

Evaluation of the animal welfare during religious slaughtering

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Abstract

Plasma cortisol and its metabolites are physiological indicators for stress assessment and slaughtering method may affect their levels, playing an important role in the correct acidification of meat. The aim of the study was to determine and compare plasma cortisol values in animals slaughtered using traditional procedures, which include stunning (using captive bolt pistol), with those in animals slaughtered using Halal method, which does not involve stunning. The study was carried out on a total of 60 Charolais male beef cattle of eight months of age, bred in free paddock outdoors. The animals were divided into two experimental groups, each consisting of 30 individuals, on the basis of the slaughtering method, *i.e.* traditional or Halal, to verify the whole production chain and to ensure that the product conformed to Muslim rules. Plasma cortisol levels (detected by Elisa test) were evaluated at two different times of animal productive life: on the farm, one week before slaughter (T0) and during bleeding (T1). The 30 calves slaughtered after stunning showed plasma cortisol values of 4.06 ± 1.94 and 43.72 ± 12.09 nmol/L, respectively on the farm and during exsanguination. Conversely, the average values found in the 30 calves subjected to ritual slaughter were 3.26 ± 1.01 and 88.81 ± 41.02 nmol/L. The study demonstrated that throughout the animal's productive life (from pasture to slaughter) the greatest variation between slaughter with and without stunning was observed during bleeding.

Introduction

Ritual slaughter often comes under discussion as it is carried out without any kind of stunning. Typical of cultures such as Judaism and Islam, it is performed in dero-

gation from the provisions of Council Regulation (EC) N° 1099/2009 on the protection of animals at the time of killing, following precise rules prescribed by the religious rite (blessings or invocations), which characterize its sacredness (Italian National Bioethics Committee, 2003).

The word *Halal* is an Arabic term that means anything or act that is permissible in accordance with Sharia law. In order to be acceptable for Muslim consumption, slaughter must meet certain conditions specified in the Qur'an and Hadith (Fuseini *et al.*, 2016). At a basic level, Halal slaughter requires animals to have a pre-slaughter rest, and to be well fed and well looked after at the time of slaughter (Rahman, 2017). They must be alive at the point of slaughter and, if pre-slaughter stunning is performed, this must necessarily be reversible and must not result in the death of the animal before slaughter. Indeed, if not slaughtered, stunned animals must be able to make a full recovery (Fuseini *et al.*, 2016). The person-performing animal bleeding must have attained the age of discretion and must be mentally stable. The slaughterer should preferably be Muslim although the Qur'an allows Muslims to consume meat slaughtered by Christians and Jews (Qur'an, 1983). The slaughterer must pronounce the name of Allah during the slaughter of each animal, which should preferably face Qibla (Qur'an, 1983). Before the throat is cut, the animal should be securely restrained, particularly the head and neck. All efforts should be made to slaughter the animal with one stroke using a very sharp knife and without leaving any blemishes or damage (Rahman, 2017). In fact, slaughter must be performed with a single movement of the knife, which must be sharpened out of sight of the animal, which should be slaughtered out of sight of other animals and shielded from the view of blood (Fuseini *et al.*, 2016). Slaughter shall be carried out from the front (towards the chest) and not from behind (towards the back) and the head should not be severed from the neck during slaughter (Riaz, 2004). The act of slaughter must begin with an incision on the animal's neck just below the glottis; the animal's trachea and oesophagus must be severed, the spinal cord should not be cut, and the head should not be completely severed, as this would induce immediate and massive haemorrhage (Fuseini *et al.*, 2016). Finally, the carcass may not be processed, *e.g.* skinning or cutting off the hocks, before the animal is completely dead (Riaz, 2004).

The word *Shechitah* is only used to describe the method of slaughtering used according to Jewish rules, which include precise precepts to define *kosher* animals

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Key words: Animal welfare, Cortisol, Halal slaughter, Stunning.

Acknowledgments: The authors thank Stefano Sportelli for providing invaluable technical assistance in the laboratory.

Contributions: GB, RB and EC designed the experiment; RB, EB and EC performed the experimental procedures; RB and RR conducted all statistical analysis. All authors contributed writing the paper.

Conflict of interests: The authors declare no potential conflict of interests.

Funding: None.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate: This research was conducted in accordance with all relevant guidelines and procedures.

