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To cite this version:

Mohammed El Khasmi, Youssef Chakir, Rita Bargaâ, Kaoutar Barka, Islah Lektib, et al.. Impact of transport distance on stress biomarkers levels in dromedary camel (Camelus dromedarius). Emirates Journal of Food and Agriculture, 2015, 27 (6), pp.507-512. 10.9755/ejfa.2015.04.058 . hal-02631512

HAL Id: hal-02631512

https://hal.inrae.fr/hal-02631512v1

Submitted on 27 May 2020

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Emirates Journal of Food and Agriculture. 2015. 27(6): 507-512
doi: 10.9755/ejfa.2015.04.058
http://www.ejfa.me/

REGULAR ARTICLE

Impact of transport distance on stress biomarkers levels in dromedary camel (*Camelus dromedarius*)

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ABSTRACT

The welfare conditions of domestic animals during transport to the slaughterhouse are largely known able to influence the level of their stress, physiology and meat quality. Furthermore, the reaction of animals to stressors depends on the duration and intensity of these stressors. The objective of the study was to investigate the effect of transport distance on some blood physiological indicators of stress and biomarkers of oxidant stress in camels. Transport distances were categorized as short (72-80km), medium (160-170km) and long (350-360km) distance. Haematocrit, haemolysis, cortisol, glucose, lactate, malondialdehyde and catalase increased gradually and significantly ($P<0.05$) with transport distance, and that over longer distance these parameters were more significant ($P<0.005$) compared with short-distance. A positive correlation ($P<0.001$) was obtained between cortisol, glucose, lactate, malondialdéhyde and catalase.As conclusion, road transport is very stressful in camel, and the effects of this stress on the relevant indicators rising much with distance. Future work should focus on the effect of transport distance on some quality indicators of camel meat.

Keywords: Camel; Haemolysis; Oxidant stress; Road transport; Stress responses

INTRODUCTION

It is well knowen that in large animals, transportation involves many stressful factors such as handling, loading, unloading, unfamiliar environments, oscillation and vibration of the mean of transport, noise, social regrouping, poor ventilation, and deprivation of both food and water. These responses changed with the duration of transportation (Padalino, 2015). Physical and psychological stress induce hypophyseal–pituitary–adrenal axis activity, causing an increased hypercortisolaemia (Snow and Mackenzie, 1977) and sympathetic–adrenal axis activation, which results in catecholamine release (Freg et al., 1985).

During environmental stress, marked changes in the levels of reactive oxygen species (ROS) scavengers occurred in the serum of camels (Kataria et al., 2010). As a very stressful factor, road transport to the slaughterhouse isable to induce hypercortisolaemia (Saeb et al., 2010; El Khasmi et al., 2013) and may lead to an increase of free radical generation (Nazifi et al., 2009) in camel.

Oxidative stress (OS) occurs when the oxidant/antioxidant imbalance results in excess production of ROS and leads to cellular and tissue damage. OS plays an important role in cancer, neurological, heart and pulmonary diseases (Dunlap et al., 2006), and may occur during and after stressful events such as transport, exercise and intensive management in both humans and animals (Kirschvink et al., 2002). In horses, a 12 hour journey was able to induce a significant increase in plasma MDA concentrations compared to baseline values (Onmaz et al., 2011). In the same species, an 8-hour journey by road transport increased the average plasma total antioxidant status soon after unloading (Niedźwiedź et al., 2013). Supplementation of diet with antioxidants prior to transporting may reduce significantly the effect of OS during transportation in goat (Adenkola et al., 2011). According to Minka and Ayo (2013), long distance travel affected the oxidant/antioxidant status of goat and decreased it's excitability and grazing behavior after unloading. However, in the same conditions, goats supplemented with vitamin C before the transport, did not show these responses after

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Received: 12 January 2015; **Revised:** 04 May 2015; **Accepted:** 05 May 2015; **Published Online:** 05 May 2015

unloading and their oxidant/antioxidant systems remained in balance after the transport (Minka and Ayo, 2013). In addition, preincubation of camel erythrocytes with ascorbic acid or vitamin E reduced significantly it's hydrogen peroxide haemolysis (Chakir et al., 2013).

The objective of the study was to investigate the effect of transport distance on some circulating indicators of stress: haematocrit (Ht), haemolysis (H%), cortisol, glucose and lactate (LAC), and twoo biomarkers of OS: malondialdehyde (MDA) and catalase (CAT) in camels.

