

## 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, malondialdehyde 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 known 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 is able 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 (Niedzwiedz 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 its 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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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 its 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 two 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  $380 \pm 50$  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 Settât (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  $1/\text{m}^2$ . During the travel they could not feed and drink. They were loaded with a tilt angle of about  $45^\circ$  to the long axis of truck and travelled in the summer season, on morning (6h am-9h 30 am, average temperature of around  $25\text{-}30^\circ\text{C}$  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 with and without EDTA. Serum was separated by centrifugation at 750g for 15min, pipetted into aliquots and then stored at  $-20^\circ\text{C}$  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:

$$\text{Ht}(\%) = [(\text{level of pellet}) / (\text{overall height})] \times 100.$$

### 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  $4^\circ\text{C}$  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 100 $\mu\text{L}$  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  $37^\circ\text{C}$  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) as follows:

$$\text{H}(\%) = (\text{Optical density of test} / \text{Optical density of distilled water}) \times 100.$$

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  $\mu\text{mol}$  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

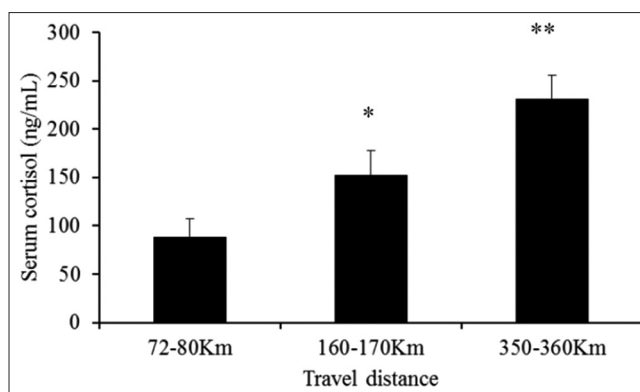
The serum levels of cortisol (ng/mL), glucose (mM) and LAC (mM) of camels travelling for medium distance (160-170 Km) were significantly ( $P < 0.05$ ) higher than those measured in camels subjected to short transport distance (72-80 Km) (respectively  $152.4 \pm 25.18$  vs  $88.32 \pm 19.4$ ;  $7.08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

### Haematocrit and haemolysis

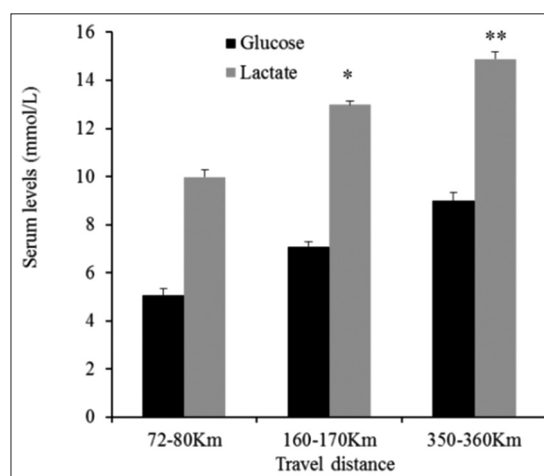
It follows from Fig. 3 that in camels transported over a long-distance, Ht(%) and H50 (mOsm/L) were significantly higher when compared to those observed in camels transported over a short-distance (respectively  $43.17 \pm 0.98$  vs  $39.17 \pm 1.17$  and  $147.65 \pm 3.99$  vs  $132.65 \pm 3.99$ ).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

### Serum malondialdehyde and catalase

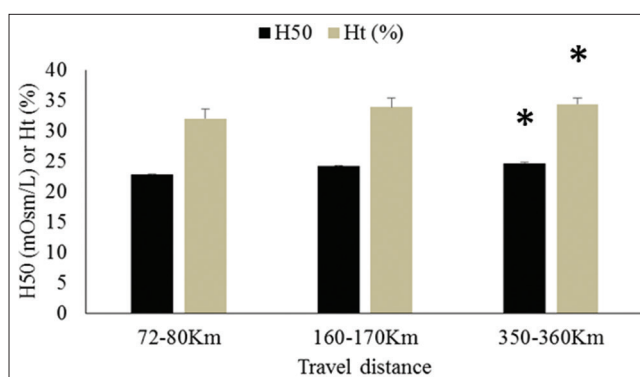
The Table 1 shows that serum levels of MDA (nM) and CAT (KU/L) increased significantly ( $P < 0.05$ ) during medium-transport distance, compared with short-distance (respectively  $3.88 \pm 0.20$  vs  $1.58 \pm 0.38$  and  $79.13 \pm 3.84$  vs



