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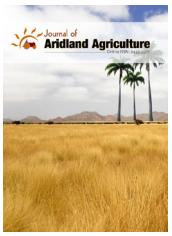

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Correlation between circulating cortisol and indicators of stress and oxidant stress during the preslaughter operations in camels

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ABSTRACT

In livestock, pre-slaughter stress begins at the farm or market, continues during transport and upon arrival at the slaughterhouse, ending at slaughter. In this investigation, a survey was conducted in the slaughterhouse of Casablanca in Morocco to record the duration of the preslaughter operations and the frequency of urination in camels. Two groups of camels were constituted, the least stressed animals (Group I, n= 12) and the most stressed animals (Group II, n= 12). Group I animals had a waiting time before loading ≤ 24 h, a loading time ≤ 15 min, an unloading time ≤ 5 min, a water and food deprivation time before slaughter ≤ 24 h, a duration of accompaniment to the slaughter room ≤ 11 min and a frequency of urination during this accompaniment < 3 times. Those in group II had higher duration and frequency values for the same parameters. In addition, serum stress [cortisol (COR)], oxidant stress biomarkers [malondialdehyde (MDA)] and activities of catalase (CAT) and superoxide dismutase (SOD) were analyzed in both groups, and correlations between these biomarkers and the durations of various preslaughter operations and the frequency of urination were established. The most stressed camels (G II) showed high serum concentrations of COR and MDA, and low CAT and SOD activities by comparison to the less stressed camels (G I) ($P<0.05$). Significant correlations were recorded between COR, MDA, CAT and SOD, and the durations of various preslaughter operations, and between COR and the frequency of urination.

KEYWORDS: Camel, Cortisol, Morocco, oxidant stress, preslaughter stress

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INTRODUCTION

The one-humped dromedary (*Camelus dromedarius*) is a species adapted to arid, semi-arid and tropical environments in Africa, Asia and Australia, where it is an important source of milk and meat (Faye, 2015; Tibary & El Allali, 2020). In farm animals, during the pre-slaughter period, physiological and behavioral responses to stress begin upon departure from the farm, continue through loading, transport, unloading, lairage at the slaughterhouse, exposure to sound and humans, and separation and mixing of animals, and end with slaughter (Terlouw *et al*., 2008). Stress induced by animal transport is a major animal welfare problem (Broom, 2008), which could be altered by loading and unloading procedures, storage density, transport duration, injury, water and food deprivation, driver behavior and road topographic conditions (Lawrie & Ledward, 2006; EFSA, 2011; Miranda-de la Lama *et al*., 2014).

High circulating levels of cortisol (COR), the most used indicator of stress, have been reported in camels exposed to different stressful situations (Saeb *et al*., 2010; El khasmi *et al*., 2010, 2015; Lemrhamed *et al*., 2018, 2019; Tabite *et al*., 2019, Mohamed *et al*., 2021). In addition, oxidative stress sets in when the body becomes unable to fight against the overproduction of free radicals or reactive oxygen species (Agarwal *et al*., 2005), following a deficiency in enzymatic (catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase) and non-enzymatic antioxidants (Lykkesfeldt & Svendsen, 2007). Known as one of the important consequences of oxidative stress, lipid peroxidation of the cell membrane releases malondialdehyde (MDA) (Sharma *et al*., 2011). In camels, pre-slaughter stress has been associated with oxidative stress marked by a significant

increase in MDA levels and adecrease in CAT activity in blood (El khasmi *et al.*, 2015; Lemrhamed *et al.*, 2018, 2019) and meat (Tabite *et al.*, 2019).

In camels, several studies have evaluated the different situations of pre-slaughter stress, such as transport (Saeb *et al.*, 2010; El khasmi *et al.*, 2010), transport distance (El khasmi *et al.*, 2015), loading density during transport (Lemrhamed *et al.*, 2018) and waiting time at the slaughterhouse (Lemrhamed *et al.*, 2019), and whose impact of stress significantly increased lipid peroxidation, drip loss and cooking loss of camel meat (Tabite *et al.*, 2019). However, to our knowledge and so far, in the same species, other stress situations before slaughter have not been studied, such as the duration of waiting before loading, loading, unloading, water and food deprivation, and accompaniment to the slaughter room. In this investigation, a survey was conducted to record all these informations and the frequency of urination, and establish correlations between them and serum levels of COR and MDA, and the activities of CAT and SOD in camels.

