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Evaluation of the Lambs' State of Consciousness Signs during Halal and Traditional Slaughtering

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Abstract: The aim of this study was to evaluate the persistence of two signs of consciousness (rhythmic breathing and corneal reflex) in lambs slaughtered according to the traditional method and Halal ritual rite. A total of 240 lambs were examined and divided into two equal groups ($n = 120$ each). Lambs of group A were subjected to the stunning phase by the action of an electric current on the brain, while lambs of group B were slaughtered according to the religious Halal method without prior stunning. Rhythmic breathing (RB) and corneal reflex (CR) were used as indicators of prolonged brain function, and their evaluation was carried out by the operators in three subsequent steps at 15 s, 30 s, and 90 s post-bleeding, respectively. The stunning of the lambs reduced the animal's state of consciousness and, consequently, reduced suffering, pain, and distress. Indeed, the lambs of group B showed longer duration consciousness than the animals stunned by electrodes. The permanence of the reflexes in Halal slaughter could be reduced by introducing a reversible stunning method to make the animal temporarily unconscious. Moreover, given that our results revealed consciousness also after 90 s post-cut, the assessment of the animal's state of consciousness in wider time intervals than those commonly used is recommended.

Keywords: lambs; consciousness; corneal reflex; rhythmic breathing; Halal slaughtering

1. Introduction

Council Regulation (EC) 1099/2009 [1] on the protection of animals at the time of killing establishes that animals shall only be killed after stunning, and the loss of consciousness and sensibility shall be maintained until their death. As reported in the previous Council Directive 93/119/EC [2], also in this Regulation, the derogation from stunning is confirmed in case of religious slaughter taking place in slaughterhouses. However, since a certain level of discretion is allowed to each member state, Sweden, Denmark, Norway, and Switzerland have already banned the slaughter of animals without pre-stunning [3].

Electric head-only stunning (EHOS) of sheep is considered compatible with Halal criteria by many Muslim representatives [4–7] because it induces a state of reversible unconsciousness and does not lead to the death of the animal [8]. Indeed, Halal slaughter requires that the animal must be alive at the point of slaughter, and the slaughter procedure must ensure the maximum blood loss, given that the Qur'an prohibits blood consumption [9,10]. The EHOS does not kill the animal pre-slaughter nor affect blood flow during bleeding, resulting in an acceptable stunning method in several slaughterhouses practicing

Halal procedure [11]. Moreover, Khalid et al. [11] reported no significant difference in final blood loss between three slaughter methods of lambs: traditional religious slaughter without stunning (TRS), electric head-only stunning (EHOS), and post-cut electric head-only stunning (PCEHOS). However, there are still many Muslims opposed to stunning in general and to electrical stunning in particular [12].

The EHOS is commonly used in the conventional slaughter of sheep, and its principle is to pass an electric current of sufficient intensity through the brain, provoking a massive depolarization of neurons in both cerebral hemispheres [8]. The technique involves placing electrodes on either side of the brain in order to disrupt the physiological rhythmic electrical activity [13]. After stunning, the animals are bled as soon as possible at the level of the neck [8].

The European Commission launched in 2006 a project called DIALREL, completed in 2010, to promote good religious slaughter practices and dialogue among key players in the industry for the protection of animal welfare [14]. To guarantee animal welfare, persons responsible for stunning have to carry out regular checks to ensure that no sign of consciousness or sensibility is presented in the period between the end of the stunning process and death [1]. These checks must be carried out, also during religious rites, on a sufficiently representative sample of animals and with a frequency established on the basis of the results of previous checks. Moreover, the slaughter of animals without pre-stunning requires highly skilled staff to guarantee both proper handling and bleeding [15]. Two of the indicators of prolonged consciousness in sheep are the resumption of the rhythmic breathing and the presence of the corneal reflex [16]. According to the Italian standard operating procedures for monitoring animal welfare at the slaughterhouse [17], the absence of the corneal reflex in sheep and goats should be evaluated between 20 s and 30 s after cutting the neck. On the other hand, according to the DIALREL project [18], sheep must be assessed to be unconscious by the slaughter persons (or the shochet) at least twice, between 15-s and 25-s post-cut. In case of prolonged consciousness of the animal (an indicator of poor procedures), corrective action must be taken without any delay.

