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ORIGINAL ARTICLE

Influence of road transportation on plasma concentrations of acute phase proteins, including fibrinogen, haptoglobin, serum amyloid A, and ceruloplasmin, in dromedary camels (*Camelus dromedarius*)

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Abstract Transportation is an inevitable husbandry practice which animals encounter in the livestock industry and raises considerable interest both in economic and animal welfare terms. With respect to the growing interest in using acute phase proteins as health and welfare indicators, the aim of the present study was to investigate the impact of road transportation on the concentrations of fibrinogen, haptoglobin, serum amyloid A, and ceruloplasmin in Iranian dromedary camels. Ten clinically healthy Iranian dromedary camels, five males and five females, were selected and subjected to a journey of approximately 300 km in a truck by road during the late summer. Blood samples were collected immediately before loading at 8:30 A.M., after 1 h transportation, at 9:30 A.M., and at the end of the journey after unloading at 1:30 PM. A final blood sample was taken 24 h after arrival. Blood concentrations of plasma proteins, including fibrinogen, haptoglobin, serum amyloid A, ceruloplasmin, total protein, and albumin, were measured using validated methods. Mean basal pre-transport concentrations of fibrinogen (3.10±0.47 g/l), haptoglobin (0.3±0.04 g/l), serum amyloid A (9.77±0.62 μg/ml), and ceruloplasmin (0.096±0.01 g/l) did not change significantly during and after road transportation. In addition, transportation had no significant effects on basal plasma concentrations of total protein (60.8±6.1 g/l) and albumin (38.3±2.1 g/l). No significant difference was observed in any parameter between sexes at each sampling time. We conclude that stressful conditions during the journey under hot summer environmental conditions were not severe enough to trigger an acute phase response. The value of the investigated plasma parameters as welfare indicators in dromedary camel over short transportation would be limited. Taken together, this plasma protein profile may be of value for future research on acute phase proteins as potential markers of health and welfare and to develop a baseline for practical applications in dromedary camels.

Keywords Dromedary camel · Road transportation · Fibrinogen · Haptoglobin · Serum amyloid A · Ceruloplasmin

Introduction

Currently, farm animal research is focused on animal welfare, and there is a substantial need to find biomarkers that can provide an objective measure of their welfare status. Transportation is an inevitable husbandry practice which animals encounter in the livestock industry. The welfare of animals during transportation to an abattoir is important both because of the impact of stress on meat quality and the value of the final product and also because of increasing consumer awareness of the ethical quality of the meat they consume (Warris 1998, 2003). Furthermore, substandard animal welfare often results in increased morbidity and mortality and poor meat and skin quantity, resulting in substantial economic loss (Minka and Ayo 2007).

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 in an extremely harsh environment (Yagil 1982; Yousif and Babiker 1989). The camel has great tolerance to

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high temperatures, high solar radiation, and water scarcity. It can survive well on sandy terrain with poor vegetation, which is not unutilized by other domestic species (Shalah 1983). Camel husbandry in Iran is localized to southern provinces, while its slaughter and meat consumption is done in most parts of the country. Sale and slaughter are the main factors for transporting camels in Iran. Handling, transport, mixing, and overcrowding are considered as predisposing factors to pneumonia in camels (Wernery and Kaaden 2002).

Acute phase proteins (APPs) are a group of blood proteins that alter in concentration in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma, or stress (Murata et al. 2004). Serum amyloid A (SAA) and haptoglobin as well as other APPs have been proposed as markers of stress in cattle and other species (Alsemgeest et al. 1995; Deak et al. 1997; Hicks et al. 1998; Arthington et al. 2003; Hickey et al. 2003; Piñeiro et al. 2007). The APPs assay may have potential for monitoring adverse environmental and/or management stressors, thus enabling better animal welfare practice (Piñeiro et al. 2007; Murata 2007). These proteins are mainly synthesized in the liver, mediated by pro-inflammatory cytokines, and their concentration can increase (positive APPs) or decrease (negative APPs) as a consequence of inflammatory stimuli. It has been suggested that APPs may be useful in the assessment of animal welfare (Eckersall 2000; Murata et al. 2004; Murata 2007). APPs and their changes due to various inflammatory and non-inflammatory conditions have been studied intensively in many animal species (Kaneko 1997; Eckersall 2000; Petersen et al. 2004; Murata et al. 2004; Murata 2007). Despite the growing interest in using APP as health and welfare indicators, no studies exist with regard to their use in dromedary camels.

