IMPACT OF THE DURATION OF STRESS STEPS BEFORE SLAUGHTER AND AT SLAUGHTER ON THE PHYSICOCHEMICAL AND BIOCHEMICAL CHARACTERISTICS OF MEAT IN CAMELS

Abstract

Animals intended for slaughter are subject to a number of stress factors abattoirs which can have negative effects on their physiological functions and on the quality of their meat. In this work, an investigation of the durations of transport, unloading, driving to the slaughter room, slaughter and bleeding was carried out, and the impact of the duration of each of these stages on physico-chemical and biochemical parameters of meat was assessed in camels. The results showed that the transport, unloading, driving to slaughter, slaughter and bleeding times were respectively, 1 ~~h~~-11 h, 3 ~~min~~ -20 min, 5 ~~min~~-20 min, 4 ~~min~~-20 min and 5 ~~min~~-10 min. Mean exudate and cooking loss values, and carbonyl and MDA contents were significantly ($P<0.05$) higher, while CAT and SOD activities were significantly ($P<0.05$) lower when transport, unloading, driving to slaughter and slaughter times were longest. Transport, unloading, driving to the slaughterhouse and slaughter times were significantly ($P<0.05$) and positively correlated with exudation, cooking loss, MDA and carbonyls, while they were significantly ($P<0.05$) and negatively correlated with CAT and SOD activities. The durations of the stages to which camels are exposed in slaughterhouse, from transport to slaughter could induce oxidation of their meat and alter its quality.

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Keywords: Preslaughter stress, Oxidative stress, Dromedary Camel, Meat composition, Morocco.

1. Introduction

Camels (*Camelus dromedarius* and *Camelus bactrianus*) play an important socio-economic role, they produce milk and meat for human populations in arid countries. World meat production reached 653,000 tonnes in 2019 [1] for an estimated camel population of 37.5 million head [2]. The handling of camels in farms, markets and slaughterhouses, the conditions of their loading into trucks, their transport, their unloading and their stabling at slaughterhouses, are potentially stressful and expose the camels to what is called "stress before slaughter" [3,4,5,6,7]. Although the welfare of farmed animals and its impact on the quality of their products has long attracted the attention of scientific researchers in developed countries [8], this research topic remains very little addressed in developing countries especially for camels.

In addition, when handled, farm animals face several potential welfare issues during and after transport, including deprivation of water and food for long periods, fatigue, injuries, diseases, mixing of different species, accelerations, vibrations, noise, space constraints, road topography, vehicle design, air pollutants and environmental conditions [9,10,11,12].

Thus, in the context of animal production, taking well-being into account is much more essential to the health of consumers, breeders and those involved in the production and trade of animal products [13,14]. The quality of carcasses and meat is affected by inappropriate antemortem handling, during loading, transport, unloading, waiting and slaughter of animals [15,16], whose durations are able to modify the quality of their meat [17]. Thus, at the end of all these pre-slaughter stress stages, the animals are kept in the lairage area before their slaughter to rest and restore their homeostasis[18]. However, if kept for a long period without access to water and food in uncomfortable enclosures, the spoilage of the meat quality of these animals will be increased [16,19,20]. To our knowledge and so far, no work has studied the effect of the duration of different preslaughter stages on the biochemical composition of meat in the dromedary camel. Thus, the objective of this work was to investigate possible correlations between durations of transport, unloading, driving to the slaughter room, slaughtering and bleeding, and the physicochemical and biochemical parameters of meat in the dromeday camel.

2.Materiels and Methods

2.1. Animals and survey

Our study was conducted on 53 male dromedaries (*Camelus dromedarius*) in good health, aged 2 to 7 years, weighing from 125 to 370 kg and having been subjected to a semi-extensive breeding method. These animals were intended for slaughter at the municipal slaughterhouse in Casablanca, Morocco. In order to assess the duration of the different stages of pre-slaughter stress from the arrival of camels at the slaughterhouse until bleeding, a survey was carried out during the hot season. This survey focused on the duration of transport, unloading, driving to the slaughter room, slaughtering and bleeding. Possible correlations between these factors and the physicochemical and biochemical parameters of the meat were researched.