**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.

$60.08 \pm 3.18$ ) and that over longer distance, these factors were more significant ( $P < 0.005$ ) (respectively  $6.44 \pm 0.52$

**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 0.000	0.988 0.000		
MDA	0.974 0.000	0.994 0.000	0.981 0.000	
Catalase	0.970 0.000	0.992 0.000	0.988 0.000	0.984 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 Kariyaji, 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 then an 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-distance. During the road transport of our camels with a load density of 1/m<sup>2</sup>, 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  $\alpha$ -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 may 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, due to the biochemical composition of camel RBC membrane (Al-Qarawi and Mousa, 2004).

### Oxidant stress

In this study, serum levels of MDA and CAT increased gradually 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.

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## 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.

## REFERENCES

- Adenkola, A. Y., J. O. Ayo, A. K. B. Sakey and A. B. Adelaiye. 2011. Modulatory role of ascorbic acid on behavioural responses of pigs transported by road during the Harmattan Season. *Niger. J. Physiol. Sci.* 26: 61-65.
- Aebi, H. 1974. Catalase. In: *Method of Enzymatic Analysis*. Bergmeyer, H. U., editor. Academic Press, New York, NY, USA, Pp. 673-684.
- Al-Qarawi, A. and H. M. Mousa. 2004. Lipid concentrations in erythrocyte membranes in normal, starved, dehydrated and rehydrated camels (*Camelus dromedarius*), and in normal sheep (*Ovis aries*) and goats (*Capra hircus*), *J. Arid Environ.* 59: 675-683.
- Bernabucci, U., B. Ronchi, N. Lacetera and A. Nardone. 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *J. Dairy Sci.* 85: 2173-2179.
- Broom, D. M. 2003. Transport stress in cattle and sheep with details of physiological and other indicators. *Dtsch. Tierarztl. Wochenschr.* 110: 83-89.
- Carlson, G. P. 1990. Clinical chemistry tests. In: Smith, editor. *Large Animal Internal Medicine*. C. V. Mos by Co., St. Louis, M. O., p. 393.
- Chakir, Y., M. E. L. Khasmi, M. Farh, R. Bargaâ, F. Riad, A. Safwate, E. H. Tahri, N. E. L. Abbadi, R. Abouhafs and B. Faye. 2013. Effects of vitamin E and vitamin C on hydrogen peroxide-induced hemolysis in Moroccan dromedary camels (*Camelus dromedarius*). *Green. J. Med. Sci.* 3: 111-120.
- De Silva, P. H. G and A. Kalubowila. 2012. Relationship of transport distance, sex on live weight loss of pigs during transit to slaughter house. *Vet. World.* 5: 150-154.
- Dodge, J. T., C. Mitchell and D. J. Hanahan. 1963. The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. *Arch. Biochem. Biophys.* 100: 119-130.
- Dronjak, S. L., D. Gavrilović and M. B. Filipović. 2004. Radojčić, immobilization and cold stress affect sympatho-adrenomedullary system and pituitary-adrenocortical axis of rats exposed to long-term isolation and crowding. *Physiol. Behav.* 8: 409-415.
- Dunlap, K. L., A. J. Reynolds and L. K. Duffy. 2006. Total antioxidant power in sled dogs supplemented with blueberries and the comparison of blood parameters associated with exercise. *Comp. Biochem. Phys.* 143: 429-434.
- Khasmi, E. L. M., F. Riad, A. Safwate, E. H. Tahri, M. Farh, N. E. L. Abbadi, V. Coxam and B. Faye. 2010. Effects of preslaughter

