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REGULAR ARTICLE

Evaluation of stress responses induced by the loading density in dromedary camel (*Camelus dromedarius*)

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ABSTRACT

The intensity of stress depends on several external factors, such as distance and conditions of transport, climate change, the nature of the journey and the vehicle used, etc. Our research aims to study the effect of loading density on certain physiological, hematological, biochemical and hormonal parameters in camels. Sixteen male animals belonging to the municipal slaughterhouse of Casablanca (west of Morocco) were divided into two groups of 8 camels to study the effect of 2 loading densities: 1camel/2-3.6 m^2 (Group I) and 1camel/1.44-1.80 m^2 (Group II). Hct, NLR, H% and biochemical parameters were analyzed in our laboratory (LPGM) at the Ben M'Sik Faculty of Sciences in Casablanca, while the hormones were analyzed by radioimmunology at the National Center of Energy, Sciences and Nuclear Techniques of Maâmoura, Morocco. In Groups I and II, rectal temperature, heart and respiratory rates, hemolysis, neutrophil/lymphocyte ratio and plasma levels of Glu, COR, T_3 and T_4 showed a significant increase ($P<0.05$) at the end of transport just after unloading by comparison to those measured before loading and transport. All these parameters were significantly ($P<0.05$) higher in Group II than those observed in Group I. An increase of the loading density (1camel/1.44-1.80 m^2) during road transport is considered as a stressful factor that could alter the physiology of the dromedary and influence the *postmortem* quality of its meat. The impact of this factor on the antioxidant status of this species will be studied later.

Keywords: Dromedary; Hormones; Leukocyte formula; Loading density; Preslaughter stress

INTRODUCTION

Because of its expected dietetic quality, camel meat could have an advantage for human consumers (Kadim et al., 2013). But, the steps preceding the slaughter of an animal for human consumption need a rigourous control to maintain product quality as well as for protecting animal welfare (Shimshony and Chaudry, 2005). During the pre-slaughter phases, domestic animals are exposed to stress-inducing factors (Terlouw et al., 2005; Deiss et al., 2009). Such stressful situation was evaluated by behavioural and physiological reactions in cows (Bourguet et al., 2010), lambs (Deiss et al., 2009), pigs (Terlouw et al., 2005) and camels (El Khasmi et al., 2013; 2015). There is good scientific evidence that many factors related to preslaughter stress can affect the well-being and meat quality of animals, and contribute indirectly to increasing the risks to food safety (Nielsen et al., 2011). Among these factors handling, loading, movement, immobilization, transport, unloading and waiting should be considered, because it compromises the welfare of animals and increases their vulnerability to disease (Rostagno, 2009; Broom, 2014).

Stress effects can be evaluated by using physiological, hematological, biochemical and hormonal approaches. Thus, enzymes, hormones, glycolytic potential, proteins, oxidative stress, lipid peroxidation and tissue damage were estimated to assess stress responses (Grandin, 2013).

On the other hand, slaughter methods, if not optimally employed, can adversely affect quality, operator and food safety and as a result cause downgrading of carcass and meat (Shimshony and Chaudry, 2005). Several works had reported that stocking density influenced the welfare of animals (Dalla Costa et al., 2007; De la Fuente et al., 2010), but scarce informations are available regarding this in the

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camel. The present study evaluates the preslaughter stress induced by loading density by analyzing rectal temperature (RT), heart rate (HR), respiratory rate (RR), hematocrit (Hct), neutrophile to lymphocyte ratio (NLR), hemolysis, and circulating levels of glucose (Glu), total protein (TP), cholesterol (CT), urea, creatinine, aminotransferase (ALAT), aspartate aminotransferase (ASAT), calcium (Ca), phosphorus (Pi), magnesium (Mg), cortisol (COR), total triiodothyronine (T_3) and total thyroxine (T_4) in the dromedary camel. These physiological, heamatological, biochemical and hormonal parameters are considered as indicators of stress responses in the camel (El khasmi et al., 2010; 2013; 2015).

MATERIALS AND METHODS

Animals

The study was carried out at Casablanca Municipality slaughterhouse in the West of Morocco (North of Africa, latitude 33°34'42.44" N, longitude 7°36'23.89" O) using 16 adult one-humped male camels (*Camelus dromedarius*), aged 4–7 years. These animals were living under similar conditions, fed with some barley concentrate and dry hay straw and exposed to the same preslaughter conditions. After arrival in the slaughterhouse, they were placed in the waiting station and were slaughtered following routine commercial slaughterhouse procedures according to Halal method. These camels were divided into 2 groups of 8 camels to study the effect of low and high loading densities: respectively 1camel/2-3.6 m^2 (Group I) and 1camel/1.44-1.80 m^2 (Group II). The densities were related to the space available within the truck and were determined as total number of animals divided by area of truck.