Consent for publication: The manuscript does not contain any individual person's data in any form.

Received for publication: 11 July 2019.
Revision received: 2 December 2019.
Accepted for publication: 12 December 2019.

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Italian Journal of Food Safety 2020; 9:8387
doi:10.4081/ijfs.2020.8387

and to select those suitable for religious slaughter, even during the breeding phase (Farouk *et al.*, 2014). Acceptable animals are slaughtered by a specially trained religious slaughter-man (*shochet*) using a special knife called *chalef* (Regenstein *et al.*, 2003). The cutting procedure, capable of causing a quick drop in brain blood pressure, rapid exsanguination of the animal and loss of consciousness, must be performed with a back-and-forth motion and it renders the animal insensitive to pain (Rosen *et al.*, 2004). When bleeding is finished, a post-procedure inspection follows, in which the animal is checked for the presence of

lesions, especially in the lungs and liver. According to the number and type of these pulmonary and hepatic injuries, the carcasses will then be classified as *cholak* (or *glatt*), *kosher* and *terif* (Mast *et al.*, 1983). After organ inspection, some portions of fat and organs such as the kidneys, the intestines and the sciatic nerve, are removed through a process called *nikkur*. All large arteries and veins, as well as any bruised meat or coagulated blood, are also removed because the blood is not considered edible. At the end of this selection, the meat is purged of all remaining blood through the koshering process (Farouk *et al.*, 2014).

There are many similarities in the principles and practice of Halal and Kosher slaughters (Gregory, 2007), though also many differences. Indeed, during Kosher pre-slaughter, the animal must be alive and conscious, while the Halal rite only requires the animal to be alive. Any form of stunning that renders the animal insensible to pain prior to slaughter is not acceptable for Kosher, while for Halal, non-stunning is preferred, but reversible forms of stunning are widely accepted and practised. The Halal method also tolerates post-slaughter stunning, so as to prevent uncontrolled movements after animal exsanguination. The Jewish religion requires a special knife (*chalef*) for each species of animal, but blessing is not required for each animal. By contrast, for Islam, there is no special knife and it can be used for different species, but blessing is required for each animal. Although in both religious rites the cut must be performed with a single movement, even a single mistake during kosher slaughtering means the meat is regarded as unfit for human consumption, which is not the case in Halal slaughter. Finally, one last important difference is that for Jewish religion certain parts of the carcass cannot be eaten, while all edible parts of the carcass are considered Halal (Regenstein *et al.*, 2003). Furthermore, the global volume and value of trade in Halal and Kosher meat is vast and has especially increased after the rise in the number of Islamic communities around the world (Lada *et al.*, 2009). The global and European market for Halal food is estimated to be worth around 547 and 77 billion dollars per year, respectively (Lever *et al.*, 2010).

Plasma cortisol and its metabolites are physiological indicators for stress assessment (Manteca, 2009) and the slaughter method used may affect the levels of stress indicators, playing an important role in the correct acidification of the meat (Nakyinsige *et al.*, 2014). The pattern of plasma cortisol, measured in order to monitor stress in beef cattle slaughtered accord-

ing to the rules imposed by the religious Jewish rite (Farouk *et al.*, 2014), revealed significant changes particularly during bleeding, when compared to traditional slaughter. In agreement with a previous study (Bozzo *et al.*, 2018), among the parameters used as stress indicators, plasma cortisol was the most useful, showing a statistically significant variation. The animals selected for kosher slaughtering showed lower levels of cortisol before and after transportation to the slaughterhouse, likely because their more docile temperament was the rationale for their selection by the Rabbis (Bozzo *et al.*, 2018). Therefore, plasma cortisol levels increased considerably at the exsanguination phase in these animals, as they were not stunned and were restrained using a rotary pen (Ceci *et al.*, 2017; Bozzo *et al.*, 2018).

The aim of the study was to determine and compare plasma cortisol values in animals slaughtered using traditional procedures, which include stunning (using a captive bolt pistol), with those of Halal-slaughtered animals, which does not involve stunning (as mentioned in the holy Qur'an). Since animals are often handled roughly before and during transportation and given that transportation can cause injuries and fatigue to animals (Rahman, 2017), regardless of the slaughter method, we only monitored two times, *i.e.* pasture and bleeding. Furthermore, for this study we used the same experimental conditions applied in a previous study (Bozzo *et al.*, 2018) to evaluate the differences between the two religious slaughtering procedures, Halal and Kosher.