MATERIALS AND METHODS

Animals and Survey

Our study was conducted on 24 male dromedaries (*Camelus dromedarius*) in good health, aged 3 to 7 years, weighing from 215 to 370 kg and having been subjected to a semi-extensive breeding method in the region of Settat located in western Morocco (North Africa, latitude 33° 00' 3.71" N, longitude -7° 36' 59.83" W). These animals were intended for slaughter at the municipal slaughterhouse in Casablanca. They were exposed before slaughter to road transport by truck for 2 hours, then were detained for 12 to 16 hours in the waiting area.

In order to assess the intensity of stress during the pre-slaughter operations in camels from their road transport, then their arrival at the slaughterhouse until bleeding, a survey was carried. This survey focused on the duration of waiting before loading, loading, unloading, water and food deprivation and accompaniment to the slaughter room, and the frequency of urination during this accompaniment. To explain this late operation, the animals were guided together from the waiting station to the slaughter room, to bring them in one by one. They were pushed from behind with one of the front legs bent and tied with a rope at the knee. On the basis of the data obtained, two groups of animals of 12 individuals each were formed: the least stressed group (G I) and the most stressed group (G II) (Table 1). Correlations between these pre-slaughter stressors investigated and serum concentrations of COR and MDA, and the activity of CAT and SOD were determined.

Table 1: Distribution of camels according to different factors of pre-slaughter stress

	Group I (least stressed, n=12)	Group II (most stressed, n=12)
Duration of waiting at the farm before loading	≤ 24 h	≥ 25 h
Duration of loading at the farm	≤ 15 min	≥ 16 min
Duration of unloading at the salughterhouse	≤ 5 min	≥ 15 min
Duration of water and food deprivation from farm until slaughter	≤ 24 h	≥ 25 h
Duration of accompaniment to the slaughter room after the rest	≤ 11 min	≥ 12 min
Frequency of urination during the accompaniment to the slaughter room after the rest	≤ 3 times	> 3 times

Collection of Blood Samples

The blood was collected after a rest period of 12 to 16 hours just before slaughter, around 7 a.m. by puncture of the right jugular vein in dry tubes. Samples were transported for 10 min in a cooler at 4°C to the Laboratory of Physiopathology and Molecular Genetics, at the Ben M'Sik Faculty of Sciences. The blood was centrifuged at 750xg for 15 minutes to distribute the serum into aliquots which were stored at -80°C until subsequent analyzes of COR, MDA, CAT and SOD which were performed in duplicate.

Analysis of Cortisol

The analysis of COR was carried out using Biosource radioimmunoassay kits (Biosource Europe SA., Belgium), at the National Center for Energy, Sciences and Nuclear Techniques of Maâmoura, Morocco. We evaluated specificity, selectivity, linearity, sensitivity, accuracy, precision, stability, and dilution tests.

Determination of Malondialdehyde

Lipid oxidation was assessed by measuring colorimetrically substances reactive with Thiobarbituric acid (SR-TBA) according to the method of Lynch and Frei (1993). This method evaluates the quantity of non-volatile aldehydes (MDA) produced during oxidation. One mL of the serum was mixed with 1 mL of a solution containing 1% thiobarbituric acid, 30% trichloroacetic acid and 0.25 M hydrochloric acid. After incubation for 15 minutes at 100°C, the mixture was transferred to an ice bath to stop the reaction. After centrifugation at 1000 g for 10 min, the supernatant was read at 535 nm.