2.2 Chemicals

2.2. Collection of meat samples

After the animals were slaughtered around 7 a.m., followed by a veterinary inspection, meat samples (*oblique muscle*) were taken around 10 a.m. using a sharp knife at a depth varying from 2 to 3 cm. These samples were transported at 4°C for 15 min, in a cooler from the slaughterhouse to the physiology and molecular genetics laboratory of Ben M'Sik faculty of sciences in Casablanca. The samples were were kept at 4°C until 24 hours*postmortem*, then were divided into 4 portions: the first to measure the water, dry matter and ash contents, the second for the pHu determination, the third for the losses analysis in exudate and cooking, and the last to analyze malondialdehyde (MDA) and carbonyl contents, and the activity of catalase (CAT) and superoxide dismutase (SOD).The portions of meat have been stored at -80°C until analysis.

2.3.Ultimate pH (pHu), exudate and cooking losses

Postmortem muscle pHu was measured using a pH meter. After removal of external fat and connective tissue, 2 g of meat was ground and homogenized with 18 ml of 5 mM sodium iodoacetate using an ultrasonic sonicator-homogenizer, then the mixture was filtered and the pH value was measured at 18°C. Use of a standardized glass electrode connected to a digital pH meter. The pH meter was previously calibrated with pH 4 and pH 7 standards. The readings were recorded in triplicate for each measurement.
To measure losses by exudate, 8g of meat were weighed before and after 24 hours of storage at 4°C. During storage, the samples were suspended by a nylon cord in a plastic bag, ensuring that the meat had no contact with the juices in the bag. The exudation was calculated as a percentage of the ratio of the weight of the juice to the weight of the initial sample.
To assess cooking loss, meat samples were placed in polythene bags, and fully immersed in a 70°C water bath for 90 minutes, with no ingredients added. The internal temperature of the meat (97°C) was monitored using a thermometer during boiling. After cooking, the samples

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were cooled at room temperature for 40 min, in their exuded fluids, then removed and lightly dried with filter paper and reweighed. Cooking losses were calculated as the difference in mass of the sample before and after cooking, expressed as a percentage of the initial mass of the sample.

2.4. Moisture, dry matter, ash and protein

The chemical composition of camel meat was analyzed according to the standard methods of AOAC-Association of Official AnalyticalChemists[21].

2.5. Determination of malondialdehyde (MDA) and carbonyls

Lipid oxidation was assessed by measuring colorimetrically substances reactive with Thiobarbituric acid (SR-TBA) according to the method of Lynch and Frei [22]. This method evaluate the quantity of non-volatile aldehydes (MDA) produced during oxidation. One ml of the extract 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.
The evaluation of protein oxidation was obtainedby assaying protein carbonyls using Dinitrophenylhydrazine (DNPH) according to the method of Oliver et al. [23]. The production of protein carbonyls is due to the oxidation of basic amino acids and threonine in meat.

2.6. Measurement of CAT and SOD 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, by the variation of the optical density following the disproportionation of H_2O_2 at an incubation temperature of 25°C. CAT activity in the crude extract was determined spectrophotometrically using the method of Aebi [24]. The activity of SOD in the extract was quantified according to the method of Paoletti et al. [25]. 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 crude extract in the 50 mM potassium phosphate buffer. Reaction by adding 0.27 mM NADH as the final concentration.

2.7. Statistical analysis

The survey data was analyzed using SPSS software. The values of the physicochemical and biochemical parameters were expressed as mean (M) ± standard error of the mean (SEM). A parametric test (correlation of Pearson's analysis) was carried out to detect correlations of the duration of transport, unloading, driving to the slaughter room, slaughtering and bleeding, with the physicochemical and biochemical parameters of meat. P<0.05 was considered statistically significant.

3. Results

3.1. Survey data

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The dromedaries usually arrived every Friday and Sunday transported in trucks at different times of the day, usually tied up, in a crouching position and deprived of water and food. The duration of transport was 10~~h~~-11h in 67.93% and 1~~ h~~-2h in 32.07% of the animals. After unloading, which lasted 3~~ min~~-4 min, 5~~ min~~-10 min and 11~~ min~~-20 min respectively, in 16.98%, 32.07% and 50.94% of the animals, the camels were kept in the stall without access water and food. Then, they were led inside the slaughter room, for 5~~ min~~-6 min, 7~~ min~~-10 min, 11~~ min~~-20 min respectively, for 22.64%, 35.85% and 42.50% of the animals. They were usually pulled by ropes and pushed. The animals were finally slaughtered according to the *halal* procedure without stunning within 4~~ min~~-5 min, 6~~ min~~-10 min and 11~~ min~~-20 min respectively, for 16.98%, 37.74% and 46.27% of the camels, while the duration from bleeding to loss of consciousness was less than 5~~ min~~-6 min, 7~~ min~~-8 min and 9~~ min~~-10 min respectively, in 16.98%, 28.30% and 55.71% of animals.

