

RESEARCH ARTICLE

Relationship between circulating levels of cortisol at slaughter and changes of some parameters of the camel meat during ageing

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ABSTRACT

Relationship between serum levels of cortisol at slaughter and changes of some parameters of meat was investigated in 50 male camels, during ageing for 10 days at $4 \pm 1^\circ\text{C}$. Blood was sampled at slaughter to determine serum levels of cortisol and oblique abdominal muscle was collected after slaughter to analyze pH, electrical conductivity (EC), drip loss (DL), cooked loss (CL), moisture, solids, ashes, protein total, calcium (Ca), phosphorus (Pi), magnesium (Mg), sodium (Na), potassium (K), catalase activity (CATa), malondialdehyde (MDA), shrinkage, R-value and total haem pigment (THP) at days 0 (four hours *postmortem*), 5 and 10 of refrigerated storage. The camels were divided into 3 groups (Gr) with different serum levels of cortisol at slaughter (low, high and very high levels): Gr1 (13.07-67.9 ng/mL, n = 24), Gr2 (80.29-107.21 ng/mL, n = 7) and Gr3 (133.7-198.04 ng/mL, n = 19). Compared to Gr1, Gr2 and Gr3 had a significant ($P < 0.05$) increase of pH, EC, DL, CL, THP, dimensional shrinkage and MDA, and a significant ($P < 0.05$) decrease of CATa at the 5th and 10th postmortem days. However, the bleeding efficiency, moisture, ashes, R-value and levels of Ca, Pi, Na, K and total protein showed no significant differences between two cortisol groups. The *antemortem* circulating levels of cortisol were positively correlated with *postmortem* pH, EC, DL, CL, THP, dimensional shrinkage and MDA, and negatively correlated with CATa. The analysis of circulating cortisol at slaughtering may predict the quality and oxidant stress status of camel meat during *postmortem* cold storage.

Keywords: Cortisol; Oxidant stress; Meat quality; Minerals; Proteins; Camel

INTRODUCTION

The stress of transport and handling, as well as waiting at the slaughterhouse induce an increase of cortisolemia, and creatine kinase and lactate in muscle (Kannan et al., 2000). Preslaughter stress (handling, transport, unloading and lairage) is responsible for a glycogen depletion in the muscle that can reduce the quality of meat (Immonen et al., 2000; Warriss, 2003).

Compared to other red meats, that of the dromedary is characterized by low levels of fat and cholesterol, and it is therefore beneficial for human health (Gheisari and Ranjbar, 2013). Currently, researchers are much more interested in improving the quality parameters of

camelina meat, such as its water retention capacity (WHC), tenderness, color and juiciness (Engy, 2017). The ultimate pH of muscle measured 24 h *postmortem* (pHu) is the important indicator of the meat quality at the commercial level. The pHu is able to impact color, WHC and tenderness of meat, and it's influenced by the antemortem levels of glycogen and the postslaughter levels of lactate in the muscle (Gregory, 2003). Like other species, the quality of meat in the dromedary camel, includes fat content, pHu, color, WHC and tenderness (Kadim et al., 2008). In this species, pre-slaughter stress (transportation, novelty, fasting, loading, unloading, stockage density and lairage) had effects on metabolism as demonstrated by high circulating levels of adrenaline and cortisol resulting in a

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depletion of glycogen in the muscle, causing high ultimate pH, drip loss and lipid peroxidation (Saeb et al., 2010; El Khasmi et al., 2010; 2015; Barka et al., 2016; Lemrhamed et al., 2018a; 2018b).

During ageing the camel meat showed a significant lipid oxidation associated to a high levels of malondialdehyde (MDA) and low catalase activity (CATa) (Tabite et al., 2018). This oxidation interferes with the integrity and safety of meat through the formation of potentially toxic compounds (Silva et al., 1999) such as MDA. Cold preservation of meat is known by an imbalance between antioxidants and prooxidants leading to an exaggerated formation of reactive oxygen species in front of an antioxidant system that is not effective (Hur et al., 2004). The aim of this work was to investigate the relationship between the circulating levels of cortisol at slaughter and some parameters of the camel meat such as pH, electrical conductivity (EC), drip loss (DL), cooked loss (CL), moisture, solids, ashes, proteins, calcium (Ca), phosphorus (Pi), magnesium (Mg), sodium (Na), potassium (K), MDA, CATa, shrinkage, R-value and total haem pigment (THP) during ageing for 10 days at $4\pm 1^\circ\text{C}$.