The aim of the present study was to investigate the impact of road transportation on concentrations of fibrinogen, haptoglobin, serum amyloid A, and ceruloplasmin in Iranian dromedary camels.

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 approximately 300 kg, were selected for the study. The camels had been reared at the Camel Research Institute in the 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 during the late summer. Transportation of the camels was conducted between the Camel Research Institute in Baphgh to Yazd and back to Institute on smooth roads in an open truck and took 5 h (about 300 km). Stocking density was about 1 m^2 per animal. Environmental temperature and relative humidity during the journey were 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 8:30 A.M. (T1), after 1 h of transportation at 9:30 A.M. (T2), and immediately at the end of the journey after unloading and *n* arrival at the Institute (T3) at 1:30 P.M. A final blood sample was taken 24 h after arrival (T4). Blood samples were collected by jugular venepuncture into vacuum containers containing EDTA (Becton Dickinson, NJ, USA). The blood samples were placed on ice until plasma was separated (<2 h). The samples were centrifuged at 750×*g* for 20 min, and then the plasma was pipetted into different aliquots and stored at −20°C until analysis. Total plasma protein was measured by the Biuret method and albumin by the bromocresol green method. SAA was measured using a solid-phase sandwich enzyme-linked immunosorbent assay (Tridelta Development Plc, Wicklow, Ireland). Haptoglobin (Hp) was measured according to preservation of the peroxidase activity of hemoglobin, which is directly proportional to the amount of Hp (Tridelta Development Plc, Wicklow, Ireland). The analytical sensitivities of these tests in plasma have been determined as 0.3 µg/ml for SAA and 0.0156 mg/ml for Hp by the manufacturer. Fibrinogen was measured by heat precipitation-refractometry method as described by Duncan et al. (1994). Ceruloplasmin activity was measured according to its phenylenediamine oxidase activity (Sunderman and Nomoto 1970).

Statistical analysis

The data are presented as mean ± standard error (SE). A two-way (time and 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 least-significant-difference procedure. All calculations were performed using SPSS/PC software.

Results

The mean (±SE) circulating concentrations of selected plasma proteins related to measurements taken before, during, and after transportation are shown in Table 1. The mean concentrations of fibrinogen and haptoglobin in the basal pre-transport conditions were 3.10±0.47 and 0.3±0.04 g/l and ranged from 2.2 to 3.6 and 0.11 to 0.5 g/l, respectively. The mean concentration of SAA in the basal pre-transport conditions was 9.77±0.62 μg/ml and ranged between 6 and 12 μg/ml. The mean ceruloplasmin concentration in the basal pre-transport conditions was 0.096±0.01 g/l and ranged from 0.041 to 0.130 g/l. The mean concentrations of total protein and albumin in the basal pre-transport conditions were 60.8±6.1 and 38.3±2.1 g/l, respectively, and ranged between 46.3–87.2 and 25.5–48.3 g/l, respectively.

No significant difference was observed in any parameter between the sexes at any sampling time. The main effect of transportation and time×gender interaction was not significant for all studied parameters ($P<0.05$).

Discussion

During transportation, animals were exposed to a variety of potential stressors such as motion of the vehicle, noise, vibrations, centrifugal forces, rapidly changing light conditions, heat, cold, poor air quality, deck height, mixing of unfamiliar groups, poor road conditions, and the possible lack of water and feed. All of these factors can be highly stressful and compromise welfare and may also reduce an animal's productivity and the quality of its products (Broom and Johnson 1993; Parrott et al. 1998; Broom 2000; Hurtung 2003). For these reasons, many researchers have attempted to quantify the severity of the stress imposed by the various stages involved in transportation and to identify acceptable conditions and methods to minimize the adverse effects of transportation. 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).

Due to the relative ease with which they can be obtained, plasma proteins have been studied extensively in both humans and animals. Alterations in their amounts and metabolism under many different conditions have also been investigated. APPs with their blood concentration increasing (positive APPs) or decreasing (negative APPs) in response to infection, inflammation, or stress have recently been proposed as welfare indicators (Eckersall 2000; Murata et al. 2004). Species variations in APPs occur because of inherent variations in synthesis and in the rates of entry and exit of these APPs from the circulation (Kaneko 1997). The mechanism behind the stress-induced APP response is not known, but a hypothesis based on a neuroendocrine-immune network concept has recently been put forward, indicating that non-inflammatory and psycho-physical stressors activate the combined action of the sympatho-adrenal axis and the hypothalamic–pituitary–adrenal axis. This would affect both the immunity-related cells and the release of glucocorticoids and would ultimately lead to the production and release of APPs (Murata 2007). Glucocorticoids have been shown to induce or facilitate hepatic APP synthesis in vitro (Higuchi et al. 1994; Alsemgeest et al. 1996).