Materials and Methods

Ethical statement

The experimental procedures were approved by the ethical committee of the Department of Veterinary Medicine at Bari University in Italy (Protocol no: 1235/18 - 17 May 2018).

Sampling protocol

The study, resulting from a partnership between the Food Safety Section of the Department of veterinary medicine at Bari University and a slaughterhouse in the Apulia region (Southern Italy), was conducted between March and May 2018. The study was carried out on a total of 60 Charolais male beef cattle of eight months of age, bred in the same free paddock outdoors. The transport took place on two consecutive days, during the same time of day

and had the same duration in the two groups (about 45 min). After transport, all the animals arrived at the same slaughtering establishment, where they were kept in the lairage facilities, for about 30-45 min, until slaughter. The animals were divided into two experimental groups, each consisting of 30 individuals, to verify the whole production chain and to ensure that the product conformed to Muslim rules. Thirty animals (group A) were selected by the responsible of the Muslim religious community and were slaughtered on the day after the animals slaughtered with the conventional procedure, at the exact same time of day.

In agreement with Regenstein *et al.* (2003) and Farouk *et al.* (2014), a Muslim or a practicing Jew (follower of Judaism) or Christian performed the Halal slaughtering. At the slaughterhouse, the authorized slaughter-men restrained the animals in a full inversion rotary pen. After this step, the animals were slaughtered by a perfectly clean incision, using a knife through the structures at the front of the neck: trachea, oesophagus, carotid arteries and jugular veins. In this study, the cattle were slaughtered using a conventional low neck cut (between the second and the fourth cervical vertebra), although the exact position on the neck is not specified in the Qur'an. Conversely, the other 30 animals (group B) were slaughtered after stunning by captive bolt gun, which causes immediate loss of consciousness, making the animals insensible to pain until death occurs due to exsanguination, as required by Council Regulation (EC) N° 1099/2009 on the protection of animals at the time of killing. These 30 calves were from the same farm as the animals slaughtered by the Halal rite and were selected at random by the operators. Plasma cortisol levels were evaluated at two different stages in the animal's productive life: on the farm, one week before slaughter (time 0) and during bleeding (time 1). Blood samples of time 0 were collected from the jugular vein, both at 6:00 a.m. to exclude a circadian variation. In time 1, the blood samples were collected during the exsanguination phase, which was carried out 30 min after the animal discharge. The blood samples were collected in vacutainer test tubes containing ethylenediaminetetraacetic-acid (EDTA) and stored in ice at 0°C for no longer than 60 min, avoiding freezing, before being transferred to the reference laboratory.

Elisa test

Plasma cortisol was determined as described in a previous study (Ceci *et al.*, 2017). Briefly, the cortisol ELISA immunoassay test (Bovine Cortisol ELISA

Kit; My-Bio-Source, San Diego, CA, USA) was used following the manufacturer's guidelines.

All reagents were kept at room temperature (25-28°C) for 30-40 min before being reconstituted. Enzyme conjugate was stored at -20 °C until use. Highly concentrated samples were diluted with sample diluent (e.g. 1:5 or 1:10) to obtain a readable range on the curve. In the first step, 50 µL of standard was added to each standard well, 50 µL of plasma to each sample well and 50 µL of sample diluent to each blank/control well. Standards, samples and diluent were added in duplicate to the plate. In the second step, 100 µL of HRP% (Horseradish Peroxidase) conjugate reagent was added to each well and incubated for 60 min at 37 °C. In the third step, the plate was rinsed 4 times with a wash solution (250-300 µL per well) and then residual liquids were carefully removed. In the fourth step, 100 µL of TMB (Tetra-Methyl-Benzidine) colour reagent was added to each well and the plate was incubated for 30 min at 18-25 °C without shaking. The reaction was stopped by adding 100 µL of H₂SO₄ 1M to each well and mixing gently for 1-2 min. Shortly after stopping the reaction, the optical density (OD) of each was determined using a micro plate reader with a wavelength of 450 nm, 540 nm or 570 nm. The mean of the readings of duplicates for each standard and sample was calculated, and the average OD of the blank was subtracted. A standard curve was created using a computer software capable of generating a four-parameter logistic (4-PL) curve-fit.

