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EVALUATION OF PRESLAUGHTER STRESS RESPONSES DURING WAITING TIME AT LAIRAGE IN DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

Stress responses were evaluated during waiting time at lairage at the slaughterhouse by analysing some physiological, haematological, biochemical and hormonal parameters in the camel. Sixteen male animals belonging to the municipal slaughterhouse of Casablanca (west of Morocco) were divided into 2 groups according to their waiting periods before slaughter: short period (12hs≤time<16hs, Group I, n=8) and long period (16hs≤time≤20hs, Group II, n=8). In groups I and II, neutrophil/lymphocyte ratio, haemolysis and circulating levels of cortisol, triiodothyronine and thyroxine were measured before waiting (at the end of transport and after unloading) and these were significantly ($P<0.05$) lower than those observed after the 2 waiting periods. After waiting time, all these parameters and plasma levels of glucose remained significantly ($P<0.05$) lower in Group II than those analysed in Group I. In the same conditions, rectal temperature, heart rate, respiratory rate and circulating levels of calcium, phosphorus and magnesium, showed no significant variation either between the stages (before and after waiting), or between the 2 waiting periods. In the camel, the waiting period at the slaughterhouse could be an important preslaughter stress factor, capable of altering the animal's physiology and the post-mortem quality of its meat. After transport and unloading, a waiting period of 16h to 20h could be considered less stressful than that of 12h to 16h in the camel. The impact of waiting period on antioxidant status will be evaluated later.

Key words: Dromedary, haemolysis, hormones, lairage period, leukocyte formula, preslaughter stress

Stress is completely defined as the combination of mental and biological responses of an animal to novel and threatening physical and psychological stimuli (Broom, 2008). Several responses, *i.e.*, increased heart rate (HR), respiratory rate (RR), adrenal activity and reduced immunological response, have been considered as stress indicators (Broom, 2014). These responses involve activation of the sympathetic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis, inducing release of catecholamines, corticotrophin releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and cortisol. In camels, investigation of stress responses had used cortisol measurement in blood (El khasmi *et al,* 2010; 2013; 2015; Lemrhamed *et al,* 2018), urine (El khasmi *et al,* 2010), saliva (Majchrzak *et al,* 2014), hair and faeces (Sid-Ahmed *et al,* 2013; Bargaa *et al,* 2016) as an indicator for the activity of the HPA axis. Other neuroendocrine systems are also involved, such as thyroid hormones in the camel (Saeb *et al,* 2010; El khasmi *et al,* 2010; Lemrhamed *et al,* 2018) and several domestic animals (Ferlazzo *et al,* 2018).

The steps preceding the slaughter of an animal for human consumption need to maintain product quality as well as protecting animal welfare (Shimshony and Chaudry, 2005; Broom, 2014). Currently, during the pre-slaughter period, domestic animals are exposed to various potentially stress-inducing factors of psychological origin, such as social disturbances, handling, transportation and novelty, or physical origin such as food and water deprivation, pain or fatigue (Terlouw *et al,* 2008; Ferlazzo *et al,* 2018). In the dromedary camel, stressful situations induced by transport, travel distance and loading density was evaluated by physiological [rectal temperature (RT), respiratory rate (RR), heart rate (HR)], haematological [haematocrit (Hct), neutrophil/lymphocyte ratio (NLR), haemolysis (H%)], endocrine [cortisol (COR), triiodothyronine (T3), thyroxine (T4)] and biochemical [glucose, malondialdehyde,

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catalase activity] responses (El Khasmi *et al,* 2010, 2013, 2015; Saeb *et al,* 2010; Lemrhamed *et al,* 2018). Pre-loading handling, transportation and unloading are crucial steps in the production chain of camel destined for slaughter and may lead to several stress reactions. Thus, the best conditions to reduce stress and promote the maintenance of good welfare of camel must be respected. In present study, the stress responses induced by waiting period before slaughter in the camel were studied, *i.e.* stress-related physiological (RT, RR, HR) and blood variables (Hct, NLR, haemolysis, COR, T3, T4, glucose, Ca, Pi and Mg).