3.2 Characteristics of the meat

The analysis of the different physicochemical and biochemical characteristics of the meat studied, according to the duration of each of the pre-slaughter stress stages~~, are grouped in~~ (**Tables 1** and **2**)~~below~~. Results showed that the losses of exudate and cooking, and the contents of carbonyls and MDA, were significantly (P<0.05) higher, while CAT and SOD activities were significantly (P<0.05) lower corresponding to the higher ~~when the~~ duration of transport, unloading, driving to the slaughter and slaughter~~were higher~~ (**Tables 1**and**2**). On the contrary, pHu values and protein, water, dry matter and ashes contents did not show any significant variation with the duration of each of the pre-slaughter stress stages studied (**Tables 1**and **2**).

Table 1 : Distribution of the physicochemical characteristics of meat according to the duration of transport, unloading, transport to the slaughtering room, slaughtering and bleeding. (M±SEM, *P<0.05; comparison to the shortest duration of each pre-slaughter stress period)

Duration of preslaughter steps	pHu	Exsudate (%)	Cooking loss (%)	Moisture (%)	Dry matter (%)	Ashes (%)
Transport						
1-2 h (17)	5.56±0.18	0.47±0.12	21.36±2.64	75.25±1.53	24.75±1.53	0.98±0.11
10-11 h (36)	5.23±0.15	0.76±0.13*	30.23±3.51*	72.67±1.65	27.33±1.65	1.11±0.13
Unloading						
3-4 min (9)	5.51±0.15	0.43±0.12	19.85±2.36	73.45±1.54	26.55±1.54	1.07±0.11
5-10 min (17)	5.53±0.16	0.45±0.13	20.53±2.56	74.51±1.46	25.49±1.46	0.97±0.11
11-20 min (27)	5.41±0.17	0.75±0.13*	28.27±3.42*	72.57±1.55	27.43±1.55	1.11±0.12
Driving to the slaughter						
5-6 min (12)	5.54±0.18	0.41±0.11	20.33±2.52	73.65±1.64	26.35±1.64	0.99±0.12
7-10 min (19)	5.49±0.17	0.47±0.13	21.52±2.56	74.55±1.63	25.45±1.63	0.96±0.11
11-20 min (22)	5.33±0.15	0.74±0.13*	29.23±3.53*	73.48±1.56	26.52±1.56	1.12±0.13
Slaughter						
4-5 min (9)	5.53±0.17	0.44±0.11	21.08±2.71	72.51±1.81	27.49±1.81	1.07±0.12
6-10 min (20)	5.51±0.18	0.46±0.12	23.73±2.78	75.21±1.44	24.79±1.28	0.96±0.12
11-20 min (24)	5.41±0.16	0.77±0.12*	30.07±3.15*	73.67±1.27	26.33±1.42	1.09±0.13

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Bleeding						
5-6 min (9)	5.52±0.17	0.45±0.13	19.81±2.76	75.23±1.34	24.77±1.34	0.98±0.11
7-8 min (15)	5.57±0.16	0.48±0.13	22.43±2.75	76.03±1.62	23.97±1.62	1.13±0.12
8-9 min (29)	5.43±0.15	0.56±0.13	24.11±3.27	74.17±1.67	27.33±1.67	1.07±0.13

In brackets the number of animals ( ).

Table 2 : Distribution of the physicochemical characteristics of meat according to the duration of transport, unloading, transport to the slaughtering room, slaughtering and bleeding. (M±ESM, *P<0.05; comparison relative to the shortest duration of each pre-slaughter stress period)