- stress on meat quality and phosphocalcic metabolism in camels (*Camelus dromedarius*). *Journal of Camelid Science* 3: 33-38.
- El Khamsi M., Y. Chakir, F. Riad, A. Safwate, E. H. Tahri, M. Farh, N. El Abbadi, R. Abouhafs and B. Faye. 2013. Effects of Transportation Stress during the Hot-Dry Season on Some Haematological and Physiological Parameters in Moroccan Dromedary Camels (*Camelus dromedarius*). *J. Life Sci. (USA)*. 7: 13-25.
- Flerov, M. A. and A. V. V'iushina. 2011. Free radical oxidation of lipids in the rat hypothalamus under stress after cortisol pre-treatment, *Russ. Fiziol. Zh. Im. I. M. Sechenova*. 97: 898-902.
- Fraukner, W. R. and J. W. King. 1970. *Manual of Clinical Laboratory Procedures*. Cleveland, Ohio. p. 354.
- Freg, J. L., S. P. Tzankoff and E. G. Lakatta. 1985. Age-related, augmentation of plasma catecholamines during dynamic exercise in healthy male. *J. Appl. Physiol.* 59: 1033-1039.
- Gornall, A. G., C. J. Bardawill and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177: 751-766.
- Gupta, S., B. Earley and M. A. Crowe. 2007. Effect of 12-hr road transportation on physiological, immunological and haematological parameters in bulls housed at different space allowances. *Vet. J.* 173: 605-616.
- Ishizaki, H. and Y. Kariyaji. 2010. Road transportation stress promptly increases bovine peripheral blood absolute NK cell counts and cortisol levels. *Vet. Med. Sci.* 72: 747-753.
- Kataria, N., A. K. Kataria, N. Pandey and P. Gupta. 2010. Serum biomarkers of physiological defense against reactive oxygen species during environmental stress in Indian dromedaries. *H. V. M. Bioflux*. 2: 55-60.
- Kim, C. Y., J. S. Han, T. Suzuki and S. S. Han. 2005. Indirect indicator of transport stress in hematological values in newly acquired cynomolgus monkeys. *J. Med. Primatol.* 34: 188-192.
- Kirschvink, N., B. De Moffart, N. Smith, D. Marlin, C. Roberts and P. Lekeux. 2002. Relationship between markers of blood oxidant status and physiological variables in healthy and heaves-affected horses after exercise. *Equine Vet. J.* 34: 159-164.
- Malena, M., E. Voslárová, P. Tomanová, R. Lepková, I. Bedánová and V. Vecerek. 2006. Influence of travel distance and the season upon transport-induced mortality in fattened cattle. *Acta Vet. Brno*. 75: 619-624.
- McCarty, R., K. Horwatt and M. Konarska. 1988. Chronic stress and sympathetic-adrenal medullary responsiveness. *Soc. Sci. Med.* 26: 333-341.
- Minka, N. S. and J. O. Ayo. 2010. Physiological responses of erythrocytes of goats to transportation and the modulatory role of ascorbic acid. *J. Vet. Med. Sci.* 72: 875-881.
- Minka, N. S. and J. O. Ayo. 2013. Physiological and behavioral responses of goats to 12-hour road transportation, lairage and grazing periods, and the modulatory role of ascorbic acid. *J. Vet. Behav.* 8: 349-356.
- Monin, G., W. Przybylski and M. Koćwin-Podsiadła. 2003. Glycolytic potential as meat quality determinant. *Anim. Sci. Pap. Rep.* 21: 109-120.
- Montane, J., I. Marco, J. Lopez-Olvera, X. Manteca and S. Lavín. 2002. Transport stress in roe deer (*Capreolus capreolus*): Effect of the short acting antipsychotic. *Anim. Welfare*. 11: 295-303.
- Mormède, P. 2007. Variabilité génétique de l'axe corticotrope chez le porc: Mécanismes moléculaires et conséquence sur la production de viande. *Bull. Acad. Vét. France*. Tome. 160. 2: 79-84.