Road transport

All camels were transported by truck for 72 to 80 km at an average 60-65 km/h speed. All camels were clinically healthy and feed deprived overnight. They were transported in a side-facing position and squatting position holding the forelegs tight by a rope at the knees. During transportation, the camels could not feed and drink, and the road was asphalted until the arrival to the slaughterhouse. They were carefully unloaded on arrival at the abattoir to avoid stress and were calmly guided into the waiting station.

Physiological parameters measure

Physiological parameters (RT, HR and RR) were measured just before the loading and at the end of transport just before the unloading of camels. RT was measured by an ordinary thermometer introduced the rectum of the animal. HR (beats/min) was determined by auscultation of the heart area. Wheras, RR (breaths/min) was determined by auscultation of the trachea.

Blood sampling

Just before the loading and at the arrival just before the unloading of animals, blood samples were collected from each camel by venipuncture from the left jugular vein. These samples were taken directly in 2 test tubes: one ethylene diamine tetra acetic acid-dipotassium (EDTA-K2) Vacutainer tube and one heparanized tube. EDTA blood was used for Ht, NLR and hemolysis measure, wheras heparin blood was used for the determination of plasma levels of all biochemical and hormonal parameters. The plasma was separated by centrifugation at 750×g for 15 min at 4°C, pipetted into aliquots and then stored at -20°C until analysis.

Hematocrit measure

The Hct was determined on whole blood with capillary tubes and centrifuged (hettich Haematokrit D-7200) using a microhematocrit reading device, and was expressed as follows:

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

Neutrophil/lymphocyte ratio determination

To determine the leukocytes differential distribution (%), blood smears were stained with May-Grunwald-Giemsa, i.e., 5 min of May-Grunwald and 5 min of Giemsa tenth diluted in water. On 100 leukocytes, the percentage of neutrophils (neutrophils, eosinophils, basophils), lymphocytes and monocytes, and the neutrophile to lymphocyte ratio (NLR) were determined.

Hemolysis test

Percent hemolysis (H%) was determined by plotting the percent hemolysis with the concentration of salt solutions (Almizraq et al., 2013; El khasmi et al., 2015).

Biochimical parameters analysis

Plasma Glu, TP, urea, creatinine, ASAT, ALAT, CT, TG, Ca, Pi and Mg concentrations were measured using a spectrophotometric (JENWAY 6320D Spectrophotometer, Model 6320D) procedure from commercially available kits (CHRONOLAB, Switzerland) according to the manufacture procedures.

Hormonal parameters analysis

Plasma COR, T_3 and T_4 levels were analyzed by radioimmunoassay (RIA) method in the National Center of Science and Nuclear Technical Energy in Maâmoura, Morocco, by using commercially available coated RIA tubes. The hormones were quantified according to the manufacturer's instructions. These kits using 125I radio-labelled COR, T3 or T4 proved efficient in previous experiments in dromedary camels (El Khasmi et al., 2010; 2013; 2015), and was purchased from DIAsource

(Immunoassays S.A., Nivelles, Belgium). The areas of validation for cortisol assays included limits of detection, and precision in the standard curve following sample dilution, inter- and intra-assay coefficients of variation results were considred.

Statistical analysis

The data were expressed in SI units as mean and standard error (SE), and were analyzed by the Freidman test for comparison between the values determined in different groups and at different stages. $P<0.05$ was seen as statistically significant.