The minimum detectable dose of bovine cortisol (sensitivity) was ≥ 0.049 ng/mL. The detection range was 0.049-200 ng/mL. No significant cross reactivity or interference between bovine cortisol and analogues was observed. Intra-Assay Precision was <8%. Inter-Assay Precision was <10% (manufactured in an ISO 13485:2003 and EN ISO 13485:2012 Certified Laboratory).

Statistical analysis

A statistical descriptive analysis based on central tendency and concentration indexes was carried out for the two groups of animals to provide a framework of the changes in plasma cortisol concentration on the farm and during bleeding. Moreover, the increasing rates from pasture to exsanguination were calculated, starting from an observation of the different cortisol concentration tendencies between the two groups of animals. The mean values of the two groups of animals and of the stress parameter monitored in this study were compared by one-way analysis of variance.

Results

Mean values, standard deviation and significance of the differences in plasma cortisol are shown in Table 1. The results revealed that cortisol was lower in group A than in group B in time 0 (F: 4.026; P=0.049); on the other hand, cortisol levels were much higher in animals slaughtered by Muslim rite (group A) than in group B (F: 33.354; P=0.000) during bleeding. These data showed the greatest variation between farm (time 0) and exsanguination phase (time 1) in Halal-slaughtered animals, being 27.52 times higher, compared with those slaughtered by a traditional procedure, which had an average increase of 12.85 times (Table 2).

Discussion

In this study, we evaluated the stress to which the animal is submitted on the farm and during slaughtering by quantifying an objective indicator of stress, *i.e.* plasma cortisol, comparing traditional slaughter to the

procedures required by the religious Islamic rite. Given that Mormède *et al.* (2007), showed a range of baseline cortisol values between zero and around 20 ng/mL, with a mean lower than 5-10 ng/mL, the average plasma cortisol values observed in this study were lower than those reported in the literature but very similar to the values reported in our previous work (Bozzo *et al.*, 2018).

Plasma cortisol showed significant changes between the two times considered, *i.e.*, during pasture (time 0) and during bleeding (time 1). The differences in the values detected on the farm were predictable due to the choice of the most docile and gentle animals by the responsible of the Muslim religious community (Farouk *et al.*, 2014; Bozzo *et al.*, 2018). On the other hand, plasma cortisol levels immediately after exsanguination (time 1) were much higher in the Halal-slaughtered animals than in those slaughtered after traditional stunning.

Also, the study which we used as our starting point (Bozzo *et al.*, 2018) to compare Halal and Kosher rites, showed the greatest variation in plasma cortisol levels

Table 1. Mean values (M), Standard Deviation (SD) and Significance Level (P) between groups. The values are expressed in nmol/L.

Plasma cortisol	Cattle farm (time 0)	Exsanguination (time 1)
Religious Muslim rite		
M	3.26	88.81
SD	1.01	41.02
Traditional slaughter		
M	4.06	43.72
SD	1.94	12.09
Between groups, P	0.049	0.000

Statistical significance: P<0.05.

Table 2. Mean values (M), Standard Deviation (SD) and Standard Error (SE) of the increases between the two times. M from time 0 to time 1 expresses the difference between the parameter recorded in time 1 and 0, divided by the level found in time 0. The values are expressed in nmol/L.

Plasma cortisol	From time 0 to time 1
Religious Muslim rite	
M	27.52
SD	11.91
SE	2.175
Traditional slaughter	
M	12.85
SD	8.50
SE	1.552
Total sample	
M	20.18
SD	12.65
SE	1.633

Statistical significance: P<0.05.