Measurement of Catalase and Superoxide Dismutase Activities

CAT catalyzes the dismutation of hydrogen peroxide (H_2O_2) into water and oxygen. The activity of CAT was measured by colorimetry at 240 nm (JENWAY 6320D Spectrophotometer, Model 6320D), by the variation of the optical density following the disproportionation of H_2O_2 at an incubation temperature of 25°C, using the method of Aebi (1974). The activity of SOD in the serum was quantified according to the method of Paoletti *et al.* (1986). The oxidation of NADH by superoxide radicals is monitored at 340 nm in the reaction mixture containing 5 mM of EDTA, 2.5 mM of $MnCl_2$, 3.9 mM of 2-mercaptoethanol and 10 µL of the serum in the 50 mM potassium phosphate buffer. Reaction by adding 0.27 mM NADH as the final concentration.

Statistical Analysis

The survey data was analyzed using SPSS software. The values of the serum concentrations of COR and MDA, and the activity of CAT and SOD were expressed as mean (M) ± standard error of the mean (SEM). The multiple comparison test Student Newman-Keuls (SNK) wasused for the criteria of the separation of the two groups, and the statistical analysis test used for the comparison between the values stress and OS indicators between the two groups was the one-way analysis of variance (ANOVA). A parametric test (correlation of Pearson's analysis) was carried out to detect correlations of these parameters, with the durations of waiting before loading, loading, unloading, water and food deprivation, accompaniment to the slaughter room, slaughtering and bleeding, and frequency of urination. $P<0.05$ was considered statistically significant.

RESULTS

Survey Data

The results of the investigation on the intensity of the stress having been induced by the various preslaughter operations to which the dromedaries were exposed before the slaughter, are presented in the Table 2, and made it possible to distinguish two groups of animals, one was the most stressed (G II) and the other was the least stressed (G I). In G II, the duration of waiting before loading, loading, unloading, water and food deprivation, and accompaniment to the slaughter room, and the frequency of urination, were higher compared to G I (respectively, 25-36 hrs vs 12-24 hrs, 16-35 mins vs 5-15 mins, 6-15 mins vs 3-5 mins, 25-72 hrs vs 10-24 hrs, 12-20 mins vs 9-11 minsand 3-5 times vs <3 times) (Table 2).

Cortisol, Oxidant/Antioxidant Biomarkers

The concentrations of COR and MDA, and the activities of CAT and SOD in the two groups of camels are presented in the Figure 1. In the most stressed camels (G II), serum concentrations of COR (ng/mL) and MDA (µmol/L) were significantly ($P< 0.05$) higher, however, CAT (UI/L) and SOD (UI/mL) activities were significantly ($P< 0.05$) lower than those observed in the less stressed camels (G I) (respectively, 98.21±9.45 vs 34.43±3.27; 21.69±1.75 vs 13.03±1.04; 18.79±1.02 vs 25.45±1.03 and 3.01±0.11 vs 5.67±0.12) (Figure 1).

Correlation between Cortisol, Oxidant/Antioxidant Biomarkers and Stress Factors during the Various Preslaughter Stages

Significant correlations were reported between COR, MDA, CAT and SOD, and stress factors during the various preslaughter stages (Table 3).Duration of waiting before loading, loading, water and food deprivation, and accompaniment to the slaughter room were positively correlated with COR and MDA,and negatively correlated with CAT and SOD activities (Table 3). MDA showed a positive correlation with COR and frequency of urination and a negative correlation with CAT and SOD activities. Finally, a positive correlation between these enzymatic activities was observed (Table 3).

Table 2: Distribution of the camels according to the intensity of stress during the pre-slaughter operations

	Group I (n=12)	Group II (n=12)
Duration of waiting before loading	12-24 h	25-36 h
Duration of loading	5-15 min	16-35 min
Duration of unloading	3-5 min	6-15 min
Duration of water and food deprivation before slaughtering	10-24 h	25-72 h
Duration of accompaniment to the slaughter room	9-11 min	12-20 min
Frequency of urination during the accompaniment to the slaughter room	<3 times	3-5 times