MATERIALS AND METHODS

Animals

This investigation was carried out during April and May, the average ambient T° was 20°C , the average humidity was 71%, and the average wind speed and rainfall were 18km/h and 1mm respectively. In the municipal slaughterhouse of Casablanca located to the West of Morocco (North of Africa, latitude $33^\circ 34' 42.44''$ N, longitude $7^\circ 36' 23.89''$ O), 50 male camels in total (5 to 8 years, 300 to 400 kg) were used. According to their serum levels of cortisol, the camels were divided into 3 groups (Gr) which exhibited different serum levels of cortisol at slaughter (low, high and very high levels): Gr1 (13.07-67.9 ng/mL, $n= 24$), Gr2 (80.29-107.21 ng/mL, $n= 7$) and Gr3 (133.7-198.04 ng/mL, $n= 19$). These animals were road transported by truck for a distance of 72 to 80 km and an average speed of 60-65 km/h. The loading density was $2.6 \text{ m}^2/\text{camel}$. By using the formula cited by the Farm Animal Welfare Council (FAWC, 1991), the area in square metres is equal to 0.0315 multiplied by the live weight of animal in kg to the power of 0.67. According to the calculated value (2.5 m^2), the loading density used in this investigation has respected the animal welfare. The trucks used to transport the camels were devoid of ceiling and roof, and its floor and surrounding walls were made of iron. The camels were transported without any bedding materials. During the transport, they did not have access to water and food, they were placed in the trucks in a squatting position and

turned to the side, and the forelegs were tightened by a rope at knee level. The road was paved from the start until the end of the journey. On arrival, the dromedaries were carefully unloaded to avoid any stress, and were then calmly taken to the stall area at the slaughterhouse.

On arrival and after unloading, the camels were subjected to a rest period for 13 to 18 hs in the waiting zone under a stocking density of $4 \text{ m}^2/\text{camel}$. They were not isolated from noise and human activity and didn't have access to water and food. After the waiting duration, the animals were guided inside the slaughter room at 7:00 am, to be slaughtered according to the routine *Halal* procedure without any prior stunning. They were placed in a squatting position on the floor with the forelegs tied with a rope at the knees. The heads were fixed in a caudal position and an adult Muslim made the animals quickly bleed by section of the jugular veins, carotid arteries, trachea and esophagus without severing the spinal cord. After slaughter, the animals were suspended, the skin, head, legs and viscera were isolated, and the carcasses were placed in the refrigerator at 4°C and in the dark for 12 h.

Blood sampling

To determine the serum levels of cortisol, blood was collected at slaughter (07 am) directly in dry tubes which were kept in ice. Serum was separated within 40 min of collection by centrifugation at 5°C for 15 min at 750xg, then stored in 1.5 ml Eppendorf tubes at -20°C until analysis.

Muscle removal

After slaughtering and veterinary inspection, approximately 450 g of oblique abdominal external muscle (*musculus abdominis obliquus externus*) were collected at 10h a.m within 3 h *postmortem* from the right side of carcasse of each animal. The muscle samples were packed in zipped plastic bags and then transported to 4°C for 10 min in the cooler to the laboratory. Fat and connective tissue were removed and lean meat samples were divided into 3 portions: P4h, P5d and P10d which were packaged separately in plastic bags and kept in a refrigerator ($4\pm 1^\circ\text{C}$) for 4h postslaughter, 5 d and 10 d *postmortem* respectively. All meat portions were divided into 4 parts: p1 (42 g) to analyze pH, DL, CL and EC; p2 (5g) to analyze moisture, solids, ashes, Ca, Pi, Mg, Na and K; p3 (17 g) to determine protein, MDA and CATa, and p4 (7.5 g) for estimate R-value, shrinking and THP at each *postmortem* ageing time. These parts were packaged in a sterile polythene bags, labelled and then were placed at $4\pm 1^\circ\text{C}$ for 10 d and all measurements were assessed in duplicate of each parameter.

To analyze total protein, MDA and CATa at different ageing times, meat samples were added to a phosphate buffer solution (0.1 M, pH 7.4) (500 mg/5 ml), then were

homogenized at 4 ° C for 30 s. The homogenates were centrifuged at 5000 rpm for 15 min, and the supernatants were stored at -80 ° C until assay.

Cortisol analysis

At the national center for nuclear science and technical energy in Maâmoura, Morocco, serum cortisol concentrations were measured by kits marketed using radiolabelled cortisol ¹²⁵I (DIAsource, Immunoassays S.A., Nivelles, Belgium). These kits have proven to be effective in previous investigations on camels (El Khasmi et al., 2013; 2015; Lemrhamed et al., 2018a). Limits of detection were included in the areas of validation for cortisol analysis, and precision in the standard curve following sample dilution, inter- and intra-assay coefficients of variation results were considered.

pH and electrical conductivity measurement

To measure the meat pH, samples (2 g) were crushed and homogenized by a porcelain mortar using 20 mL neutralized 5-mM sodium iodoacetate. The pH meter was calibrated with pH 4 and 7 standards, and the pH value was measured at 18–20°C by a standardized glass electrode. The EC was measured with a conductivity meter (model Hanna EC 215 connected to an electrode of the four rings HI 76303) which is microprocessor type LF 196 equipped with a conductivity cell. The device is calibrated with potassium chloride solution (0.01 mol/L) whose the EC is known for a reference temperature of 25 °C. 5 g of muscle were ground and the homogenates were centrifuged at 18,000 x g for 30 min. The volume of juice recovered is supplemented to 20 mL with pure water to have a sufficient volume to plunge the conductivity cell. The EC values were adjusted automatically in relation to the temperature of the sample. The values of Measurements made on pure water used to dilute meat juices were deduced from the conductivity values and were expressed in mS/cm.

