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Serum enzyme and mineral levels in dromedary camels during transport.

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ABSTRACT

Nazifi S, Baghshani H, Saeb M, Saeb S, Serum enzyme and mineral levels in dromedary camels during transport, Online J Vet Res., 13 (2):84-92, 2009. This study was conducted with the aim of investigating the effect of truck transportation during heat stress on serum enzyme and mineral levels in dromedary camels. Five males and females were used. Blood samples were collected before loading, at 1 h, after unloading and 24 h after. Mean lactate dehydrogenase, alanine aminotransferase, alkaline phosphatase, gammaglutamyl transferase, sodium, potassium, calcium, magnesium, copper, zinc and selenium values did not change significantly. However, creatine kinase and aspartate aminotransferase activity increased. The results suggest that handling and preloading muscle stress may be responsible for the rise in these 2 enzymes.

Key words: Dromedary camel, Transportation stress, Minerals, Enzymes, Serum

INTRODUCTION

The intensity and specialization of livestock production and the demand for livestock to be marketed and slaughtered outside places where they are being produced have necessitated animal transport all over the world (Kannan et al., 2000; Warriss, 2004). Transportation is considered as a major stressor for farm animals and might have deleterious effects on the health, wellbeing, performance and ultimately on product quality (von Borell and Schaffer, 2005). The complexity and suite of behavioral and physiological changes due to stress response can differ markedly from species to species, individual to individual and stressor to stressor and can vary according to prior experience and hormonal status (Cook et al., 2000).

The dromedary camel is one of the most important domestic animals in the arid and semi arid regions as it is equipped to produce high quality food at comparatively low costs under extremely harsh environments. The camel has great tolerance to high temperatures, high solar radiation and water scarcity. It can survive well on sandy terrain with poor vegetation and may chiefly consume feeds unutilized by other domestic species (Kadim et al., 2009). Camel husbandry in Iran is almost localized in southern provinces, while its slaughter and meat consumption is done in most parts of this country. Sale and slaughter are the most usual factors for transporting camels in Iran.

Stress represents the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, often with detrimental effects (Hicks et al., 1998). Different parameters have been used to assess animal welfare during transport, but this can only be properly evaluated if a number of measures are considered (Grandin, 1997). Several clinical, biochemical, hormonal, and immunological effects of transportation stress have been documented in farm animals (Becker et al., 1985; Dobson, 1987; Knowles, 1998; Broom, 2003; Fazio and Ferlazzo, 2003; Sporer et al., 2008; Fazio *et al.,* 2008). Our previous report (Saeb et al., 2009) has shown that subjecting camels to road transportation stress caused strong physiological response with increased concentrations of serum cortisol, thyroid hormones and physiological variables of metabolism. Indeed acute stressors may elicit large changes in other measurable components of blood such as serum enzymes and electrolytes (Galyean et al., 1981; Cole et al., 1988; Schaefer et al., 1990; Apple et al., 1993; Pollard et al., 2002; Adenkola et al., 2009). The objective of the current study was to investigate the impact of road transportation on serum enzyme and mineral levels in the dromedary camels, as no information is currently available in this species as far as we are aware.

MATERIALS AND METHODS

Animals and transportation:Ten clinically healthy Iranian dromedary camels (*Camelus dromedarius*), five males and five females, ranging in age from 3 to 4 years and weighing about 300 kg were selected for the study. The camels had been reared at the Camel Research Institute in Yazd province of Iran, which is supervised by an experienced veterinarian. Preliminary procedures (handling, physical restraint, loading, and unloading) were undertaken by the same staff and blood sampling was always carried out by the same operator. The journey took place on one day in August 2008. Transportation of the camels was conducted between Camel Research Institute in Baphgh to Yazd and back to Institute, on smooth roads in an open truck which took 5 hours (about 300 Km). Stocking density was about 1m^2 per animal. Environmental temperature and relative humidity during the journey was 32-36°C and 17-25%, respectively. The camels had similar feeding and watering conditions ad libitum before and after the journey. During the journey there was no feed, water, or unloading for rest.

Blood sampling and processing:Blood samples were collected immediately before loading, at 08:30 A.M. (T1), after 1 h transport, at 09:30 A.M. (T2) and immediately after transport termination and unloading, on arrival at Institute (T3) at 01:30 P.M. Final blood sample was taken 24 h after arrival (T4). Blood samples were collected from jugular vein under aseptic conditions directly into test tubes without any anticoagulant and were kept in ice until serum was separated within 2 h of collection by centrifugation at 4°C for 10 min at 3000 rpm. Any haemolysed samples were discarded. Serum samples were stored at - 20°C until analyzed.