Materials and Methods

Animals

The study was carried out at Casablanca Municipality slaughterhouse (West of Morocco, 33.35° N, 7.36° W and 27 m altitude) during the winter season, from January to February, with temperatures ranged between 11-16°C. Sixteen adult one-humped male camels (*Camelus dromedarius*), aged 4–7 years were used. These animals were maintained under similar conditions, fed with some barley concentrate and dry hay straw and exposed to the same preslaughter conditions. All the camels were transported by truck from the market to the slaughter house within 2h and spent approximate 12h to 20h in the lairage before slaughtering. Prior to transportation, these animals were ensured as clinically healthy and were deprived of water and food for 1 to 3 hours before being loaded. These 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. On arrival at the abattoir, the animals were carefully and calmly unloaded to avoid stress and were guided into the waiting station before slaughtering. These were divided into 2 groups of 8 animals according to their waiting period at lairage: short period (12h≤time<16h, Group I) and long period (16h≤time≤20h, Group II). During waiting, the animals were kept together without mixing of different groups. Each animal had enough space to stand up, lie down and turn around. Water and feed was not available to the animals on their arrival and throughout the waiting time.

Physiological parameters

Physiological parameters (RT, HR and RR) were measured before waiting (just after transport and unloading) and just at the end of waiting before slaughtering.

Blood sampling

Blood samples were collected from each camel before waiting (just after transport and unloading) and just at the end of waiting before slaughtering. EDTA blood was used for Hct, NLR and haemolysis (H%) measure, whereas heparinised 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.

Haematocrit

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

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

Neutrophil/lymphocyte ratio

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 10th diluted in water. Of 100 leukocytes, the percentage of neutrophils (neutrophils, eosinophils, basophils), lymphocytes and monocytes and the neutrophil to lymphocyte ratio (NLR) were determined.

Haemolysis test

The profile of H% was analysed by using the slightly modified method of O'Dell *et al* (1987). A 100 µl aliquot of blood was added to test tubes containing 5ml of various concentrations of buffered salt solutions (BSS, pH 7.4) ranging from 0.1 to 0.9%. The contents of these tubes were gently mixed by inverting them five times and were allowed to stand at 37°C for 30 min. Thereafter, 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 540 nm using a spectrophotometer. The 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 per cent haemolysis was calculated according to Faulkner and King (1970) as follows:

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

H(%) curve was obtained by plotting per cent haemolysis against the saline concentrations. H50 was determined as the saline concentration responsible for an haemolysis of 50% of red blood cells.

Biochemical and hormonal parameters analysis

Plasma Glu, Ca, Pi and Mg concentrations were measured using a spectrophotometric procedure from commercially available kits. Plasma levels of COR, T3 and T4 were analysed by radioimmunoassay (RIA) method in the National Centre 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 proved efficient in previous experiments in dromedary camels (El Khasmi *et al,* 2013, 2015; Lemrhamed *et al,* 2018) 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 considered.

Statistical analysis

Statistical Analysis System (SAS, Version 9.0) was used. The data were classified into 2 groups according to waiting periods at lairage (short period, 12hs≤time<16hs, n=8; and long period, 16hs≤time≤20hs, n=8). Blood samples were analysed by one-way (general linear model procedure) analysis of variance. A difference of $P < 0.05$ was considered statistically significant.

Results and Discussion

In Groups I and II, NLR, H50 (mOsmol/L) (Fig 1) and plasma levels of COR (ng/mL), T3, T4 (nM) (Fig 2) measured after waiting time showed a significant ($P<0.05$) increase when compared with those observed before waiting (at the end of transport and after unloading) (1.31±0.1 *vs* 0.91±0.1; 139.6±3.5 *vs* 121.6±3.9; 54.33±5.33 *vs* 25.21±2.67; 3.56±0.25 *vs* 1.42±0.17 and 117.8±16.93 *vs* 66.50±6.86, respectively in Group I and 0.93±0.1 *vs* 0.86±0.1; 131.1±3.2 *vs* 123.1±3.1; 43.67±5.74 *vs* 30.34±5.52; 2.71±0.21 *vs* 1.34±0.19 and 81.445±8.24 *vs* 53.62±7.21, respectively in Group II). By comparison to glucose levels (mM) measured after waiting, those analysed before rest were significantly ($P<0.05$) higher in Group I and lower in Group II (8.14±0.02 *vs* 7.28±0.03 and 6.23±0.04 *vs* 7.37±0.05, respectively) (Fig 3).