Duration of preslaughter steps	Protein (%)	Carbonyls (nmol/mg)	MDA (nmol/kg)	SOD (µmol/min/mg)	CAT (UI/kg)
Transport					
1-2 h (17)	19.15±3.53	1.15±0.11	112.74±15.53	8.67±0.14	364.13±19.34
10-11 h (36)	17.11±3.32	1.87±0.13*	157.23±18.41*	7.21±0.13*	301.53±17.48*
Unloading					
3-4 min (9)	17.18±3.63	1.13±0.12	103.81±16.57	8.53±0.15	371.22±20.15
5-10 min (17)	18.15±3.67	1.14±0.13	123.45±17.43	8.32±0.15	350.31±19.35
11-20 min (27)	17.81±3.58	1.76±0.13*	151.56±16.76*	7.33±0.13*	312.42±18.21*
Driving to the slaughter					
5-6 min (12)	18.18±3.26	1.16±0.13	121.25±17.46	8.61±0.15	358.27±19.25
7-10 min (19)	20.13±3.63	1.15±0.13	133.57±18.19	8.53±0.15	333.31±19.48
11-20 min (22)	17.45±3.37	1.83±0.14*	163.19±19.08*	7.44±0.14*	308.65±18.83*
Slaughter					
4-5 min (9)	19.21±3.27	1.16±0.11	110.33±15.21	8.45±0.15	361.31±20.12
6-10 min (20)	17.25±3.36	1.25±0.12	126.74±16.42	8.27±0.15	345.26±20.13
11-20 min (24)	18.16±3.41	1.85±0.12*	161.21±18.41*	7.28±0.13*	310.71±18.53*
Bleeding					
5-6 min (9)	20.11±4.07	1.18±0.12	123.13±16.25	8.50±0.16	357.81±21.71
7-8 min (15)	19.23±3.49	1.21±0.13	131.12±16.21	8.46±0.15	340.31±19.58
8-9 min (29)	18.19±3.51	1.29±0.13	139.34±17.32	8.54±0.16	337.86±20.35

In brackets the number of animals ( ).

Furthermore, the analysis of the correlations between the duration of each of the pre-slaughter stress stages studied and the meat characteristics determined, for each animal, showed that the durations of transport, unloading, driving to the slaughter and slaughter, were significantly (P<0.05) and positively correlated with exudation, loss on cooking, and MDA and carbonyl contents (**Table 3**), whereas they were significantly (P<0.05) and negatively correlated with CAT and SOD activities (**Table 3**). On the contrary, no significant correlation (P>0.05) between these durations and the pHu and the water, dry matter, ash and protein contents was found (**Table 3**).

Table 3 : Correlations between the physicochemical and biochemical parameters of meat and the duration of transport, unloading, driving to the slaughtering room, slaughtering and bleeding

Meat parameters	Duration of preslaughter steps				
	Transport	Unloading	Driving to the slaughter	Slaughter	Bleeding
pHu	r=-0.391 p=0.098	r=-0.273 p=0.095	r=-0.206 p=0.084	r=-0.262 p=0.094	r=-0.254 p=0.091
Exsudate	r=0.586 p=0.032	r=0.449 p=0.041	r=0.561 p=0.033	r=0.557 p=0.034	r=0.257 p=0.401
Cooking loss	r=0.573 p=0.031	r=0.606 p<0.029	r=0.603 p=0.028	r=0.553 p=0.035	r=0.313 p=0.291
Moisture	r=0.355 p=0.242	r=0.268 p=0.441	r=0.277 p=0.423	r=0.342 p=0.245	r=0.343 p=0.245
Dry matter	r=0.121 p=0.684	r=0.212 p=0.454	r=0.143 p=0.637	r=0.211 p=0.454	r=0.119 p=0.686
Ashes	r=0.323 p=0.288	r=0.252 p=0.431	r=0.314 p=0.291	r=0.243 p=0.447	r=0.317 p=0.287
Protein	r=0.219 p=0.453	r=0.329 p=0.286	r=0.245 p=0.446	r=0.297 p=0.412	r=0.311 p=0.292
Carbonyls	r=0.446 p=0.041	r=0.448 p=0.040	r=0.463 p=0.038	r=0.456 p=0.039	r=0.360 p=0.237
MDA	r=0.565 p=0.016	r=0.576 p<0.015	r=0.651 p=0.048	r=0.634 p<0.049	r=0.348 p=0.243
CAT	r=-0.686 p=0.018	r=-0.594 p=0.016	r=-0.581 p=0.018	r=-0.678 p=0.016	r=-0.381 p=0.095
SOD	r=-0,573 p=0.031	r=-0,763 p=0.026	r=-0,722 p=0.024	r=-0,754 p=0.025	r=-0,367 p=0.098

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4. Discussion

In dromedaries, stressful situations before slaughter, such as the duration of transport, unloading, driving to the slaughter room and slaughter, significantly influenced the losses to exudation and cooking, and antioxidant status of meat. Indeed, at the level of the meat, the water losses and the contents of MDA and carbonyls increased significantly, while the activities of CAT and SOD decreased significantly with the increase of these durations. According to Terlouw and Bourguet[8], preslaughter and slaughter stress influences animal behaviour, physiology and meat quality. In our country, as in the Middle East and