Drip loss and cooking loss

DL was measured using the method of Pohja and Niinivaara (1957). Meat samples weighing 5 g were placed between two filter papers (Whatman No. 1) and pressed at 10 kg for 5 min. Then, meat samples were separated from the filter papers and reweighed. The percentage of weight loss after cold storage was determined from the difference in muscle weight before and after ageing.

To analysis the CL (Honikel, 1998), meat samples (30 g) were placed in polyethylene bag and totally immersed without adding ingredients or fat in a water bath at 70°C for 90 min. The internal temperature monitored using a thermometer during boiling was 97°C. After cooking, the samples were cooled to room temperature for 40 min in its exuded fluids and then removed and dried slightly with blotting paper and reweighed. CL (%) were determined by

the difference between the sample mass before cooking and mass after cooking and was expressed as a percentage of the initial weight of sample.

Moisture, solids, ashes and minerals analysis

The chemical composition of camel meat was analyzed according to the standard methods of Association of Official Analytical Chemists (AOAC, 2000).

Ca, Pi and Mg were colorimetrically analyzed in ashes after acidification (JENWAY 6320D Spectrophotometer, Model 6320D) using commercially available kits (CHRONOLAB, Switzerland). Na and K were determined in ashes after acidification by flame photometry using standard solutions prepared by dilution of stock solutions of Na and K.

Analysis of total protein, malondialdehyde and catalase activity

The total protein (g protein/100 g sample) was measured in meat samples of 10 g by the Kjeldahl method using the factor 6.25 for conversion of nitrogen to crude proteins.

The MDA was estimated by the method of Botsoglou et al. (1994). Half a mL of the homogenate having been prepared from a sample of 5 g was mixed with 0.5 ml of trichloroacetic acid (TCA) 20% and 1 ml of thiobarbituric acid (TBA) 0.67%. The mixture was incubated in a water bath at 100°C for 15 min, then cooled, and after addition of 4 ml of n-butanol, the mixture was centrifuged for 15 min at 3000 rpm. The absorbance of the supernatant was measured at 530nm against the blank. The concentration of MDA was calculated according to the formula:

$$C = 103OD/\epsilon\chi LD \quad (1)$$

where C: concentration of MDA in $\mu\text{mol/mL}$; OD: optical density read at 530 nm; ϵ : molar extinction coefficient of the MDA = $1.56 \times 10^5/\text{M/cm}$; L: optical path length = 0.779 cm; χ : volume of the sample (mL); D: dilution factor.

The CAT activity in meat samples was estimated by the method of Aebi (1974). An enzyme fraction was prepared from 2 g of the sample which was cut and homogenized in three volumes of phosphate buffer (0.1 M, pH 7.4) by homogenizer. The homogenate obtained was centrifuged at 2,000 rpm for 30 min at 4 °C and on the final supernatant the activity of the CAT was analyzed. The disappearance of H_2O_2 was assisted colorimetrically at 240 nm for 2 min according to the following formula:

$$(2.3033/T) \times (\log A1/A2) \quad (2)$$

Where A1: absorbance at the first minute; A2: absorbance at the second minute; T: time interval in minutes.

The CAT activity was defined as the decrease of H_2O_2 /min/g of muscle using a molar extinction coefficient of 0.041/mM/cm.

Estimation of R-value, shrinkage and total haem pigment

To evaluate meat R-value (Honikel and Fisher, 1977), 2 g of muscle samples were homogenized in 10 mL of 1M perchloric acid and filtered. The filtrate (0.1 mL) was diluted with 4.9 mL of 0.1M phosphate buffer (pH 7.0). The optical density at 250 nm (for IMP) and that at 260 nm (for ATP) were spectrophotometrically determined (JENWAY 6320D Spectrophotometer, Model 6320D) and the R-value was evaluated by the ratio of 250:260.

To estimate meat shrinkage, width and length of the same meat samples of 5 cm (3 g) steaks were measured before and after cooking, to measure the reduction in width and length, then the dimensional shrinkage according to the standard methods of (AOAC, 2000).