In the same conditions, at the end of waiting, NLR, H50 (mOsmols/L) (Fig 1) and plasma levels of COR (ng/mL), T3, T4 (nM) (Fig 2) and glucose (mM) (Fig 3) remained significantly ($P<0.05$) higher in group I (short waiting time) than those analysed in group II (long waiting time) (1.31±0.1 *vs* 0.9±0.1; 139.6±3.5 *vs* 131.1±3.2; 54.33±5.33 *vs* 43.67±5.74; 3.56±0.25 *vs*

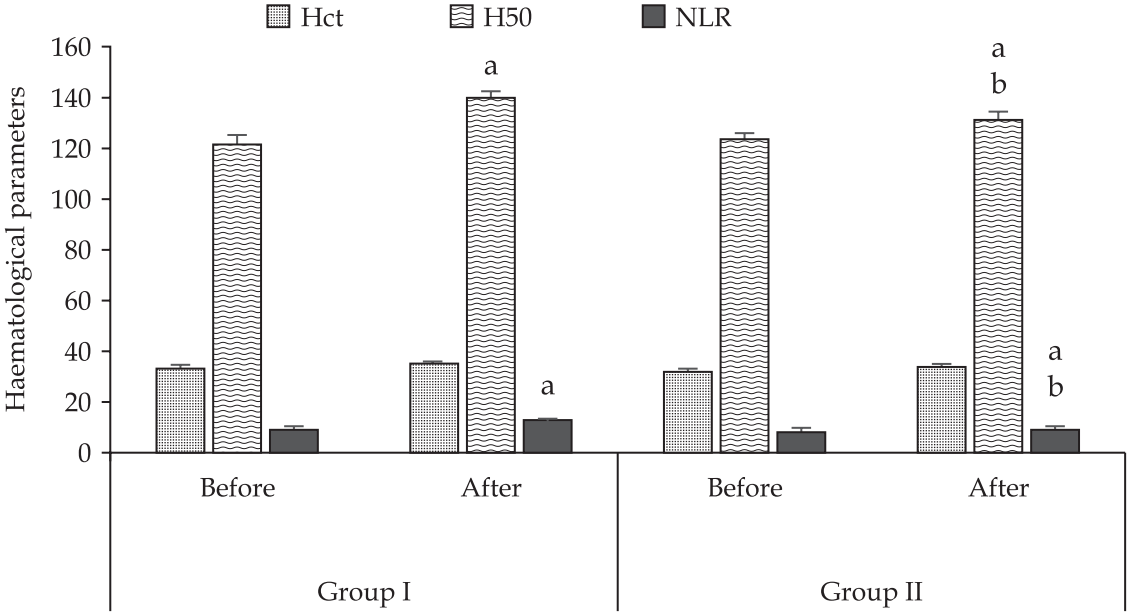

Fig 1. Haematocrit (Hct) (%), concentration of saline solution (mOsmol/L) inducing the haemolysis of 50% of erythrocytes (H50) and neutrophil/lymphocyte ratio (NLR) ($x10^{-1}$) before waiting (just at the end of transport after unloading) and after 2 different waiting periods: short period (12h≤time<16h, n=8, Group I) and long period (16h≤time≤20h, n=8, Group II). (Means±SE, $^{a}P<0.05$, comparison before and after waiting for the same group, $^{b}P<0.05$, comparison after waiting between Groups I and II).

2.71±0.21; 117.8±16.93 *vs* 81.44±8.24 and 8.14±0.02 *vs* 6.23±0.04, respectively).