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Africa, OIE animal welfare standards are often not respected. Indeed, the transport of dromedaries is not sufficiently regulated by law and is not subject to any official control on the welfare of these animals during their transport.
In domestic animals, the pre-slaughter stress stages begin at the farm, rearing site, and market, continue with loading, transport, unloading and waiting at the slaughterhouse, and end at slaughter[26,27]. Indeed, in camels, transport could have induced hypercortisolemia [28,29] and an activation of the generation of free radicals [30]. Similarly, rectal temperature, heart and respiratory rates, hemolysis, neutrophil/lymphocyte ratio, and plasma glucose and cortisol levels after transport and unloading were significantly higher than those analyzed before transport, and been positively correlated with distance traveled [3,29], stocking density [4] and housing conditions [5]. In goats, it has been reported that road transport for 2 h induced an increase in circulating levels of cortisol and adrenaline, pHu and losses by exudation and cooking of meat [31]. Similarly, in sheep, road transport on an uneven route for 4 hours induced a significant increase in the pHu of the meat [32].
The rest period before slaughter, gives the animal the opportunity to eat, drink, sleep and rest from the stress of transport, if it takes place in the absence of pathogens, mixing with ~~d~~ other animal species and additional handling [18]. According to Cooke et al. [33], a 2 h rest improved welfare in preconditioned calves, while a 5 h rest was more beneficial than 10 or 15 h rest in newly weaned calves [34]. On the contrary, the duration of rest did not induce any significant difference in morbidity, mortality and certain welfare indicators in conditioned calves that were rested for 0, 4, 8 or 12 h [35].
Although the dromedary is a large animal and difficult to handle, during transport, unloading, driving to the slaughter room and slaughter, vehicles are often not adopted for this animal, it lacks the equipment and the means and the technicians have no information on stress and animal welfare. Thus, all stages of handling the dromedary before slaughter require experience [36], and any extension of the duration of each of these stages will be more stressful for the animal. In addition, in camels, slaughter is done by the halal procedure without stunning during which the animal should be restrained in a squatting position, tying its front legs with a rope at the level at the knees. Once the animal's head was fixed and turned towards the tail, a quick incision with a knife would cut between the base of the neck and the thorax, to cause the animal to bleed rapidly by sectioning the jugular vein, the carotid arteries, the esophagus and trachea, without severing the spinal cord. Indeed, in camels, Lemrhamed et al. [6], reported that at slaughter, blood levels of MDA, glucose and cortisol were higher, while CAT activity was lower, than those observed just before slaughter. Thus, increasing the preparation time of the animal for the traditional slaughter procedure will have a much more pronounced impact on its antioxidant status.
Other works have reported that the elevation of MDA content and the decrease of CAT and SOD activities in meat, have been considered as reliable indicators of oxidative stress [37,38]. The production of free radicals during the oxidation of meat induces peroxidation of lipids and proteins. Zhu et al. [37] had demonstrated in a study carried out in ducks, that the group of animals which was slaughtered after transport for 2 hours showed a significantly higher level of MDA compared to the control group which was not transported. Similar results were reported by Shao [39] who reported a significant increase in MDA after 2 hour transport in pigs. On the other hand, Barka et al. [38] studied the effect of transport distance on certain physicochemical and biochemical parameters in 3 muscles (triceps, oblique and diaphragm) in camels. The authors found a significant decrease in glycogen content and a significant increase in pHu in these muscles as transport distance increased, with no significant variation in protein, ashes, dry matter, and moisture. On the contrary, MDA content increased and CAT activity decreased significantly with increasing transport distance. In addition, in camels, Tabite et al. [7] investigated the relationship between blood cortisol levels at slaughter

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according to pre-slaughter stress intensity and post-mortem physicochemical composition, quality characteristics and indicators of antioxidant status of meat stored in cold. These authors found that cortisol levels were positively correlated with pH, exudation, cooking loss, and MDA, and were negatively correlated with CAT activity.

5. Conclusions

As with other domestic animals, the dromedary cannot escape the stressful conditions that continue with loading, transport, unloading, reception, stabling, driving to the slaughterhouse and slaughter. The handling of this animal will be much more stressful due to the lack of means adopted and the training of breeders, drivers and slaughterhouse technicians. This favors alterations of the homeostasis of the dromedary and the quality of its meat, endangering the health of the consumer and food safety. Guaranteeing the welfare of the dromedary camel throughout the stages preceding slaughter requires good environmental conditions, access to water and food, good health and normal behavioral reactions, while respecting appropriate stocking density with comfortable lairage, animal health and bedding conditions. After all, the dromedary needs legislation on its well-being during all these *antemortem* stages according to international standards.

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