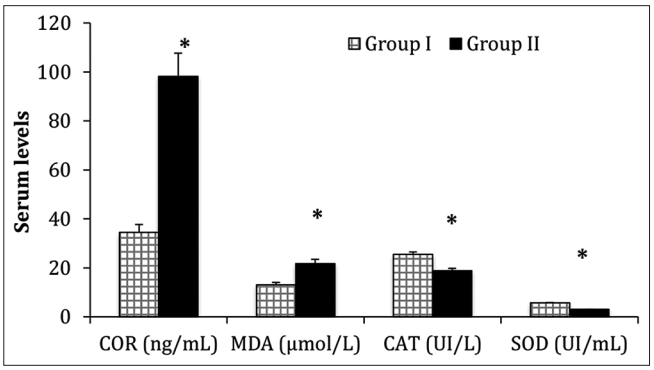

Figure 1: Serum concentrations of cortisol (COR), malondialdehyde (MDA), and activities of catalase (CAT) and superoxide dismutase (SOD) in the less stressed camel group (G I, n=12) and the most stressed one (G II, n=12). (M±SEM, *P<0.05; comparison between GI and GII).

The distribution of the two groups of camels according to the intensity of stress during the pre-slaughter operations was based on the duration of waiting before loading, loading, unloading, water and food deprivation and accompaniment to the slaughter, and frequency of urination during this accompaniment.

DISCUSSION

The study reported here showed that in camels, stress before slaughter induced by an increase in the duration of waiting before loading, loading, unloading, water and food deprivation, and the accompaniment to the slaughter room, was responsible for a significant increase of urination frequency, higher serum concentrations of COR and MDA, and significantly lower CAT and SOD activities. The duration of the preslaughter operations to which the camels were subjected (waiting, loading, and food and water deprivation) were positively correlated with circulating COR and MDA, and negatively correlated with oxidative stress biomarkers (CAT and SOD).

According to numerous studies, in camels, high cortisol blood concentrations are a reliable indicator of the state of physical, psychological and physiological stress (Saeb *et al.*, 2010; El

Table 3: Pearson correlation coefficient between different factors and indicators of stress before slaughter in camels (MDA: malondialdehyde, CAT: activity of catalase, SOD: superoxide dismutase).

	Cortisol	MDA	CAT activity	SOD Activity
Duration of waiting before loading	0.5301* (0.0431)	0.4145* (0.0451)	-0.5243* (0.0412)	-0.4786* (0.0181)
Duration of loading	0.5383* (0.0470)	0.4743* (0.0215)	-0.4913* (0.0154)	-0.4315* (0.0367)
Duration of unloading	0.2041 (0.4839)	0.2249 (0.4395)	-0.0682 (0.8169)	-0.0993 (0.7357)
Duration of water and food deprivation	0.5658* (0.0349)	0.4763* (0.0402)	-0.5376* (0.0474)	-0.5421* (0.0435)
Duration of accompaniment to the slaughter room	0.4678* (0.0249)	0.4813* (0.0202)	-0.5163* (0.0421)	-0.5507* (0.0411)
Frequency of urination during the accompaniment to the slaughter room	0.5607* (0.0370)	0.1150 (0.6954)	-0.1429 (0.6260)	-0.0336 (0.9093)
Cortisol (ng/mL)		0.7513** (0.0020)	-0.7588** (0.0017)	-0.7347** (0.0021)
MDA (µmol/L)			-0.7243** (0.0034)	-0.7152** (0.0036)
CAT activity (UI/L)				0.7304** (0.0032)
SOD activity (UI/mL)				

khasmi *et al.*, 2015; Tharwat *et al.*, 2015; Abd-El-Rahman *et al.*, 2017; Ebissy *et al.*, 2019). Compared to the reference values reported by Saeb *et al.* (2010), the higher cortisol concentrations mentioned in the present study in camels having been exposed to the different pre-slaughter operations for a long time, prove that the latter are more stressful. MDA, which is the last product of lipid peroxidation and enzymatic antioxidants like SOD and CAT have been widely used to estimate oxidative stress in camels (Marai *et al.*, 2009; Gaughan, 2011; El khasmi *et al.*, 2015; Abd-El-Rahman *et al.*, 2017; Lemrhamed *et al.*, 2018; Mohamed *et al.*, 2021). In the present study, the higher MDA and COR concentrations and urination frequency, the low enzyme activities of CAT and SOD, and the correlations between these parameters and the duration of the pre-slaughter operations, justify an alteration of the welfare in the most stressed group of camels. This could be due to the lack of slaughterhouse infrastructure and equipment, and the training of technicians and animal handlers. Indeed, the animals wait a long time before being transported, are loaded and then unloaded without the use of ramps, and continue to be deprived of water and food in the slaughterhouse.