Table 1 Mean (± SE) of plasma proteins in female and male dromedary camels (n=5 in each gender) before transport (T1), 1 h after transport initiation (T2), at the end of transportation (T3), and 24 h after arrival (T4)

Parameter[a]		T1	T2	T3	T4
Fibrinogen (g/l)	Female	3.02±0.56	3.14±0.60	3.24±0.50	3.02±0.30
	Male	3.18±0.42	3.24±0.39	3.32±0.43	3.34±0.41
	Total	3.10±0.47	3.19±0.48	3.28±0.44	3.18±0.38
haptoglobin (g/l)	Female	0.31±0.04	0.31±0.03	0.24±0.06	0.26±0.05
	Male	0.28±0.07	0.29±0.08	0.26±0.07	0.31±0.08
	Total	0.30±0.04	0.30±0.04	0.25±0.04	0.28±0.04
Serum amyloid A (μg/ml)	Female	9.92±0.87	9.64±0.54	10.32±0.51	8.08±0.64
	Male	9.62±0.99	10.4±1.06	10.57±1.20	9.10±0.54
	Total	9.77±0.62	10.02±0.57	10.44±0.61	9.04±0.51
Ceruloplasmin (g/l)	Female	0.084±0.017	0.108±0.016	0.107±0.015	0.080±0.011
	Male	0.109±0.006	0.114±0.013	0.122±0.011	0.102±0.014
	Total	0.096±0.010	0.111±0.010	0.115±0.010	0.090±0.010
Total protein(g/l)	Female	58.2±4.1	61.1±4.8	61.1±5.9	57±7.2
	Male	63.4±7.2	54.8±4.1	53±4.14	55±6.1
	Total	60.8±6.1	57.95±4.3	57.05±5	56±5.8
Albumin (g/l)	Female	40.0±2.30	38.90±1.40	39.80±1.30	35.10±2.50
	Male	36.5±3.50	39.69±2.65	38.10±1.70	38.30±2.20
	Total	38.3±2.10	39.29±1.91	39.00±1.10	36.60±1.70

[a] No significant difference was observed in any parameter between sexes at all sampling times. The main effect of time and time×gender interaction was not significant for all parameters ($P<0.05$)

Camels are animals exposed to a wide array of physiological and pathological stressors in harsh grazing condition. However, they acquire various biochemical adaptive patterns to withstand these conditions. In the current study, circulating levels of fibrinogen, haptoglobin, serum amyloid A, ceruloplasmin, total plasma protein, and albumin were investigated in relation to road transportation in dromedary camels.

Haptoglobin is an α_2-globulin synthesized in the liver (Feldman et al. 2000) and is a major APP in numerous species of livestock and domesticated animals. Elevated haptoglobin concentrations occur not only with inflammation but also with some conditions not generally associated with inflammation or tissue damage (Murata et al. 2004). In the present study, the mean serum basal concentrations of Hp in Iranian dromedary camels were within the reference value (0.11–0.61 g/l) reported by Nazifi et al. (2006) for serum Hp of apparently healthy dromedary camels. Results from this study show that Hp concentrations did not change significantly due to road transportation in dromedary camels and agree with the results from Averós et al. (2009) and Hicks et al. (1998) in swine and with Hirvonen et al. (1997) and Arthington et al. (2003) in cattle. No increase in Hp concentrations after long (443.5 km) and short (32.3 km) journeys were found in piglets (Averós et al. 2009), while Piñeiro et al. (2007) reported an increase in Hp concentrations following prolonged transportation in pigs. In bovine, while some studies have found an increase in serum Hp levels after transportation-induced stress (Murata and Miyamoto 1993; Earley et al. 2006; Qiu et al. 2007), Sporer et al. (2008) reported a decrease in Hp level following road transportation. Serum and bronchoalveolar lavage fluid Hp was increased following the stresses of transportation, weaning, and co-mingling with calves (Mitchell et al. 2008).