Therefore, the aim of this study was to evaluate the persistence of two signs of consciousness (rhythmic breathing and corneal reflex) in lambs slaughtered according to the traditional method and Halal ritual rite. Taking into account the time intervals considered in the Italian standard operating procedures and in the DIALREL, we monitored these signs in three different moments at 15 s, 30 s, and 90 s post-cut in order to include the 15 s reported in the DIALREL and the 30 s reported in the Italian standard operating procedures.

2. Materials and Methods

2.1. Sampling

The study, conducted in July 2020, was the result of a collaboration between the Department of Veterinary Medicine at Bari University (Italy) and a slaughterhouse practicing Halal slaughtering in the Apulia region (Southern Italy). It was carried out on 240 male lambs, belonging to the Gentile di Puglia breed, aged between six and twelve months and with an average carcass weight between 14 and 22 kg. This research did not require Ethics Board approval because data were obtained from routine procedures of the slaughterhouse.

The lambs were reared semi-intensively within the same farm associated with the slaughterhouse and fed with concentrate mixture and ad libitum cereal straw. Animals were equally managed, applying the same production disciplinary. In particular, lambs were fed the same diet formulated to meet or exceed the nutrient requirements for fattening lambs according to the National Research Council [19] recommendations. Lambs fasted about 12 h before transport.

Animals were divided into two groups ($n = 120$ each) according to the type of slaughter performed (traditional slaughter and Halal religious rite without stunning). In the first group (group A), lambs were subjected to the stunning phase by the action of an electric current on the brain (Gozlin Electronic stunner TEQ002, serial number: TEQHZZV19, year of manufacture: 2019), applying the electrodes at the level of the animals' head, delivering a voltage of $230 \text{ V} \pm 10\%$ for 5 s with a constant current of 1.1 A,

given that 1.0 A is the minimum requirement for sheep under Council Regulation (EC) 1099/2009 [1] on the protection of animals at the time of the killing. In the second group (group B), lambs were slaughtered according to the religious Halal method without prior stunning. The two groups were transported from the same farm on two consecutive days. In particular, group B was slaughtered the day after the animals were slaughtered with the conventional procedure. Both groups were transported for about one hour and, once unloaded from the truck, all the animals were kept in the lairage facilities until slaughter, with free access to water.

Lambs were directed by the slaughterhouse staff to the “pre-selection corridor” that leads to the site of jugulation (for ritual slaughters) or to the site where the stun electric gripper was present (for traditional slaughter). Subsequently, the lambs were moved from the pre-selection corridor to the “containment corridor”, which can accommodate a maximum of three lambs at a time. This corridor was composed of a movable belt that allows both to immobilize the animal, blocking its limbs, and to move it forward through a pedal knob operated by the slaughterhouse staff (Figure 1). The lambs were restrained only at the level of the limbs in both types of slaughtering, without a specific head restraint before and after neck cutting.

At this point, the procedure was different according to the type of slaughtering. In traditional slaughtering (group A), the first lamb entering the containment corridor was stunned using head-only electrical stunning (electrodes applied on both sides of the head between the eye and the ear) and subsequently jugulated by severing the structures at the front of the neck in a single cut. The heads of the lambs were wetted before stunning them to increase stun effectiveness. On the other hand, during ritual slaughtering (group B), the first lamb entering the containment corridor was jugulated, followed by the second and the third lamb based on the belt progression. Particularly, both during the Halal practice and the traditional method, trachea, esophagus, carotid arteries, and jugular veins were severed.