MATERIALS AND METHODS

Animals

18 male camels (6 to 9 years of age, average weight of 380 ± 50 kg) were divided to 3 groups: group I, group II and group III of 6 animals and were transported over different distances to Casablanca Municipality slaughterhouse. These groups were transported respectively from Settat (72-80 km), Fqih Ben Saleh (160-170 Km) and Essaouira (350-360) representing respectively 90, 200 and 400 minutes at an average 60 km/h speed. All camels were clinically healthy and feed deprived overnight.

Camels were transported in a side-facing position with a load density of 1/m^2. During the travel they could not feed and drink. They were loaded with a tilt angle of about 45° to the long axis of truck and travelled in the summer season, on morning (6h am-9h 30 am, average temperature of around 25-30°C and relative humidity of around 55-65%, without rain or wind). The animal groups were transported at different days and in different trucks which were driven by different drivers. During all transportations, the road was asphalted until the arrival to the slaughterhouse.

Blood sampling

At the end of road transport, blood samples were collected by jugular vein puncture from each camel between 11h and 13h in a tube swith and without EDTA. Serum was separated by centrifugation at 750g for 15min, pipetted into aliquots and then stored at -20°C until analysis of glucose, LAC, cortisol, MDA and CAT. Blood collected in EDTA tubes, was used to measure the Ht and prepare the erythrocytes suspensions for H% analysis.

Haematocrit measure

Ht was determined by centrifuging a precise amount of blood in calibrated haematocrit tubes (Hettich Haematokrit D-7200), the report cell mass/plasma was expressed as % by direct reading on the tube:

Ht(%)=[(level of pellet)/(overall height)]x100.

Preparation of erythrocytes suspensions

Erythrocytes were isolated by centrifugation for 20min at 1000xg. The plasma and buffy coat were carefully removed using a micropipette. The cells pellet was washed three times with 310mOsm isotonic phosphate buffer (pH 7.4), centrifuged at 1000xg for 10min and finally suspended in an equal volume of isotonic phosphate buffer. This constituted the erythrocyte suspension, which was stored at 4°C for 24h until further analysis (Dodge et al., 1963).

Haemolysis measure

The procedure of H% was a slightly modified method of O'Dell et al. (1987). A 100µl aliquot of washed erythrocyte suspension was added to test tubes containing 5mL of 0.2%, 0.3%, or 0.9% buffered salt solutions (BSS, pH 7.4). The contents of these tubes were gently mixed by inverting them five times and were allowed to stand at 37°C for 30min. Thereafter, then these tubes were centrifuged at 1270xg for 10min to pellet the cells. The supernatant was then transferred into a glass cuvette and the absorbance was measured at 540nm, measure data wave length of 540nm using a spectrophotometer by reading the absorbance. The profile of the H% of our camels was previously analyzed by using a BSS (pH 7.4) concentrations, ranging from 0.1% to 0.9%. H% in each tube was expressed as a percentage, taking as 100% the maximum value of absorbance of distilled water. BSS (0.9%) was considered as a control sample. The percent haemolysis was calculated according to Fraukner and King (1970) asfollows:

H(%)= (Optical density of test/Optical density of distilled water)x100.

H(%) curve was obtained by plotting percent haemolysis against the saline concentrations.

Glucose, lactate and cortisol dosage

Serum glucose and LAC concentrations were measured using a spectrophotometric procedure from commercially available kits. Serum cortisol levels were analyzed by radioimmunoassay method in the National Center of Science and Nuclear Technical Energy in Maamoura, Morocco, by using commercially available coated RIA tubes for human cortisol. This kit proved efficient in previous experiments in dromedary camels (El Khasmi et al., 2010; 2013), and was purchased from DIAsource (Immunoassays S.A., Nivelles, Belgium). Validation for assays included limits of detection, and precision in standard curve following sample dilution, inter- and intra-assays.

Malondialdehyde and catalase analysis

Serum TBARS were measured by a colorimetric method based on a previously described method. TBARS

values were expressed in nmol/ml MDA equivalents. Briefly, serum samples were homogenized with cold and were mixed with trichloroacetic acid (20%) and the precipitate was dispersed in H_2SO_4 (0.05 M). TBA (0.2% in sodium sulfate 2 M) was added and heated for 30min in boiling water bath. TBARS adducts were extracted by n-butanol and measured at 532 nm (Satho, 1978).