In fact, animal welfare is affected by the loading livestock into the truck, the loading and unloading time and the transport time resulting in an increase in heart rate, nervousness and agitation of the animal (EFSA, 2004; Chacon *et al.*, 2005; Padalino & Riley, 2020). Furthermore, the duration of transport affects the physiological and behavioral state (Miranda-de la Lama *et al.*, 2014), altered the immune system (Padalino *et al.*, 2017), favors infections (Cirone *et al.*, 2019) and has been found to be a major risk factor for morbidity and mortality (Gonzàlez *et al.*, 2012; Padalino *et al.*, 2015; Padalino *et al.*, 2018) in farm animals. In addition, longer transport duration increases glycogen consumption (Chulayo & Muchenje, 2017; Reiche *et al.*, 2019) and the period of water deprivation, generating conditions of greater dehydration and hemoconcentration, especially during the hot season (Tadich *et al.*, 2005; Chulayo & Muchenje, 2017). According to EFSA (2004), the duration of a road transport begins when the first animal is loaded and ends when the last animal leaves the vehicle.

In animals, separation or social isolation can cause blathering (Deiss *et al.*, 2009), while water and food deprivation and fatigue are responsible for restlessness and vocalizations of the animal making it difficult to handle (Vieira *et al.*, 2008; Dalla Costa *et al.*, 2016). However, in addition to physiological and behavioral responses, stress assessment is based on environmental context, and post-mortem carcass and meat quality traits, characteristics, rearing conditions and genetics of the animal (Terlouw & Bourguet, 2022).

Prior to slaughter, and following handling prior to loading, and during loading and unloading, animals are kept in the lairage area for rest and recovery from stress. However, a long period of housing without access to water and food could induce a significant increase in serum levels of COR and MDA associated with a decrease in CAT activity in camels (Lemrhamed *et al.*, 2019). Therefore, it is necessary to transport and unload livestock without extending the rest period by more than 4 h before slaughter in the hot season (Pérez-Linares *et al.*, 2015).

Upon arrival at the slaughterhouse, animals should be provided with good quality water and fodder, comfortable and clean enclosures, and sufficient space to rest (FAO, 2004; OIE, 2011). However, as in many developing countries, in the slaughterhouses surveyed in this study, the results recorded showed that the welfare of camels is not well respected. They have suffered poor handling during all the operations preceding their slaughter, justified by their long duration, which could have negative effects on the homeostasis of these animals.

In order to reduce the duration of the various operations performed on camels before slaughter, means of transport and loading/unloading equipment adapted to this species are required. Animals should be unloaded as soon as possible after arrival at the slaughterhouse. Waiting areas must have sufficient space to allow them to rest, drink, feed and protect themselves from extreme weather conditions. And finally, a legislation on the welfare of camels during all stages of pre-slaughter according to international standards must be developed.

CONCLUSION

All pre-slaughter operations to which camels have been exposed for a long time, were marked by high serum concentrations of COR and MDA, and low enzymatic activities of CAT and SOD, associated with increased frequency of urination. Availability of such data would be useful to monitor and protect camel welfare during the preslaughter operations. We suggest that these stressful operations before slaughter must be taken into consideration, and special attention should be paid to reduce

the durations of waiting before loading, loading, unloading, water and food deprivation, and accompaniment to the slaughter room in the camel. Further studies on camel welfare are needed to analyze these parameters with a larger number of slaughterhouses in Morocco, taking into account other risk factors, such as characteristics of the vehicles and drivers experience.

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