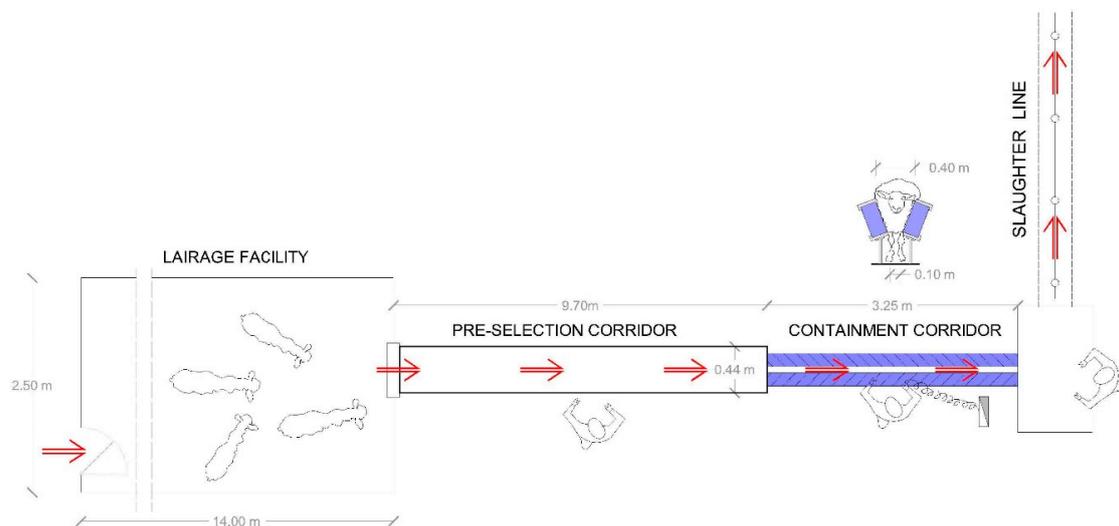


Figure 1. Plan of the slaughterhouse for the lambs slaughtering. Pre-selection corridor: leads to the site of jugulation (for ritual slaughter) or to the site where the stun electric gripper is present (for traditional slaughter). Containment corridor: can accommodate a maximum of three lambs at the time, and it is composed of a movable belt that allows both to immobilize the animal, blocking its limbs, and to move it forward through a pedal knob operated by the slaughterhouse staff.

Rhythmic breathing (RB) and corneal reflex (CR) were used as indicators of prolonged brain function, and their evaluation was carried out by the operators in three subsequent steps at 15 s, 30 s, and 90 s post-bleeding. The choice to wait for 15 s, 30 s, and 90 s was established in the health control plan of the slaughterhouse. Indeed, the DIALREL project [18] suggests checking the signs of

consciousness for sheep at least twice between 15 s and 25 s post-cut. On the other hand, according to the Italian standard operating procedures for monitoring animal welfare at the slaughterhouse, the absence of the corneal reflex in sheep and goats slaughtered without stunning should be evaluated between 20 s and 30 s after cutting the neck. The same Italian operating manual reports that, in the electrically stunned animal, it is necessary to control eye movement after 60 s. In this case, the choice to wait 90 s was established in order to avoid disturbing the flow chart of the slaughterhouse as the time between jugulation and the last evaluation of the animal's signs of consciousness was exactly 90 s. The evaluation of these signs of consciousness 15 and 30 s post-bleeding was performed when the animals were still in their natural position on the movable belt. Conversely, the assessment of the presence or absence of signs of consciousness at 90 s post-bleeding was carried out by the operator after moving the lambs from the containment corridor to a table (horizontal position).

The assessment of RB and CR was carried out, as described in a previous study [20]. Briefly, RB was tested by observing the presence or absence of inhalation and exhalation movements by the animal. The CR was tested by lightly touching the cornea and, if present, the eyeball retracted slightly, and the eyelids closed. Both RB and CR were assessed by the operator and the religious responsible for the Halal Committee, during the two types of slaughters, without any instrumental evaluation. If these signs of consciousness were still present 90 s post cutting the neck, the operators had to proceed with an irreversible stunning by a captive bolt gun. After this step, the lambs were attached to a hook vertically to proceed with the various slaughtering phases, which ended by placing the carcasses in the cold room.

2.2. Data Analysis

The signs of consciousness were presented using descriptive statistics of data. The observations were elaborated to evaluate which of the two groups of animals showed higher persistence of the signs of consciousness. All data analyses were performed using Cohort Stat (version 6.311., 2006, Cohort Software Inc., Monterey, CA, USA), and *p*-values were considered significant at *p* < 0.05. The statistical significance was determined by the χ^2 test, and when significant differences were found for a parameter, the one-way ANOVA procedure was performed at the different time points of the study.

3. Results

In group A, 15 s after the neck cut, four lambs showed CR, 48 lambs showed RB, and the other four lambs showed both reflexes, with the final involvement of 56 lambs out of 120 (46.67%). In the same group, 30 s after the neck cut, four lambs showed CR (the same four as before), and 22 lambs showed RB; no lambs showed both reflexes at the same time. Therefore, the lambs involved were 26 out of 120 (21.67%). After 90 s post-cut, only four lambs presented RB, and, consequently, they were again stunned by captive bolt gun (Table 1).