However, Hct (Fig 1), plasma levels of Ca, Pi and Mg (Fig 3), RT, HR and RR (Fig 4) showed no significant variation either between the stages (before and after waiting), or between the 2 waiting times.

The parameters analysed in this work (RT, HR, RR, H%, NLR, COR, glucose, T3, T4) were previously used by several studies as good indicators of stress responses in the dromedary camel (El khasmi *et al,* 2010, 2013), (Saeb *et al,* 2010; El khasmi *et al,* 2015; Lemrhamed *et al,* 2018). In domestic animals, the pre-slaughter stress can start in the farm, breeding site and market, continues with loading, transport, unloading, reception, conduction to the lairage area in the slaughterhouse where the animal can wait several hours and ends at the bleeding (Terlouw *et al,* 2008; Kadim *et al,* 2008; De la Fuente *et al,* 2010). In order to avoid stress at each of these stages, the International Committee of the World Organisation for Animal Health has developed recommendations for each of the pre-slaughter and slaughter processes for domestic animals, on the basis of available scientific data (Shimshony and Chaudry, 2005). So far, no recommendations concerning the dromedary welfare have been developed. However, research works had reported that this species has been more sensitive to road transport stress (El khasmi *et al,* 2010, 2013; Saeb *et al,* 2010), distance of transport (El khasmi *et al,* 2015) and loading density during transport (Lemrhamed *et al,* 2018). These stress situations were marked by a significant increase of H%, NLR, glycaemia and circulating levels of COR and thyroid hormones.

In the dromedary camel, among pre-slaughter stress factors, waiting at lairage after road transportation might be a potential source of stress, marked by significant high NLR, haemolysis and circulating levels of COR and thyroid hormones. Work shows that beyond 2 hours, increased waiting time promotes overlap in calves (Grigor *et al,* 2004). Resting animals in the lairage for before slaughter, is highly recommended for reduce transport stress and improve meat quality characteristics (Thompson, 2004). However, this requires good waiting conditions, while this period can also be a source of stress leading animals to become reactive and stressed. Furthermore, the rate energy gain by animals depend upon the amount of stress from transportation and the conditions of the lairage at the abattoir (Gregory, 2008; Grandin, 2010). In fact,