To determine THP from ground meat (Warriss (1979), samples (2.5 g) were mixed with acetone (10 ml), distilled water (0.5 ml) and HCl (0.25 ml) in a beaker of 50 ml, then the mixtures were covered with parafilm and maintained overnight at 4°C in the dark. After filtration of mixtures, the optical density was spectrophotometrically determined at 640 (JENWAY 6320D Spectrophotometer, Model 6320D), using a blank solution (80% acetone, 2% HCl and 18% H₂O). The absorbance was multiplied by 680 to estimate the concentration of THP (µg/g of meat).

Statistical analysis

The data were expressed as Mean±standard error (SE) and analysis of variance (ANOVA) of the General Linear Models procedure of the Statistical Analysis System Software (SAS, 2005) was used to analyze the data. To search if significant differences existed among groups and ageing times, Duncan’s multiple range test was used and P<0.05 was considered as the level of significance. Pearson’s correlation tests were used to analyze correlations between parameters.

RESULTS

pH, drip loss, cooking loss and electrical conductivity

Compared to low cortisol camels (Gr1), high (Gr2) and very high (Gr3) ones showed a significant (P<0.05) increase of pH, DL, CL and EC at 5 and 10 d postmortem during storage at 4 ± 1 °C. In Gr1, Gr2 and Gr3 values measured at the 5th d were respectively 5.60±0.11, 6.60±0.11 and 6.65±0.11 for pH, 9.23±1.12, 12.56±1.45 and 13.11±1.41 for DL (%), 23.78±1.53, 27.67±1.63 and 28.23±1.57 for CL (%) and 6.75±2.24, 13.16±2.52 and 13.87±2.56 for EC (mS/cm) (Fig. 1).

Moisture, solids and ashes

As showed in Fig. 2, no significant differences among Gr1, Gr2 and Gr3 were observed in moisture, solids and ashes during all cold storage period. Mean values (%) registered at 5 d postpartum were respectively 7.26±0.14, 7.34±0.17 and 7.33±0.17 for moisture, 2.74±0.14, 2.67±0.17 and 2.67±0.16 for solids and 1.14±0.1, 1.13±0.08 and 1.13±0.09 for ashes (Fig. 2).

Total protein and minerals

Groups 1, 2 and 3 showed no significant differences of meat levels of protein total, Ca, Pi, Mg, Na and K during the cold storage period (Figs. 3 and 4). In these groups, protein levels (g/100g) were 23.34±3.11, 22.42±2.16 and 23.11±2.12 respectively at 5 d postmortem (Fig. 3). At the same stage, Mg levels (mg/100g) in groups 1, 2 and 3 were 42.43±3.15, 40.72±3.42 and 40.03±3.33 respectively

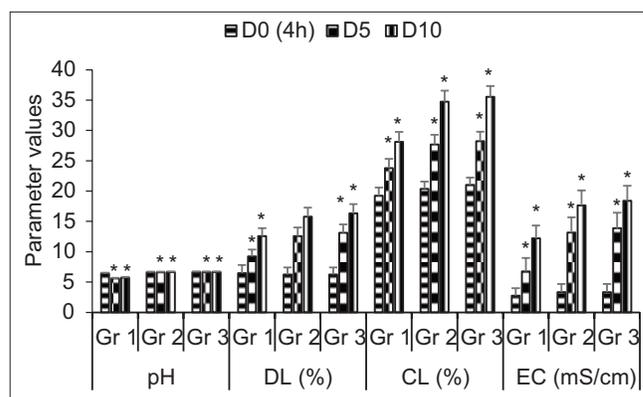


Fig 1. Impact of ageing on pH, drip loss (DL) and cooking loss (CL) in meat at 4 h postslaughter and 5 and 10 d postmortem during storage at 4 ± 1 °C, in 3 groups (Gr) of camels with different serum levels of cortisol at slaughter [Gr1 (13.07 – 67.9 ng/mL, n= 24), Gr2 (80.29 – 107.21 ng/mL, n= 7) and Gr3 (133.7 – 198.04 ng/mL, n= 19)]. (Means±SE, *P<0.05, comparison between groups at the same day of cold storage).

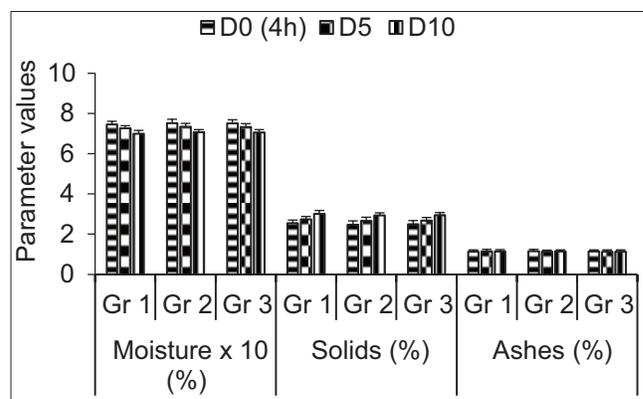


Fig 2. Impact of ageing on moisture, solids and ashes in meat at 4 h postslaughter and 5 and 10 d postmortem during storage at 4 ± 1 °C, in 3 groups (Gr) of camels with different serum levels of cortisol at slaughter [Gr1 (13.07 – 67.9 ng/mL, n= 24), Gr2 (80.29 – 107.21 ng/mL, n= 7) and Gr3 (133.7 – 198.04 ng/mL, n= 19)]. (Means±SE).