Activities of aspartate aminotransferase (EC 2.6.1.1, AST) and alanine aminotransferase (EC 2.6.1.2, ALT) were determined by the colorimetric method of Reitman and Frankel, lactate dehydrogenase (EC 1.1.1.28, LD) by the Sigma colorimetric (Cabaud Wroblewski) method, creatine kinase (2.7.3.2, CK) by the Sigma colorimetric (Modified Hughes) method, gammaglutamyl transferase (EC 2.3.2.2, GGT) by modified Szasz method, and alkaline phosphatase (EC 3.1.3.1, ALP) by the modified method of Bowers and McComb. All the enzyme activities were measured at 37°C and the results presented in U/L (Burtis and Ashwood, 1994). The samples were analysed for sodium, potassium, calcium, magnesium, copper, zinc and selenium by the atomic absorption spectrophotometer (Shimadzo, AA- 670, Kyoto, Japan).

Statistical analysis: The data are presented as mean ± standard error (SE). A two way (time, gender) repeated measures analysis of variance was applied for statistical analysis. The level of significance was set at $P < 0.05$. Significances between means were assessed using the Bonferroni test. All calculations were performed using SPSS/PC software.

RESULTS

The mean (±SE) of measured parameters of Iranian dromedary camels before, during and after transportation are shown in Table 1. No significant difference was observed in any parameter between sexes at all sampling times. Time×gender interaction was not significant for all parameters. The serum concentrations of measured minerals were not significantly changed due to transportation stress.

As shown in Table 1 CK activity was significantly higher during transportation (T2) and after transportation (T3) compared with pre-transport (T1) values and the levels remained high at the final sampling (T4). The increase in serum activities after transportation was significant for AST, but not for LD. In the current study transportation had no significant effects on serum activities of ALT, GGT and ALP.

Table 1. Mean (± SE) of serum enzyme and mineral levels in dromedary camels (n=10, five males and five females) before transport (T1), 1 h after transport initiation (T2), on the end of transportation (T3) and 24 h after arrival (T4).

Parameter	T1	T2	T3	T4
Sodium (mmol/L)	119.00±1.99	125.01±3.40	127.54±4.80	121.00±3.08
Potassium (mmol/L)	4.33±0.41	4.47±0.46	4.26±0.36	4.11±0.38
Calcium (mmol/L)	1.99±0.24	2.14±0.44	2.07±0.28	1.82±0.27
Magnesium (mmol/L)	0.95±0.32	1.20±0.48	1.07±0.54	1.16±0.58
Copper (µmol/L)	14.68±3.13	14.45±2.32	13.15±1.99	13.65±2.11
Selenium (µmol/L)	0.16±0.03	0.13±0.03	0.15±0.03	0.13±0.02
Zinc (µmol/L)	12.59±2.80	14.67±2.11	15.59±4.99	11.62±2.47
CK (U/L)	220±25^{a}	319±17^{b}	333±25^{b}	318±19^{b}
AST (U/L)	127±18^{a}	141±26^{a,b}	167±25^{b,c}	150±17^{a,b}
LD (U/L)	555±94	620±87	798±123	715±116
ALT (U/L)	15.8±1.7	15.3±1	18.2±1.5	14.6±1.7
ALP (U/L)	113±17	127±10	113±11	108±14
GGT (U/L)	85.6±9.3	83.7±9	79.8±11.8	87.6±8.7

^{a,b,c}Mean (± SE) in each row with no common superscript differ significantly ($P < 0.05$).CK, creatine kinase; AST, aspartate aminotransferase; LD, lactate dehydrogenase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gammaglutamyl transferase.

DISCUSSION

Identification of stressful situations promotes greater well-being, health and reproductive efficiency of domestic animals as well as protection of their performance and economic potential (Fazio and Ferlazzo, 2003). Transportation is thought to be very stressful because animals are exposed to unfavorable environments and it causes changes in an animal's physiological status during transportation and for some period thereafter. Many researchers have attempted to quantify the severity of the stress imposed by the various stages involved in transport and to identify acceptable conditions and methods to minimize the adverse effects of transport. Our previous results suggested that road transportation is generally a strong stressful event for dromedary camels and provided some biomarkers for stress detection in this species (Saeb et al., 2009). So it was thought of interest to assess if some other measurable components of blood would change due to the stressful stimulus of transportation in this species. Moreover, a combination of several measurements is better to assess the effects of a particular handling practice (Haresign et al., 1995).