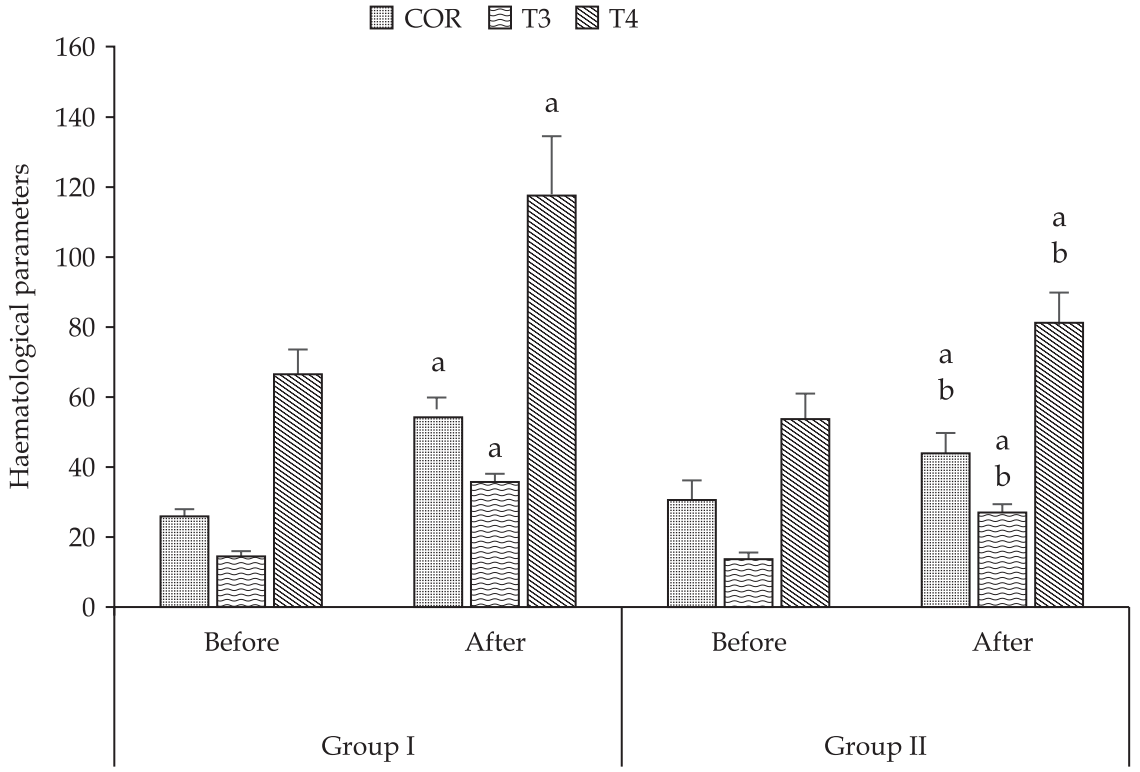

Fig 2. Plasma levels of cortisol (COR) (ng/mL), total triiodothyronine (T3) ($x10^{-1}$nM) and total thyroxine (T4) (nM) before waiting (just at the end of transport after unloading) and after 2 different waiting periods: short period (12h≤time<16h, n=8, Group I) and long period (16h≤time≤20h, n=8, Group II). (Means±SE, $^{a}P<0.05$, comparison before and after waiting for the same group, $^{b}P<0.05$, comparison after waiting between Groups I and II).

during transfer to the lairage station, animals can be exposed to various stressors such as fasting or forced exercise, breakdown of social group and the familiar environment, background noise, handling and novelty, resulting in a physical exhaustion and a psychological stress (Terlow, 2005). For example, Tume and Shaw (1992) found cortisol concentrations in cattle slaughtered in commercial abattoirs to be higher than in animals slaughtered at research abattoirs owing to the inherent noise and movements of animals and people in the yards. In pigs, a significant increase of COR levels was found in blood after more than 4h (Rey-Salgueiro *et al,* 2018) or after 9h (Pérez *et al,* 2002) in lairage and in saliva after 20h of waiting (Jama *et al,* 2016) at the slaughterhouse. According to Hambrecht *et al* (2005) and Jama *et al* (2016), in pigs, decreasing lairage duration increased significantly plasma lactate and urine and plasma cortisol, suggesting that pre-slaughter rest can alleviate stress induced by pre-slaughter handling operations. Finally, the return to basic physiological and behavioural state prior to slaughter without feed but with access to water, could be observed after waiting 24 to 48 hours in cattle (Mounier *et al,* 2006), 12–24 hours in camel (Kadim *et al,* 2013), 24 hours in steers (Tadich *et al,* 2005) and more than 17 hours in pig (Jama *et al,* 2016).

The findings reported in this work, showed an increase of number of neutrophils and a decrease of number of lymphocytes in the camels after waiting. These variations were more pronounced after the short waiting time than the long waiting time. An excessive release of neutrophils occurs through the action of endogenous COR, causing mobilisation of the marginal neutrophils of the microvasculature, as well as the induction of increased reserve release of these cells from the bone marrow (Jain, 1993). Increased release of neutrophils at stress during short waiting time may weaken the camel's immune function (Thrall, 2006), leaving them susceptible to infections and inflammatory diseases. However, in pigs, lymphopenia with neutropenia has been reported (Chacon *et al*, 2005), as well as neutrophilia accompanied by lymphopenia (Gupta *et al,* 2007). These results may be explained by an excessive release of adrenaline (Jones and Allison, 2007) as well as by emotional changes or excess muscular effort (Jain, 1993). The increased circulating levels of COR in camels during short waiting time, might reduce mobilisation of circulating lymphocytes by inhibition of production. COR decreases the number of circulating lymphocytes, particularly T-helper cells involved in response to foreign substances resulting in a decrease of all cell-mediated immunity (Earley *et al,* 2017).