RESULTS AND DISCUSSION

The impact of stress induced by transport and increasing the loading density on some physiological, hematological, biochemical and endocrine parameters in the dromedary camel has been studied in the present study. In animals transported with low (Group I) or high (Group II) density, RT, HR, RR, NLR, H50 (Table 1), and circulating levels of Glu, COR, T3 and T4 (Table 2) measured after transport, were significantly ($P<0.05$) higher than those measured before loading and transport. However, Hct (Table 1) and plasma levels of TP, urea, creatinine, ASAT, ALAT (Table 3), CT, TG, Ca, Pi and Mg (Table 4) showed no significant variations. At the end of transport before unloading, RT, HR, RR, NLR and H50 (Table 1), Glu, COR, T3 and T4 (Table 2) in camels that were transported with low density (Group I) were significantly lower ($P<0.05$) by comparison to camels that n were transported with high density (Group II). Wheras, in both groups, Hct (Table 1) and plasma levels of TP, urea, creatinine, ASAT, ALAT (Table 3), CT, TG, Ca, Pi and Mg (Table 4) were not influenced by the loading density.Stress is a non-specific phenomenon that implicates several behavioural, physiological and emotional reactions in response to a variety of environmental stimuli (Voslářová et al., 2010). Pre-slaughter stress can start with the preparation of the animal in the farm, breeding site and market, continues with loading, transport, unloading, reception, conduction to the storage area in the slaughterhouse, and ends at the bleeding (Terlouw et al., 2005; Melesse et al., 2011; Chen et al., 2013; Miranda-de la Lama, 2013). In order to avoid stress at each of these stages, the International Committee of the World Organization for Animal Health has

Table 1: Impact of loading density on rectal temperature (RT), heart rate (HR), respiratory rate (RR), hematocrit (Hct), neutrophile/lymphocyte ratio (NLR) and concentration of saline solution where 50% hemolysis occurs (H50) during road transport in the dromedary camel (Mean±SE, [a]P<0.05 comparison before and after transport of the same group, [b]P<0.05 comparison between Groups I and II after transport)

	RT (°C)	HR (beats/mn)	RR (cycles/min)	Hct (%)	NLR	H50 (mosm/L)
Group I						
Before transport	37.8±0.3	46±3	11±1	33.4±2	0.91±0.11	123.05±3.99
After transport	38.1±0.3	53^{a}±3	15^{a}±2	34.61±2.83	0.92±0.11	132.65^{a}±3.99
Group II						
Before transport	37.7±0.4	47±4	12±1	33.59±2.18	0.91±0.01	125.16±2.22
After transport	$40.2^{a,b}$±0.4	$62^{a,b}$±4	$20^{a,b}$±2	35.15±3.32	$1.31^{a,b}$±0.11	$147.65^{a,b}$±3.99

Table 2: Impact of loading density on circulating levels of glucose (Glu), cortisol (COR), triiodothyronine (T_3) and thyroxine (T_4) during road transport in the dromedary camel (Mean±SE, [a]P<0.05 comparison before and after transport of the same group, [b]P<0.05 comparison between Groups I and II after transport)

	Glu (mmoles/L)	COR (ng/mL)	T_3 (nmoles/L)	T_4 (nmoles/L)
Group I				
Before transport	6.47±0.03	28.58±0.52	1.214±0.29	63.62±4.01
After transport	6.90±0.04^{a}	35.021±1.74^{a}	1.716±0.51	81.445±7.24^{a}
Group II				
Before transport	6.50±0.03	25.21±0.67	1.426±0.174	66.50±5.86
After transport	7.20±$0.02^{a,b}$	53.33±$5.33^{a,b}$	3.569±$1.25^{a,b}$	110.8±$19.93^{a,b}$

Table 3: Impact of loading density on plasma levels of total protein (TP), urea, creatinine and transaminases (ASAT, ALAT) during road transport in the dromedary camel (Mean±SE)

	TP (g/dl)	Urea (mmoles/L)	Creatinine (µmoles/L)	ASAT (U/L)	ALAT (U/L)
Group I					
Before transport	6.25±0.16	29.62±1.8	0.73±0.03	2.74±0.12	13.15±0.47
After transport	6.21±0.10	32.23±2.5	0.75±0.023	4.22±0.14	16.23±0.51
Group II					
Before transport	6.27±0.30	29.60±0.63	0.74±0.01	2.81±0.08	13.51±0.75
After transport	6.28±0.14	32.54±2.6	0.75±0.02	4.33±0.16	16.42±0.6

Table 4: Impact of loading density on plasma levels (mmoles/L) of cholesterol (CT), triglyceride (TG), calcium (Ca), phosphorus (Pi) and magnesium (Mg) during road transport in the dromedary camel (Mean±SE)