(Fig. 3), and those of K (mg/100g) were 765 ± 71 , 774 ± 76 and 773 ± 72 respectively (Fig. 4).

Malondialdehyde and catalase activity

Compared to camels with low circulating cortisol at slaughter (Gr1), those with high (Gr2) and very high (Gr3) cortisol showed a significant increase of meat concentration of MDA at 4 h postslaughter and 5 and 10 d postmortem, and a significant decrease of CAT activity in the meat at 5 and 10 d postmortem during cold storage (Fig. 5). In Gr 1 and Gr 3, MDA levels (mg/kg) were respectively 1.23 ± 0.85 and 6.21 ± 1.11 , $P < 0.005$ and CATa (UI/g) was respectively 183 ± 27 and 113 ± 18 , $P < 0.05$ at the 5th postmortem d (Fig. 5).

R-value, shrinkage and total haem pigment

During all cold storage period and at the same stage of cold storage, no significant variations of meat R-value and pourcentage of shrinkage were observed between

groups 1, 2 and 3 (Fig. 6). At 5 d postmortem, R-value was 1.38 ± 0.07 , 1.41 ± 0.07 and 1.40 ± 0.09 respectively in groups 1, 2 and 3. In the same way, shrinkage (%) was 7.97 ± 1.55 , 6.22 ± 1.48 and 7.43 ± 1.52 respectively (Fig. 6). However, in groups 2 and 3, THP ($\mu\text{g/g}$) was significantly ($P < 0.05$) higher by comparison to Gr1 (13.39 ± 1.18 vs 10.18 ± 1.06 and 13.40 ± 1.21 vs 10.18 ± 1.06 respectively at 5 d postmortem (Fig. 6).

A significant correlation between circulating concentrations of cortisol at slaughter, and pH, DL, CL, EC, MDA, CATa and THP in camel meat during cold ageing was found (Table 1).

DISCUSSION

In this study we investigated the relationship between circulating levels of cortisol at slaughter and postmortem

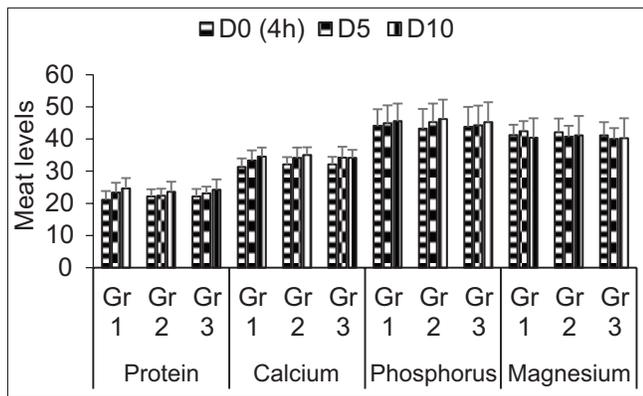


Fig 3. Impact of ageing on protein total (g/100g fresh weight), calcium, phosphorus and magnesium (mg/100g fresh weight) levels in meat at 4 h postslaughter and 5 and 10 d postmortem during storage at $4 \pm 1^\circ\text{C}$, in 3 groups (Gr) of camels with different serum levels of cortisol at slaughter [Gr1 (13.07 – 67.9 ng/mL, n= 24), Gr2 (80.29 – 107.21 ng/mL, n= 7) and Gr3 (133.7 – 198.04 ng/mL, n= 19)]. (Means \pm SE).

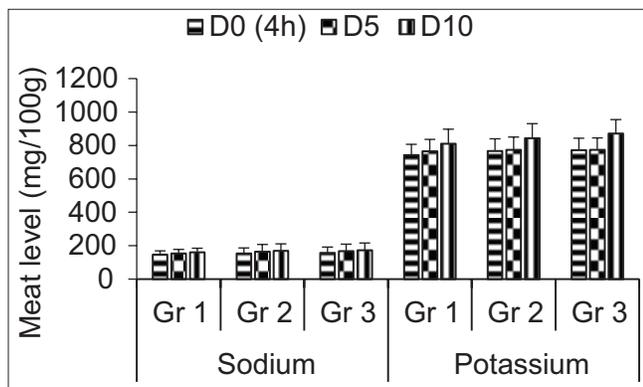


Fig 4. Impact of ageing on sodium and potassium levels (mg/100g fresh weight) in meat at 4 h postslaughter and 5 and 10 d postmortem during storage at $4 \pm 1^\circ\text{C}$, in 3 groups (Gr) of camels with different serum levels of cortisol at slaughter [Gr1 (13.07 – 67.9 ng/mL, n= 24), Gr2 (80.29 – 107.21 ng/mL, n= 7) and Gr3 (133.7 – 198.04 ng/mL, n= 19)]. (Means \pm SE).