The CAT activity was measured using the method of Aebi (1974). The disappearance of hydrogen peroxide was monitored spectrophotometrically at 240nm for 5min. A molar extinction coefficient of 0.041/mM/cm was used to determine the CAT activity. The activity was defined as the µmol decreased H_2O_2/min/mg protein. The amount of total proteins was determined using Biuret method (Gornall et al., 1949).

Statistical analysis

The data were expressed in SI units and analyzed by the Mann-Whitney U test for comparison between groups. All values were expressed as mean and standard error (SE), and P<0.05 was seen as statistically significant.

RESULTS

Serum cortisol, glucose and lactate

The serum levels of cortisol (ng/mL), glucose (mM) and LAC (mM) of camels travelling for medium distance (160-170 Km) were significantly (P<0.05) higher than those measured in camels subjected to short transport distance (72-80 Km) (respectively 152.4±25.18 vs 88.32±19.4; 7.08±0.21 vs 5.07±0.28 and 12.99±0.16 vs 9.97±0.31) (Figs. 1 & 2). These parameters became more higher (P<0.005) when camels were transported for a long-distance (350-360 Km) (respectively 231.7±23.75; 9±0.35 and 14.88±0.29) (Figs. 1 & 2).

Haematocrit and haemolysis

It follows from Fig. 3 that in camels transported over a long-distance, Ht(%) and H50 (mOsm/L) were significantly higher when compared to those observed in camels transported over a short-distance (respectively 43.17±0.98 vs 39.17±1.17 and 147.65±3.99 vs 132.65±3.99). During transport for medium-distance, these parameters didn't show any significant variation (Fig. 3).

Serum malondialdehyde and catalase

The Table 1 shows that serum levels of MDA (nM) and CAT (KU/L) increased significantly (P<0.05) during medium-transport distance, compared with short-distance (respectively 3.88±0.20 vs 1.58±0.38 and 79.13±3.84 vs 60.08±3.18) and that over longer distance, these factors were more significant (P<0.005) (respectively 6.44±0.52

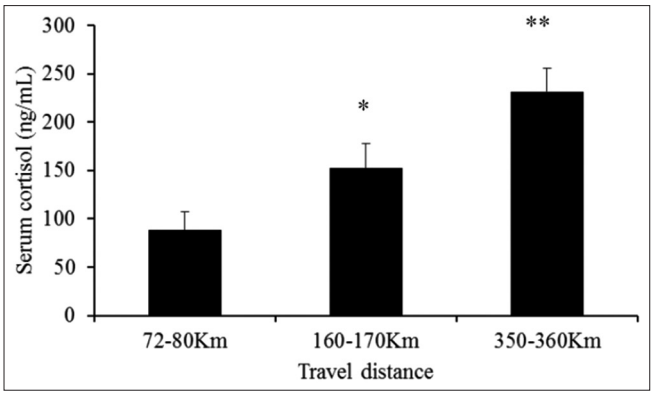

Fig 1. Effect of transport distance on serum cortisol levels in Camel. (M±ET, *P<0.05; **P<0.005; comparison of medium and long-distance with short-distance).

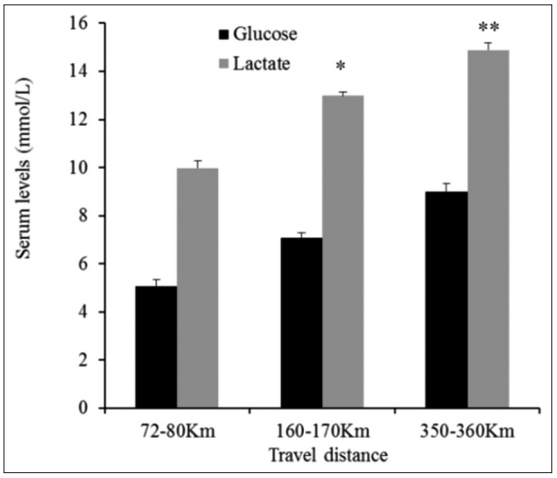

Fig 2. Effect of transport distance on serum levels of glucose and lactate in Camel. (M±ET, *P<0.05; **P<0.005; comparison of medium and long-distance with short-distance).