Table 1. Presence of corneal reflex (CR) and rhythmic breathing (RB) or both (CR + RB) in lambs at 15, 30, and 90 s post neck cutting after traditional slaughtering (*n* = 120) and Halal religious rite (*n* = 120).

Type of Slaughtering	15 s Post-Cut				30 s Post-Cut				90 s Post-Cut			
	CR	RB	CR + RB	Tot	CR	RB	CR + RB	Tot	CR	RB	CR + RB	Tot
Traditional (group A)	4	48	4	56	4	22	0	26	0	4	0	4
Halal (group B)	26	26	58	110	28	22	16	66	4	12	0	16

On the other hand, in group B, 15 s after the neck cut, 26 lambs showed CR, 26 lambs showed RB, and 58 lambs showed both reflexes, with the final involvement of 110 lambs out of 120 (91.67%). In the same group, 30 s after the neck cut, 28 lambs showed CR, 22 lambs showed RB, and 16 lambs showed both reflexes. Hence, in this step, the total number of lambs involved was 66 out of 120 (55%). After 90 s post-cut, four lambs showed CR, 12 lambs showed RB, while no lambs showed both reflexes

at the same time. Therefore, the total number of the animals still presenting reflexes 90 s post-cut was 16, and they were subjected to the stunning by a captive bolt gun. As a result, the percentage of rejected animals out of the initial number of 120 was 13.33% (16 lambs out of 120; Table 1).

In the case of CR, the difference between traditional slaughtering and religious Halal rite was significant during all three different time points of the study ($p = 0.007$, $p = 0.009$, and $p = 0.032$, respectively, during the first, second, and third steps). On the other hand, in the case of RB, the difference between the two types of slaughtering was significant ($p = 0.008$) at 15 s and 90 s post neck cutting, but it was not significant at 30 s post-cut. Regarding the simultaneous presence of both reflexes, there was a statistical difference between the two slaughtering procedures at 15 s ($p = 0.007$) and 30 s ($p = 0.025$) post neck cutting, but there was no statistical difference at 90 s post-cut. Finally, regarding the total reflexes, the difference between traditional slaughtering and religious Halal rite was significant during all three steps ($p = 0.007$, $p = 0.006$, and $p = 0.009$, respectively, in the first, second, and third steps; Table 2).

Table 2. Significance level between the two groups of animals slaughtered following traditional slaughtering ($n = 120$) or Halal religious rite ($n = 120$).

Item	15 s Post-Cut	30 s Post-Cut	90 s Post-Cut
Corneal reflex (CR)	0.007	0.009	0.032
Rhythmic breathing (RB)	0.008	NS	0.008
CR + RB	0.007	0.025	NS
Total (CR + RB + (CR + RB))	0.007	0.006	0.009

NS: not significant.

4. Discussion

Our study reports the assessment of the signs of consciousness in lambs associated with the slaughtering methods and evaluates the timeline included in the DIALREL project [18], thus increasing our knowledge about lamb consciousness during slaughter and, consequently, about animal pain.

In this research, we evaluated the persistence of the signs of consciousness in 240 lambs according to the slaughtering method. The findings showed that the presence or the absence of the stunning phase is of paramount importance in prolonging the animals' consciousness. Indeed, during Halal slaughtering, there were 110, 66, and 16 lambs presenting reflexes at 15 s, 30 s, and 90 s post-cut, respectively. On the other hand, after electrical stunning and during the same time intervals, the lambs presenting both reflexes or only one of them were 56, 26, and 4, respectively. In particular, in group A, among the 56 lambs presenting reflexes at 15 s post-cut, four showed only CR, 48 showed only RB, and four animals showed both reflexes. In the same way, among the 26 lambs presenting reflexes at 30 s post-cut, four showed only CR, and 22 showed only RB; no animals showed both reflexes. Finally, 90 s after neck cut, only four lambs presented RB (Table 1). Conversely, in group B, among the 110 lambs presenting reflexes at 15 s post-cut, 26 showed only CR, 26 showed only RB, and 58 animals showed both reflexes. In the same way, among the 66 lambs presenting reflexes at 30 s post-cut, 28 showed only CR, 22 showed only RB, and 16 animals showed both reflexes. Finally, 90 s after the neck cut, there were no animals presenting both reflexes at the same time, but four lambs presented only CR, and 12 lambs showed only RB (Table 1).