- Nazifi, S., S. Mahdi, B. Hasan and S. Saeedeh. 2009. Influence of road transportation during hot summer conditions on oxidative status biomarkers in Iranian dromedary camels (*Camelus dromedarius*). *Afr. J. Biochem. Res.* 3: 282-287.
- Niedźwiedz, A., K. Kubiak and J. Nicpoń. 2013. Plasma total antioxidant status in horses after 8-hours of road transportation. *Acta Vet. Scand.* 55: 58.
- O'Dell, B. L., J. D. Browning and P. G. Reeves. 1987. Zinc deficiency increases the osmotic fragility of rat erythrocytes. *J. Nutr.* 117: 1883-1889.
- Omer-Elfaroug, S. A., A. Sanhoury, B. E. Elwaseela, I. Fadlallah, M. Galal-Eldin Elazhari and E. Möstl. 2013. Assessment of adrenocortical activity by non-invasive measurement of faecal cortisol metabolites in dromedary camels (*Camelus dromedarius*). *Trop. Anim. Health. Prod.* 45: 1453-1458.
- Onmaz, A. C., R. Van Den Hoven, V. Gunes, M. Cinar and O. Kucuk. 2011. Oxidative stress in horses after a 12-hours transport period. *Rev. Med. Vet.* 162: 213-217.
- Orzechowski, O., P. Ostaszewski, A. Brodnicka, J. Wilczak, M. Jank, B. Balasinska, K. Grzelkowska, T. Ploszaj, J. Olczak and A. Mrowczynska. 2000. Excess of glucocorticoids impairs whole-body antioxidant status in young rats, relation to the effect of dexamethasone in soleus muscle and spleen. *Horm. Metab. Res.* 32: 174-180.
- Padalino, B. 2015. Effects of the different transport phases on equine health status, behavior and welfare: A review. *J. Vet. Behav.* 10: 272-282. doi: 10.1016/j.jveb.2015.02.002.
- Parker, A. J., G. P. Hamlin, C. J. Coleman and L. A. Fitzpatrick. 2003. Dehydration in stressed ruminants may be the result of a cortisol-induced diuresis. *J. Anim. Sci.* 81: 512-519.
- Ram Nath, V., P. S. Rekha and K. S. Sujatha. 2008. Amelioration of heat stress induced disturbances of antioxidant defense system in chicken by brahma rasayana. *Evid. Based Complement Altern. Med.* 5: 77-84.
- Saeb, M., H. Baghshani, S. Nazifi and S. Saeb. 2010. Physiological response of dromedary camels to road transportation in relation to circulating levels of cortisol, thyroid hormones and some serum biochemical parameters. *Trop. Anim. Health and Prod.* 42: 55-63.
- Sanders, V. M. and R. H. Straub. 2002. Norepinephrine, the  $\alpha$ -adrenergic receptor, and immunity. *Brain Behav. Immun.* 16: 290-332.
- Satho, K. 1978. Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method. *Clin. Chem. Acta.* 90: 37-43.
- Smiecińska, K., J. Denaburski and W. Sobotka. 2011. Slaughter value, meat quality, creatine kinase activity and cortisol levels in the blood serum of growing-finishing pigs slaughtered immediately after transport and after a rest period. *Polish J. Vet. Sci.* 14: 47-54.
- Snow, D. H. and G. Mackenzie. 1977. Some metabolic effects of maximal exercise in the horse and adaptations with training. *Equine Vet. J.* 9: 134-140.
- Stull, C. L. and A. V. Rodiek. 2000. Physiological responses of horses to 24 hours of transportation using a commercial van during summer conditions. *J. Anim. Sci.* 78: 1458-1466.
- Tateo, A., B. Padalino, M. Boccaccio, A. Maggiolino and P. Centoducati. 2012. Transport stress in horses: Effects of two different distances. *J. Vet. Behav.* 7: 33-42.
- Tauler, P., A. Aguilo, I. Gimeno, E. Fuentespina, J. A. Turand, A. Pons. 2003. Influences of vitamin C diet Supplementation on endogenous antioxidant defences during exhaustive exercise. *Eur. J. Physiol.* 446: 658-664.