during bleeding. The same trend was observed between the animals slaughtered by the Kosher method and those slaughtered after stunning by captive bolt gun (F: 16.021; P=0.000), although the Fisher F was almost half of the value recorded in this study (F: 33.354; P=0.000). Moreover, the means of the increases between the two times during religious slaughter were similar in both studies (25.51 nmol/L in the religious Jewish rite and 27.52 nmol/L in the religious Muslim rite) and overlapped in traditional procedures (12.53 nmol/L in the previous study and 12.85 nmol/L in the latest one). These data (Table 2) could be explained whereas the Jewish slaughtering involves much more restrictive procedures than those observed during the Islamic rite (Farouk *et al.*, 2014). As can be seen from Table 1, the average plasma cortisol values increase more than 10 times between the breeding and the exsanguination phases in animals slaughtered traditionally (from 4.06 to 43.72 nmol/L), while they increase almost 30 times in those slaughtered following the religious Halal rite (from 3.26 to 88.81 nmol/L). On the other hand, in the previous study on the Kosher rite (Bozzo *et al.*, 2018), the growth rate between the two phases was about 23 times (from 2.96 to 68.70 nmol/L). Furthermore, one of the main differences between the two methods of slaughter regards the knife used: Jewish precepts require the slaughtering knife (*chalef*) to be at least twice the neck width and perfectly sharp so as to ensure a single cut (Bozzo *et al.*, 2017). Consequently, as indicated by some authors (Farouk *et al.*, 2016), to achieve similar results these strict standards also need to be introduced into Halal procedures. For this reason, using the high neck cut (first cervical vertebra) instead of the conventional low neck cut (from second to fourth cervical vertebrae), ensured faster collapse of the animals and improved bleeding efficiency by reducing the frequency of false aneurysm formation and the early arrest of blood flow (Gibson *et al.*, 2015).

Regarding the slaughtering procedures, in a previous study conducted by Dunn (1990), the cortisol levels of the cattle subjected to religious slaughtering in the rotary Weinberg pen were significantly higher than those of cattle slaughtered in the pen approved by the American Society for the Prevention of Cruelty to Animals (ASPCA), where the animals were upright (Grandin, 2010). On the other hand, rotating restraint boxes are preferred by some slaughtermen to give easier access to the animal's neck (Farouk *et al.*, 2016). Because of the potential pain that could occur during the restriction process prior to slaughter, upright

slaughter of the animals should preferably replace the rotary pen as a means of restraint (Farouk *et al.*, 2016). Furthermore, during slaughter without prior stunning, complete immobilization of the head is absolutely necessary, even though the head holder causes more stress than body restraint (Grandin, 1992). Holding an animal too tightly causes it stress and can cause bruising in the meat (World Organization of Animal Health, 2013). Moreover, excessive stress to the body seemed to increase blood splash and reduce bleeding (Grandin, 1992). Many researchers (Gregory, 2008; Nakyinsige *et al.*, 2014) reported a correlation between meat quality and blood, showing the need to remove more blood as fast as possible in order to extend the shelf life of the meat and to provide safer meat for human consumption. The study confirms what was already showed by Önenç and Kaya (2004), who claimed that slaughtering method affects plasma cortisol levels, which play a central role in meat acidification. Moreover, during stressful situations, the secretion of glucocorticoids stimulates hepatic glycogenolysis, leading to an increase in glucose levels (Knowles and Warriss, 2000; Pollard *et al.*, 2002).

Conclusions

A survey of a small number of Halal consumers in the USA indicated an increased desire for better animal welfare in the production systems (Pufpaff, 2014). The Islamic ethical system takes the relationship between humans and other animal species quite seriously and argues that animals have feelings and interests of their own, of their own. Indeed, they have the right to be treated with kindness, respect and consideration (Farouk *et al.*, 2014). Therefore, to achieve this goal, stunning should be introduced during religious slaughtering. In agreement with Islamic principles, the stunning method that may be acceptable and considered humane must not kill the animal, but only temporarily render it unconscious (reversible) in order to minimise the pain and distress caused by the act of slaughter. Although not all the Islamic community allows the introduction of stunning during slaughter, there are procedures that could be accepted, according to the precepts of the Qur'an. To reduce the stress of the animal not subjected to stunning, the following arrangements could be introduced: (i) using an ASPCA pen for animal restraint at the time of slaughtering, which keeps the animals in a natural position to prevent their excessive movement inside the restraint pen and to avoid repercussions related to

reduced bleeding, (ii) employing a proper knife, with similar characteristics to that used during Kosher slaughter since animal welfare is affected by the size of the knife. Finally, (iii) cutting the neck at the level of the first cervical vertebra, so that death occurs earlier. All these procedures could improve animal welfare conditions at the slaughterhouse, increase meat quality and, importantly, are respectful of the Islamic religion. These measures may also be interpreted as a first step towards a point of balance between devotion to one's religion and respect for animal welfare.

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