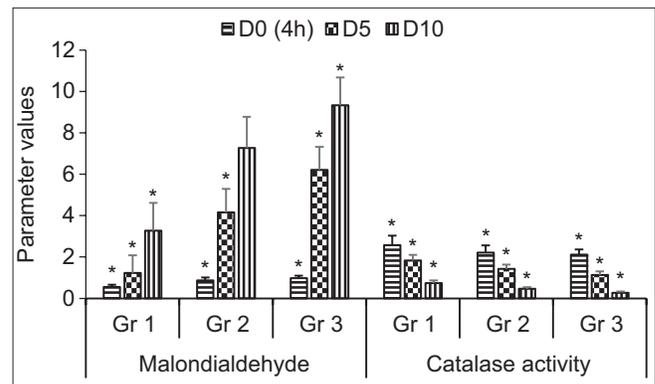


Fig 5. Impact of ageing on malondialdehyde (mg/kg) and catalase activity (x100UI/g) in meat at 4 h postslaughter and 5 and 10 d postmortem during storage at $4 \pm 1^\circ\text{C}$, in 3 groups (Gr) of camels with different serum levels of cortisol at slaughter [Gr1 (13.07 – 67.9 ng/mL, n= 24), Gr2 (80.29 – 107.21 ng/mL, n= 7) and Gr3 (133.7 – 198.04 ng/mL, n= 19)]. (Means \pm SE, * $P < 0.05$, comparison between groups at the same day of cold storage).

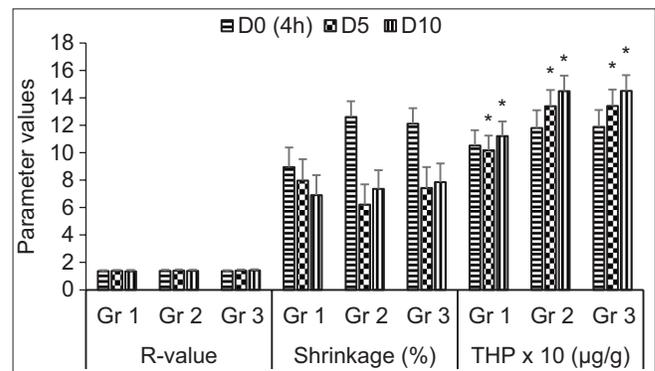


Fig 6. Impact of ageing on R-value, shrinkage and total haem pigment (THP) in meat at 4 h postslaughter and 5 and 10 d postmortem during storage at $4 \pm 1^\circ\text{C}$, in 3 groups (Gr) of camels with different serum levels of cortisol at slaughter [Gr1 (13.07 – 67.9 ng/mL, n= 24), Gr2 (80.29 – 107.21 ng/mL, n= 7) and Gr3 (133.7 – 198.04 ng/mL, n= 19)]. (Means \pm SE, * $P < 0.05$, comparison between groups at the same day of cold storage).

Table 1: Correlation coefficients between circulating levels of cortisol at slaughter and quality and stress oxidant parameters in camel met.

| | Cortisol | pH | DL | CL | EC | MDA | CATa | THP |
|----------|----------|--------------------|--------------------|--------------------|--------------------|---------------------|----------------------|---------------------|
| Cortisol | 1.0000 | r=0.781 p=0.000 | r=0.417 p=0.041 | r=0.461 p=0.033 | r=0.432 p=0.044 | r=0.711 p=0.002 | r= 0.523 p=0.017 | r=0.471 p=0.018 |
| pH | | 1.0000 | r=0.650 p=0.004 | r=0.547 p=0.014 | r=0.750 p=0.000 | r=0.621 p=0.002 | r=- 0.452 p=0.027 | r=0.894 p=0.014 |
| DL | | | 1.0000 | r=0.671 p=0.000 | r=0.598 p=0.003 | r=0.351 p=0.037 | r=- 0.521 p=0.017 | r=0.894 p= 0.013 |
| CL | | | | 1.0000 | r=0.721 p=0.001 | r=0.4713 p=0.018 | r=- 0.545 p=0.015 | r=0.511 p=0.013 |
| EC | | | | | 1.0000 | r=0.798 p=0.012 | r=- 0.436 p=0.017 | r=0.790 p=0.017 |
| MDA | | | | | | 1.0000 | r=- 0.465 p=0.019 | r=0.713 p=0.040 |
| CATa | | | | | | | 1.0000 | r=0.721 p=0.042 |
| THP | | | | | | | | 1.0000 |

DL : drip loss, CL : cooking loss, EC : electrical conductivity, MDA : malondialdehyde, CATa : catalase activity, THP : total haem pigment

physicochemical composition, quality characteristics and oxidant parameters of the camel meat during refrigerated ageing for ten days. Cortisol levels were positively correlated with *postmortem* pH, DL, CL, EC, MDA and THP, and were negatively correlated with CATa.