In this work, plasma glucose levels increased after the short waiting time and decreased after the long waiting time in the camel. The high levels of

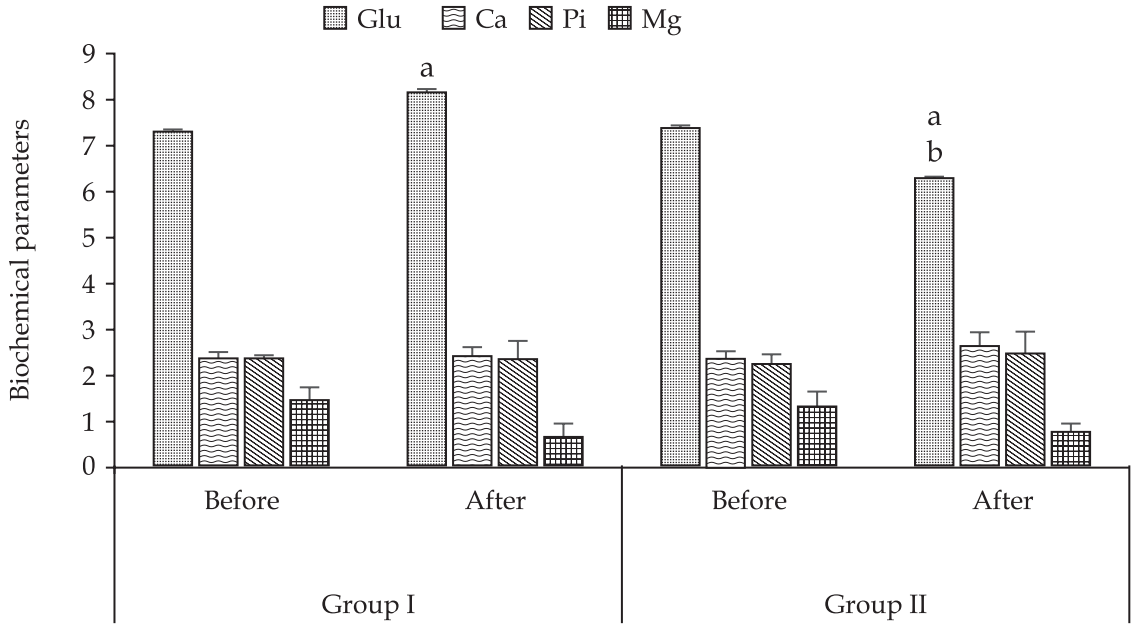

Fig 3. Plasma levels (nM) of glucose (Glu), calcium (Ca), phosphorus (Pi) and magnesium (Mg) before waiting (just at the end of transport after unloading) and after 2 different waiting periods: short period (12h≤time<16h, n=8, Group I) and long period (16h≤time≤20h, n=8, Group II). (Means±SE, $^{a}P<0.05$, comparison before and after waiting for the same group, $^{b}P<0.05$, comparison after waiting between Groups I and II).

glucose observed just before slaughter, might impact the meat industry, since glycogen which is a precursor of glucose, is essential during the postmortem transformation of muscle into meat. The requirement of muscle glucose during waiting period at lairage by using glycogen can directly affect the blood concentration of glucose and quality of the meat, which are directly dependent on all pre-slaughter stages (Minka and Ayo, 2010; Gruber *et al,* 2010; Chulayo *et al,* 2016). According to Jama *et al* (2016) muscle glycogen content could therefore increase with waiting time, leading to a decrease of circulating levels of glucose in pigs. Tadich *et al* (2005) reported in the steers that had been transported for 3 h, glucose levels started to recover after 24 h of lairage. In comparison, a decline in glucose levels was observed in cattle that had been transported for up to 31 h even after 24 h in lairage (Mounier *et al,* 2006). Lairage duration might affect the circulating levels of glucose, however, the magnitude of waiting stress response is highly dependent on micro-ambient temperature and humidity leading to increased production of biochemical reactions and reduced blood levels of glucose (Chulayo *et al,* 2012, 2016; Romero *et al,* 2014). In addition, new environment where camels were mixed with unfamiliar animals, increase of probability of interactions between camels, waiting stressful conditions and adrenaline and COR secretion might be responsible for increased levels of circulating glucose and glycogen breakdown in the muscle (Terlouw *et al,* 2008).