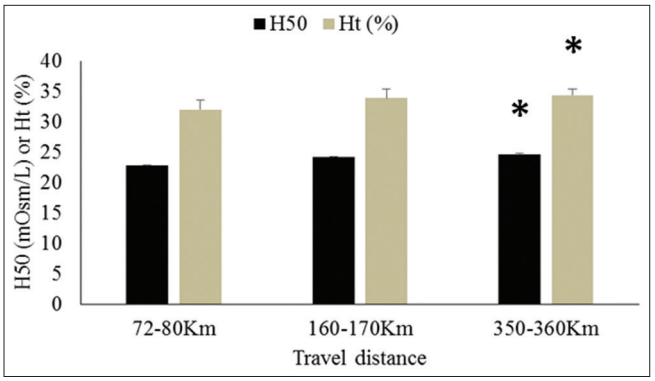

Fig 3. Effect of transport distance on haemolysis 50 (H50) and haematocrit (Ht) in Camel. (M±ET, *P<0.05; **P<0.005; comparison of medium and long-distance with short-distance). H50 is the Phosphate buffered saline concentration able to induce an haemolysis of 50% of erythrocytes.

Table 1: Effect of transport distance on serum levels of malondialdehyde and catalase in Camel

	Transport distance		
	72-80 km	160-170 km	350-360 km
Malondialdehyde (nmol/mL)	1.58±0.38	3.88±0.20*	6.44±0.52**
Catalase (KU/L)	60.08±3.18	79.13±3.84*	93.95±3.62**

M±ET, *P<0.05; **P<0.005; comparison of medium and long-distance with short-distance

Table 2: Correlation of Pearson between cortisol, glucose, lactate, malondialdehyde and catalase

	Cortisol	Glucose	Lactate	MDA
Glucose	0.966			
	0.000			
Lactate	0.950	0.988		
	0.000	0.000		
MDA	0.974	0.994	0.981	
	0.000	0.000	0.000	
Catalase	0.970	0.992	0.988	0.984
	0.000	0.000	0.000	0.000

Value of P

and 93.95±3.62). In addition, a positive correlation (P<0.001) was obtained between Cor, Glu, Lac, MDA and Cat (Table 2).

DISCUSSION

Cortisol, glucose and lactate

In our study we have evaluated the effects of three transport distances (short: 72-80 Km, medium: 160-170 Km and long: 350-360 Km) on stress and OS indicators in camels. The increase in the circulating levels of stress biomarkers such as cortisol, glucose and LAC was associated with the increasing travel distance, has been reported by other investigations in other domestic species (Malena et al., 2006; DeSilva and Kalubowila, 2012). Cortisol levels were higher in blood samples collected after transport than in samples collected before transport, in camel (El Khasmi et al., 2010), sheep (Parker et al., 2003), pig (Smiecińska et al., 2011), horse (Tateo et al., 2012), goat (Minka and Ayo, 2010), bull (Gupta et al., 2007), calf (Bernardini et al., 2002), black steer calf (Ishizaki and Kariyaj, 2010) and monkey (Kim et al., 2005). In our camels, blood samples were taken at the same time interval at the slaughterhouse, so, the cortisol levels measured in the study were slightly influenced by the circadian rhythm.

The gradual and significant increase of serum cortisol, glucose and LAC with transport distance observed in this work may be explained by an activation of hypothalamo-hypophyso-adrenal axis (HHAA) during exposition to road transport stress (Saeb et al., 2010; El Khasmi et al., 2013). Cortisol, released by the adrenal cortex in response to the pituitary hormone ACTH (Omer-Elfaroug et al., 2013), is able to induce peripheral catabolism, particularly of tissue proteins and stimulates the neoglucogenesis in the liver (Mormède, 2007). Road transportation of animals is a potent stressor before the slaughter, which results from vehicle motion, noise and vibration, and can change several of the animal's physiological systems (e.g. cardiovascular, immune and endocrine via the activation of the HHAA (Broom, 2003).

The increase of plasma glucose levels under road transportation observed in our camels agrees with the results found in horse (Stull and Rodiek, 2000), goat (Minka and Ayo, 2010) and Holstein calf (Bernardini et al., 2002). This hyperglycaemia may be primarily due to catecholamines secretion induced by an activation of the sympathetic nervous system (Sanders and Straub, 2002), resulting muscle and liver glycogenolysis thenan increase of circulating glucose and lactate related to fuel homeostasis (Monin, 2003; (Dronjak et al., 2004).