Various cruel practices such as rough handling, loading, transport, environmental conditions, soon and unloading are able to impact the animal welfare, resulting in body injuries, body weight shrinkage, muscle injury and fatigue, and degradation of meat quality (Teke et al., 2014). Transport, high stocking density and waiting period before slaughter are an integral part of stress responses which are mediated by an activation of the hypothalamic-pituitary-adrenal axis (HPA) in livestock in general (Swanson and Morrow-Tesch, 2001), and dromedary camel in particular (El khasmi et al., 2013; 2015; Lemrhamed et al., 2018a; 2018b). Transport induces an increase of blood cortisol and the animal could only restore normal value after a rest period, but, a long lairage time could increase cortisol concentrations again in sheep (Liu et al., 2011), calves (Bernardini et al., 2012), camel (Lemrhamed et al., 2018b) and rabbit (Skladanowska-baryza et al., 2018).

The biological mechanism of animal stress responsible for high circulating levels of cortisol and mediated by the activation of HPA axis, involves a release of corticotrophin-releasing factor by hypothalamus. This factor, stimulates the adenohypophysis to secrete adrenocorticotrophic hormone (ACTH) in blood circulation which in turn stimulates the secretion of cortisol by the adrenal gland (Moberg, 2000). Stress responses are initiated by an activation of the sympathetic system, resulting in a release of noradrenaline and adrenaline (Moberg, 2000). According to Ferguson and Warner (2008), these hormones might influence several muscle or meat parameters, such as, glycogen levels,

pHu, metabolic and structural modifications, proteolytic processes, water holding capacity (WHC), tenderness and flavor.

High circulating levels of cortisol at slaughter tended to increase pH, DL, CL and EC of meat camel during *postmortem* cold storage. Significantly higher DL and CL might be due to higher pHu observed in meat samples during ageing. According to Naveena et al. (2011), the increase in pH could result from the large number of hydrophilic sites on which several water molecules cling to hydrogen and also ionic bonds at the hydrophilic sites located on the polypeptides.

In pigs, it has been shown that transport stress combined with a short rest period induced an increase in EC of 80%, whereas after the same transport followed by sufficient rest increased EC only by 56% compared to non transported animals (Cygan-Szczegielniak and Janicki, 2012). In rabbits, a longer transport increased the pH, and the levels of moisture and DL in the meat compared to the shorter transport (Dal Bosco et al., 1997; Trocino et al., 2003).

In pig, preslaughter stress had been found able to reduce the pork quality by decreasing muscle glycolytic potential (muscle glycogen content before slaughter), tenderness and WHC (Gregory, 2003), and increasing plasma lactate, cortisol, muscle temperature, *postmortem* acidification, and pHu (Hambrecht et al., 2005). It was reported that transport and waiting before slaughter are responsible for a depletion of glycogen then a low levels of *postmortem* acidity in the muscle (Gregory, 2003; Campo et al., 2010). This depletion impacts meat pHu, tenderness, WHC and color (Apple et al., 1995; Mounier et al., 2006). In cattle, Teke et al. (2014) have reported a lowest muscle pHu after a rest period of 72 h, and a highest pHu after 24 h waiting

duration. In addition, in bulls, Mounier et al. (2006) have studied the effects of three periods of rest (1, 17 and 40 h) before slaughter, and have found a negative correlation between pHu and lairage time. In pig, transport stress decreased significantly the *antemortem* levels of muscle glycogen (Leheska et al., 2003), however, in the same species, Brown et al. (1999) found no effect of stress transport on the muscle glycogen.

Other authors think that muscle depletion of glycogen during long preslaughter feed and water restriction had been able to increase the meat pHu (Warris, 1990). So, according to Gallo et al. (2003) a long period of rest had altered several parameters of meat quality. In fact, after transport, the meat pHu increased after a lairage time of 48 h in sheep (Toohey and Hopkins, 2006), and 9 h in pig (Pérez et al., 2002). In the same way, meat of lambs who had waited 24 h in lairage had been darker than that of lambs who had waited 1 h (Jacob et al., 2005). These authors explained their findings by a possible depletion of glycogen stores in the muscle during the waiting period and food privation before slaughter.