Muscle damage and change in cell permeability resulting from injury, bruising, exertion or exhaustion can be monitored using plasma creatine kinase (CK) (Kent, 1997). Increased CK activity due to transportation stress has been reported in cattle (Sporer et al., 2008; Knowles et al., 1999, Cole et al., 1988). Galipalli et al. (2004) reported that in Boer goats, plasma CK activities increased as a result of 6 h transportation, and further increased during overnight holding. In another work by Kannan et al. (2007), plasma CK activities increased following 6 h transportation but did not increase further when measured after overnight holding, although the levels remained higher than the pre-transport levels. In pigs a small non-significant increase in CK after a journey of eight hours and a decrease after journeys of 16 and 24 hours have been reported (Brown et al., 1999). Grigor et al. (1997) observed that high levels of CK in transported red deer decreased after 18 h of lairage. In dromedary camel, as the results showed, significant increase in CK activity was observed in T2, indicating that the camels in the current study may have experienced muscle damage and physical stress due mainly to prejourney handling and loading. This increased activity was peaked at T3, suggesting that the animals were also physically fatigued during the journey. At T4 CK activity was not significantly changed in comparison with T3 values suggesting that CK clearance and/or animals' full recovery had not taken place after 24 hours.

Cattle subjected to transportation stress had increased serum concentrations of glutamic oxaloacetic transaminase (SGOT) and alkaline phosphatase (ALP) (Galyean et al., 1981; Schaefer et al., 1990). Transport did not cause significant effects on enzyme activities of LD and AST in rats (van Ruiven et al., 1998). Transport significantly increased the levels of ALT, AST and CK in three genetic lines of turkeys (Huff et al., 2008). Increased AST, ALT, CK and LD activities following transport stress have been reported in adult mouflon (Marco et al., 1997). Transport for 12 h did not affect AST, ALT and LD activities while transport for 24 h resulted in increase in ALT and LD activities and tended to increase AST activity in feeder calves (Cole et al., 1988). According to the results of this study, 5 h road transportation resulted in increase in AST activity at T3 and tended to increase LD activity at T3 and T4 ($P < 0.09$) in comparison with T1 values. Although CK is a more specific marker of muscle damage than AST, AST is frequently used to complement CK changes (Kaneko et al., 1997).

The results of this study show that 5 h transportation under summer conditions did not affect serum sodium, potassium, calcium, magnesium, copper, zinc and selenium concentrations in camels. Wegner and Stott (1972) reported changes in sodium, potassium, calcium and magnesium following the injection of corticotrophin (ACTH). Within 30 min after ACTH injection, sodium increased and potassium decreased; within 1 hr calcium and magnesium decreased and continued to decline throughout a 24-hr study (Wegner and Stott, 1972). An increase in plasma sodium concentration as a consequence of stress has been reported in white-tailed deer (Kocan et al., 1981). Indeed, Bornez et al. (2009) described an increase in plasma sodium and potassium concentration in suckling lambs in relation to stress response. No significant differences were reported due to transportation stress in plasma concentrations of calcium and magnesium in goats (Al-Kindi et al., 2005). Indeed serum Ca, K and Na concentrations were not significantly affected by transport stress in calves (Galyean et al., 1981; Cole et al., 1988). No significant changes were observed in sodium and potassium concentration following 4 h transportation stress during the harmattan season in pigs (Adenkola et al., 2009). Decreased sodium concentrations following transportation stress has been reported in cattle (Schaefer et al., 1990). Transportation stress significantly decreased the concentration of magnesium in desert sheep and goats (Ali et al., 2006). Magnesium treatment has been shown to ameliorate the incidence of stress (Donoghue et al., 1990). Concentrations of serum Ca, P, and Mg were higher in transported pigs than in nontransported controls (Tang et al., 2009).

Discrepancies between results of various studies mentioned above may be due to several factors, including species differences, duration of the journey, whether or not animals were fasted before transportation, handling procedures and other factors related to journey quality.

Concerning the effect of sex on serum enzyme activities in camels, contradictory results are reported in the literature. According to Eldirdiri et al. (1987) sex had no significant influence on serum AST, ALT and LD activities but AP and GGT activities were higher in females than in males. Kataria and Bhatia (1991) reported that serum AST and ALT activities were significantly higher in males than in females. Whereas, Bengoumi et al. (1997) reported that sex had no significant influence on serum AST and ALT activities. However as the results of the present work show sex had no significant effect on serum enzyme activities of AST, ALT, LD, CK, ALP and GGT in Iranian dromedary camels. Disagreements between literatures about the effect of sex on serum enzyme activities in dromedaries may be due to nonconsideration of physiological state of the females and males, average age of the groups sampled and seasonal changes.

In conclusion, we have determined that 5 hour transportation in dromedary camels increase concentrations of CK and AST and tend to alter LD activity, while had no significant effect on other measured parameters. The pattern of enzyme changes indicated the effect of physical stress on muscles especially due to the early portions of transport such as prejourney handling and loading. It seems that (1) applying better handling procedures may alleviate this muscular damage and (2) not all parameters could be useful as stress and welfare indicators in dromedary camels over short road transportation.

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