous study of 9 h of transportation increased concentrations of cortisol was reported (3). In racehorses, short-term (300 km) transportation or an exercise effected similar serum enzyme and metabolic changes (5). The increases in serum total protein, Na, K and Cl levels after transportation ($P<0.05$) indicate that the present horses may have had hypertonic dehydration. Although the horses had had water available in their boxes, during a stressfull condition such as transport

dehydration may occur. In addition Hct levels exhibited variations with increases after transportation ($P<0.05$) in this study.

WBC counts were deemed as a general indicator of health and the ability of the horses handling stress during transport. The WBC counts showed increases during 24 hours transport in horses (20), however the WBC counts did not exhibit a variation and remained within the reference range for horses during this study. Regarding other relevant hematological indices RBC, Hb and Hct levels exhibited variations with increases after transportation ($P<0.05$) and MCHC exhibited variations with slight decrease after transportation ($P<0.05$).

As a result of horses undergoing 19 h transportation showed patophysiological responses that involve changes in stress indices, hematological and serum biochemical parameters, and dehydration. Changes in PCV (Ht) values may suggest that the horses did not consume sufficient water. The rise in cortisol concentration following transport may have an influence on the immune system. In addition the alterations in glucose concentration may have effect on energy metabolism.

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