The stress responses observed during waiting in the camels used in this investigation, might be explained by an activation of the hypothalamic-pituitary adrenal axis, inducing the release of catecholamines, glucocorticoids and other hormones that may alter the blood biochemical and cellular physiology, metabolism and immune function (Stanger *et al,* 2005). In the same conditions, the hypothalamus-pituitary-thyroid (HPT) axis may be involved in stress responses in animals (Joseph-Bravo *et al,* 2015), which underlines the evaluation of iodothyronines and notably of T3, as markers of their welfare and stress (Ferlazzo *et al,* 2018).

In camel, homeostatic changes due to waiting period at lairage were observed, with effects on immune function, integrity of erythrocyte membrane and corticotropic and thyrotropic axis. These pre-slaughter stress responses could alter the animal's physiology and the post-mortem quality of its meat. After transport and unloading, a waiting period of 16h to 20h could be considered less stressful than that of 12h to 16h in the camel. Nevertheless, our data do not enable us to recommend a specific waiting time, however, good waiting conditions are recommended aiming to reduce stress and improve camel welfare. The impact of waiting period on the blood and muscle antioxidant status will be evaluated later in this species.

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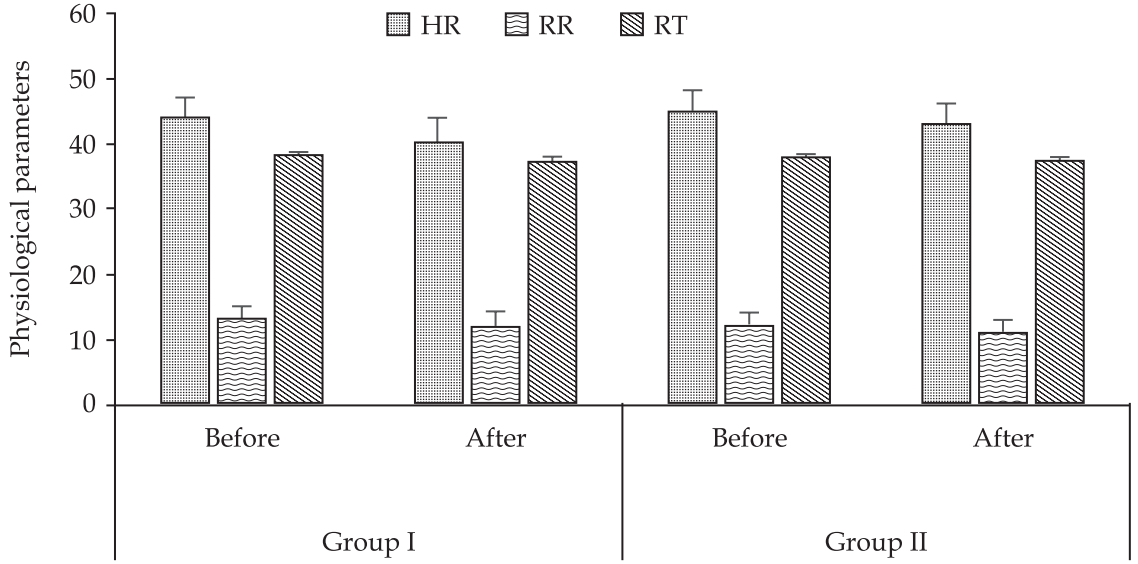

Fig 4. Heart rate (HR) (beats/mn), respiratory rate (RR) (cycles/mn) and rectal temperature (RT) (°C) before waiting (just at the end of transport after unloading) and after 2 different waiting periods: short period (12h≤time<16h, n=8, Group I) and long period (16h≤time≤20h, n=8, Group II). (Means±SE).

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