	CT	TG	Ca	Pi	Mg
Group I					
Before transport	0.92±0.01	0.42±0.03	2.3±0.2	2.2±0.2	1.3±0.3
After transport	0.95±0.07	0.43±0.02	2.4±0.2	2.3±0.4	1.2±0.3
Group II					
Before transport	0.93±0.03	0.41±0.013	2.35±0.18	2.25±0.092	1.35±0.23
After transport	0.98±0.02	0.44±0.03	2.6±0.3	2.4±0.5	0.7±0.2

developed recommendations for each of the pre-slaughter and slaughter processes for domestic animals (Shimshony and Chaudry, 2005), without any specific one in the camel. All values of stress parameters analyzed here in the camel increased after loading and road transportation. As a very stressful factor, road transport to the slaughterhouse had been able to induce in the camel, hypercortisoleamia (Saeb et al., 2010; El Khasmi et al., 2013) and an activation of free radical generation (Nazifi et al., 2009). In this species, we had previously reported that H% and circulating COR, thyroid hormones and Glu levels measured after transport were significantly higher than those observed in samples collected before transport and were positivly correlated with travel distance (El Khasmi et al., 2010; 2015). In addition to the physical effort required during transport, loading involves manipulations of camels and their exposure to new environments. These steps induce an increase in heart rate as it had been observed in calves (Lensink et al., 2001, Van de Water et al., 2003) and sheep (Baldock and Sibly, 1990), coupled with an increase in the levels of cortisol and creatine kinase in pigs (Kim et al., 2004, Brown et al., 2005). In bovine species, exposure to transport stress induced a significant increase of HR, NLR, and circulating levels of COR, Glu, lactate and heat shock protein 70 (Grigor et al., 2004; Chacon et al., 2005; Mounier et al., 2006; Chulayo et al., 2016). Furthermore, Jama et al. (2016) had found that in pigs, levels of saliva and urine cortisol and serum creatine kinase activity before transportation were lower than those analyzed at arrival at the abattoir.

During loading and road transportation, truck vibration, movement, acceleration and noise expose animals to stress, which compromises their physiological and biochemical processes then their well-being (Bourguet et al., 2011; Chulayo et al., 2012; Miranda-de la Lama et al., 2014). Under these conditions, animals showed an increase of COR and catecholamines secretion (Sporer et al., 2008; Muchenje et al., 2009; Rosado et al., 2010; Chulayo et al., 2012; 2013) resulting in increase of plasma levels of heat shock protein 70 and glucose, which were positively correlated with preslaughter distance travelled (Chulayo et al., 2016).

The impact of road ransport had been the most studied parameter in the camel (El khasmi et al., 2010; 2013; 2015) since it is the source of a large number of stressors, sometimes with extreme consequences. It had been accepted that several factors associated with transportation including physical discomfort, social character, handling, novelty of environment, adverse weather conditions, thirst, tiredness and finally deprivation of water and food, accentuate the preslaughter stress responsiveness as shown in animals (Brown et al., 1999; Karaca et al., 2016; Knowles and Warriss, 2000; Tarrant and Grandin 2000; Muchenje et al., 2009; Bourguet et al., 2011; Njisane and Muchenje, 20013) resulting in important economic losses. For example, in lambs, pre-slaughter stress induced by fasting for 24h or more, reduced the meat quality in lambs (Karaca et al., 2016) and induced liveweight losses estimated at 8% in cattle and sheep (Knowles and Warriss, 2000, Tarrant and Grandin, 2000). In addition, isolation of animals from their congeners constitutes additional sources of prelaughter stress (Deiss et al., 2006, Terlouw et al., 2008).

Many research works are increasingly interested in the welfare of domestic animals and in particular the stress induced by the transport, whose impact on their physiology and the quality of their meat is very pronounced (Chacon et al., 2005; Mounier et al., 2006). We found that among physical stressors, an increase of loading density during road transportation was a potential source of preslaughter stress in the camel. In cattle, for example, levels of cortisol and creatine kinase, as well as the number of bruises observed on carcasses, increase with loading density (Tarrant et al., 1992; Terlouw et al., 2005). However, in cattle, high stocking densities may allow animal to lean against one another, which requires high effort to maintain standing and not fall (Tarrant, 1990; Knowles, 1999). In addition, in the sheep, a high loading density in the truck decreases the risk of bruising (Jarvis and Cockram, 1994). Compared to other domestic animals, the loading and transport of camels is difficult for the operator and difficult for the animal because of its large size and its aggressiveness when it is stressed. In our investigation, the camels were transported in a squatting position holding the forelegs tight by a rope at the knees. They require experience in all stages of preslaughter processes including the loading and road transport (Kadim et al., 2013).

CONCLUSION

In the dromedary camel, the loading and stocking density may be considered as preslaughter stressfull factors capable of altering physiological, hematological, biochemical and hormonal profiles and the welfare of the animal, and would be able to influence the *postmortem* quality of its meat. Preslaughter stress responses observeed here in the camel, are a shared responsibility between different actors. The impact of this factor on the antioxidant status of this species will be studied later.

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