Haematocrit and haemolysis

In our study, long-distance transport increased significantly Ht and H% compared with short-distancc. During the road transport of our camels with a load density of $1/m^2$, the increase of Ht was previously observed as a stress response in camel (El Khasmi et al., 2013) and may be explained by a water loss by thermoregulation and urination, and/or a splenic contraction (Carlson, 1990). The release of catecholamines into the circulation by an activation of the sympathetic-adrenal medullary system under stress (McCarty et al., 1988), could rise Ht by a splenic contraction and then a release of erythrocytes into the circulation. This mechanism may be induced by the action of catecholamines on α-adrenergic receptors located in the splenic capsule (Montane et al., 2002; Tauler et al., 2003). In our investigation, the total protein were not analyzed, so, as we have demonstrated any increase of total protein, we can not suggest a relative increase of the Ht.

Long-distance transport increased significantly the H% of camel's red blood cells (RBC). In camel, we had previously determined the potential use of H% as a diagnostic tool in road transportation stress (El Khasmi et al., 2013) which my induce oxidant alterations of plasmic membrane of RBC and then increase H%. Camel's RBC are characterized by a significant increase of osmotic resistance and cell membrane stability by comparison with those measured in other mammalian species, du to the biochemical composition of camel RBC membrane (Al-Qarawi and Mousa, 2004).

Oxidant stress

In this study, serum levels of MDA and CAT increasedgradually and significantly with transport

distance. One of the explicative factor of these oxidant indicators seems to be a higher stress activity during the road transportation as suggested by the higher levels of serum cortisol. In fact, it had been reported in rats that administration of glucocorticoid hormones caused lipid peroxidation by decreasing the nonenzymatic antioxidant capacity and suppressing the enzymatic antioxidant systems in erythrocytes (Orzechowski et al., 2000) and hypothalamus (Flerov and V'iushina, 2011).

An increase in free-radical generation as a result of stress may be responsible of the high levels of MDA and Cat observed here, resulting oxidative damage of the erythrocytes membrane (Ramnath et al., 2008; Nazifi et al., 2009).

The ROS are compounds with high potential to damage almost all types of cellular constituents by increasing lipid peroxidation, resulting induction and/or amplification of a number of tissular lesions (Bernabucci et al., 2002). The mammalian RBC are able to defend themselves against these compounds, by an effective and complex antioxidant system, including protective enzymes and biological antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione, AA, and vitamin E (Ramnath et al., 2008). In camel, oxidative alterations of camel erythrocytes induced hydrogen peroxide, may be attenuated by incubation with vitamins A and/or C (Chakir et al., 2013).

As conclusion, in camel, the significant gradual increase of circulating levels of biomarkers analyzed here e.g. cortisol, glucose, LAC, MDA and CAT with transport distance, could be explained at least in part with a higher stress activity during the road transportation. These stress conditions could induce metabolic changes enhancing the formation of reactive oxygen species then lipid peroxidation which may be responsible for haemolysis. However, to confirm this hypothesis, other factors must be taken in consideration such as absence of blood sampling before transport, use of animals handled differently, and the differences of trucks, drivers, days travel and road topographies. The increase of transport distance might be very stressful in camel, so, the maximum care should be taken during this process. Future work should focus on the effect of transport time on the stress oxidant in camel meat.

ACKNOWLEDGMENTS

The authors thank the President of urban municipalities of Casablanca and Dr. ABOUHAFS Rachid the responsible for prefectural veterinary service of Casablanca, to carry out this work.

Author contributions

M. E. made a major contribution to this article, was involved in conception and design of study and redaction. Y. C. was involved in documentation, tissue and blood sampling and analysis of results. R. B. was involved in physicochemical analysis, acquisition of data and interpretation. K. B. was involved in blood sampling, physicochemical analysis, acquisition of data and interpretation. I. L. was involved in documentation, physicochemical analysis, acquisition of data and interpretation. N. E. was involved in radioimmunological analysis of cortisol, acquisition of data and interpretation. A. B. was involved in processing and statistical analysis of experimental results. B. F. was involved in revision of experimental design.

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