SAA proteins comprise a superfamily of apolipoproteins and are considered one of the major acute phase reactants in vertebrate. SAA proteins are highly conserved across evolutionary distinct vertebrate species with respect to both their sequence and inductive capacity, and it is therefore generally assumed that they have a crucial, yet ill-identified, protective role during inflammation (Uhlar and Whitehead 1999). Elevated serum SAA levels are found following inflammation and also under conditions unrelated to inflammation such as physical stress or at parturition (Murata et al. 2004). Arthington et al. (2003) showed that transportation journeys of 344 km could increase SAA concentration in beef calves (48.9 vs. 33.4 µg/ml for transported and non-transported calves, respectively). Lomborg et al. (2008) demonstrated marked SAA responses in healthy adult cattle after exposure to complex stressors (transportation, tie stall housing, slippery floors, and social isolation). In the present study, mean baseline levels of SAA in dromedary camels obtained before transportation were lower than values reported in adult cattle (14 mg/l) by Lomborg et al. (2008). Road transportation had no significant effect on SAA concentration in dromedary camel.

Ceruloplasmin (Cp) is a blue multi-copper oxidase, which is found in the plasma of vertebrate species. The protein is synthesized in the liver as a single polypeptide chain and secreted into the plasma with six atoms of copper bound per molecule (Fleming and Gitlin 1990). Ceruloplasmin is present in the α_2-globulin fraction of vertebrate plasma, and 95% of plasma copper is bound to it. Purification and partial characterization of camel Cp revealed that the physico-chemical parameters of camel Cp did not differ considerably from those of other mammals Cp levels in other studies (Essamadi et al. 2002). The mean basal pre-transport concentration of plasma Cp of Iranian dromedary camels were higher than values reported in the serum of bactrian camel (54.4± 9.2 mg/l) by Zongping (2003) and approximately similar to the values reported by Kincaid et al. (1986) for cattle.

Transportation did not change Cp concentration in Iranian dromedary camel and is in agreement with results obtained by Arthington et al. (2003) in beef calves. However, some studies have reported an increase in Cp concentration due to road transportation in calves (Qiu et al. 2007).

Plasma fibrinogen in all species is synthesized in the liver and is released into the circulation as a molecule consisting of three non-identical polypeptide chains. Hyperfibrinogenemia, which is characteristic of a broad range of bacterial infections and other inflammatory conditions, has been reported in a wide range of vertebrates (Gentry 2004). Mean basal fibrinogen concentration obtained in the present study was greater than values (241.2±15.4 mg/dl) reported by Hussein et al. (1992) but within the range (200–400 mg/dl) reported by Higgins and Kock (1984) for dromedary camels. Road transportation had no significant effect on plasma fibrinogen concentration in dromedary camel and is in agreement with results obtained by Earley et al. (2006) in young cattle but differ from results obtained by other workers (Phillips 1984; Qiu et al. 2007; Mohammadi et al. 2007) who reported an increase in plasma fibrinogen following road transportation in calves.

Road transportation has been reported to cause dehydration and may manifest itself as a hyperproteinemia (Atkinson 1992; Schaefer et al. 1992). In the current study, plasma albumin and total protein did not change significantly due to stress, suggesting no dehydration during a 5-hour transportation journey in dromedary camel. This is different from previously reported data in cattle (Knowles et al. 1999; Mohammadi et al. 2007), sheep (Knowles et al. 1996), goat (Kadim et al. 2006), and the horse (Smith et al. 1996), which all showed a significant increase in total protein and albumin levels in relation to transportation stress. No signs of dehydration during transportation in dromedary camels in relatively high temperatures might reflect the camel's ability to utilize internal water due to their physiological behavior (Mehrotra and Gupta 1989).

The literature concerning the effects of transportation on APP concentrations in livestock is variable, as described above. In addition, duration of the journey (Piñeiro et al. 2007; Averós et al. 2008, 2009) and both the conditions and transportation quality (Piñeiro et al. 2007) can influence the acute phase response due to transportation. More investigation into APP changes during transportation and other stressful conditions is essential to discriminate these changes from changes due to inflammatory conditions and also to assess if they can be used as welfare indicators in various species. Although the results obtained in this work did not reveal any significant changes during a 5-h road journey in the various plasma proteins in dromedary camels, these results are of value for further research.

We conclude that stressful conditions during the journey under hot summer environmental conditions were not severe enough to trigger an acute phase response in the dromedary camels. This might be due to the camel's ability to withstand harsh conditions. To sum up, the value of APPs as welfare indicators in the case of dromedary camels for short transport periods would be limited. Nevertheless, due to the lack of scientific literature concerning APP in dromedary camels, further research is needed to define the usefulness of APPs as potential markers of camel health and welfare and to develop a baseline data for practical applications.

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