In cattle, the DL of meat from animals that have spent 24 h in lairage was lower than that from animals that have been resting for 48 h or 72 h (Teke et al., 2014). However, in the same animals and conditions, the lairage time showed no influence on CL, WHC and tenderness (Teke et al., 2014). The DL and pHu as an important quality parameters are influenced by *antemortem* muscle content of glycogen, and increase significantly in meat when fasting time and lairage period increase before slaughter (Terlouw and Rybarczyk, 2008).

In goat, it had been reported that a road transport for 2 h in a warm atmosphere induced higher circulating levels of cortisol and adrenaline, and higher pHu, expressed juice, CL percentage and shear force in the meat (Kadim et al., 2006). Similarly, in sheep, a rough road transport for 4 h (Ruiz-De-La-Torre et al., 2001), or a restraint-stress (Apple et al., 1995) induced a significant increase of meat pHu. Furthermore, Miranda-de la Lama et al. (2011) had also reported that when the road was unpaved, transported lambs showed a significant increase of circulating cortisol, lactate and glucose, and meat CK levels and pHu compared with lambs transported on paved roads. These responses might be explained by an impact of cortisol. In fact, in lambs, injection of cortisol induced a significant increase of glucose metabolism, temperature, pH and WHC of fresh meat (Pighin et al., 2013). In addition, WHC, proteolysis, lipid oxidation and color of meat are largely influenced by the *Postmortem* pH and temperature kinetics of muscle, and are able to determine the sensory aspects of meat, then the satisfaction of the consumer (Bee et al., 2007; Ferguson

et al., 2008). Furthermore, according to Offer and Knight (1988), the variations of *postmortem* pH and temperature in the muscle could impact shrinking myofibrils and therefore the WHC during ageing of meat.

The preslaughter stress and cold ageing showed no effect on the chemical composition of the camel meat. Barka et al. (2016) have studied the effect of transport distance on some physicochemical parameters in 3 muscles (triceps, oblique and diaphragm) in the camel. The authors have found a significant decrease of glycogen content and a significant increase of pHu in these muscles when the distance of transportation increased, without any significant variations of proteins, ashes, dry matter and moisture. On the contrary, meat from rabbits having been road transported for a long journey showed a significant increase of moisture compared to rabbits having submitted to short transport (Dal Bosco et al., 1997).

In the study reported here, MDA contents increased significantly, whereas CAT activity decreased significantly during the postmortem stages of cold storage, but, the camels with high circulating cortisol at slaughter showed the highest levels of MDA and the lowest activities of CAT in their meat. In the camel, Barka et al. (2016) have evaluated the impact of stress induced by transport distance on muscle biomarkers, and have found that the levels of MDA increased while the CAT decreased significantly in the muscle when the transport distance increased. The oxidation of oxymyoglobin and lipids, as well as microbial contaminations leads to discoloration, DL and the production of potentially toxic compounds (Aidani et al., 2014). In the camel meat, the continuous increase of MDA levels observed during cold storage (Tabite et al., 2018), may be explained by formation of free short-chain fatty acids and unstable lipid hydroperoxide by microbial enzymatic hydrolysis (Gheisari et al., 2009). Lipid peroxidation of raw and cooked meat products of camel is one of the main causes of deterioration in the quality during cold and frozen ageing (Abdelhadi et al., 2013). In camels, Maqsood et al. (2015) studied protein and lipid characterization of fresh meat during 9 d of cold ageing at 4°C. They found that camel meat undergoes a lipid oxidation at a more pronounced level on the 3rd day of cold ageing, then a low peroxidation index, the 9th day.

The results showed no significant differences in *postmortem* R-value and THP contents during cold storage of meat, between camel groups with different circulating levels of cortisol at slaughter. As an indicator of ATP level, R-value is defined as the ratio of adenine nucleotidase (ATP) to inosine nucleotidase (IMP) (Khan and Frey, 1971). It is

largely known that muscle levels of THP are recognized to impact the lightness of meat (Fletcher, 2002), thus, the levels of THP were negatively correlated with lightness in poultry meat (Sirri et al., 2009) and meat products (Guzman et al., 1995). In the dromedary camels, the high and very high cortisol groups showed high meat levels of THP when compared with the low cortisol group. So, the meat of camels with high cortisol at slaughter might be darker than that in low cortisol ones.

CONCLUSION

Taken together, our results showed that in the dromedary camel, high and very high circulating levels of cortisol at slaughter influenced the *postmortem* pH, antioxidant status and quality characteristics of its meat during cold ageing. It could be suggested that measuring serum cortisol at exsanguination may be useful to predict the *postmortem* storage conditions and quality value of camel meat.

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Author's contributions

R. T. was involved in conception and design of study and redaction. A. L. was involved in documentation, blood sampling and analysis of results. M. F. was involved in blood sampling, physicochemical and radioimmunological analysis. N. E. was involved in radioimmunological analysis. A. B. was involved in processing and statistical analysis of results. B. F. was involved in revision of experimental design and redaction. M. E. made a major contribution by supervising this work.

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