It is a very interesting highlight that, in group A, there was a great difference between the occurrence of the two types of reflexes. Indeed, the four lambs presenting CR 15 s post-cut showed the same reflex, also, 30 s post-cut and no reflex after 90 s. On the other hand, the number of lambs presenting RB decreased among the three steps (48, 22, and 4 animals 15, 30, and 90 s post-cut, respectively). It is clear that the animals slaughtered by traditional stunning showed many more times RB than CR in the three steps and, mainly, 15 and 30 s after neck cut. This situation can be explained considering a different involvement of the central nervous system (CNS) and of the peripheral nerve in the occurrence of the two reflexes. Indeed, the corneal reflex is caused by a loop between the

trigeminal nerve (V) and the facial motor nerve (VII) [21]. A sensory stimulus that comes into contact with free nerve endings or with mechanoreceptors located within the epithelium of the cornea causes the reflex activation. Through the ophthalmic division of the trigeminal nerve, sensory information is transmitted to the synapse within the spinal trigeminal nucleus in the brainstem [21]. Damage to the central or peripheral nervous system can cause dysfunction of the corneal reflex [22].

On the other hand, at the origin of the rhythmic breathing, there is a group of neurons, known as the central pattern generator (CPG), interconnected in the medulla of the brainstem [23]. Each group of these neurons (about six) is activated at different times and controlled by a series of positive and negative feedback systems on each other. The breathing rhythm may not be affected, even if several cells in each group are damaged or inhibited [23]. Moreover, the ventral medulla contains receptors that perceive the concentration of carbon dioxide, bicarbonate, and acid-base balance [24]. In fact, the medulla is able to detect a fall in pH caused by carbon dioxide in the blood, which diffuses freely across the blood–brain barrier and dissolves to form a weak acid called carbonic acid [23]. For this reason, the control of breathing involves the interaction of both chemical and neural receptors of the peripheral and central nervous system as well as end organs. The neural receptors are found in the upper airway, in the major respiratory muscles (diaphragm, internal/external intercostals, and abdominal musculature), lungs, and pulmonary vessels [25]. The peripheral chemoreceptors include the carotid and aortic bodies, which are the primary sites responding to changes in PaO₂, PaCO₂, and pH [26]. Increases in CO₂ or decreases in pH have been associated with increases in ventilator response by multiple mechanisms [27–29].

Therefore, our results could be explained considering that after electrical stunning (group A), the damage at the central and peripheral nervous system leads to a negative response to CR (only eight lambs out of 120 and four lambs out of 120 presented CR 15 s and 30 s after neck cutting, respectively). On the other hand, in group B, 84 lambs out of 120 and 44 lambs out of 120 showed CR at 15 s and 30 s post-cut, respectively. This means that, without stunning, the consciousness of the animal (tested by lightly touching the cornea) remains for longer than the animals stunned by electrodes, probably because the neuronal pathway is not damaged or is damaged later. Indeed, in the case of CR, the difference between traditional slaughtering and religious Halal rite was significant during all three different time points of the study (Table 2).

Regarding RB, this sign of consciousness is much more present than CR in both the slaughtering methods (52, 22, and 4 lambs of group A in the three steps, respectively, and 84, 38, and 12 lambs of the group B in the three steps, respectively). Indeed, in the case of RB, the difference between the two types of slaughtering was not significant at 30 s post-cut. This could be explained considering that, with electrical stunning, there is damage at the level of the central and peripheral nervous system, resulting in a lower number of positive responses to RB in the animals of group A. On the other hand, after neck cutting and regardless of the use of stunning, there are many other mechanisms linked to chemical and mechanical variations, which lead to the activation of the major respiratory muscles (diaphragm, internal/external intercostals, and abdominal muscles) to cope with pressure variations [30].

Indeed, the catastrophic fall in blood pressure, leading to a fall in cardiac output, is caused by the severance of the main blood vessels in the neck. Kirton et al. [31] reported that electrical stunning increased blood pressure by an average of 3.5 times within about 11 s from the stun. In fact, one of the actions of stunning by electrodes is cardiac inhibition via parasympathetic stimulation mediated by the vagus nerve. After the electrical stunning, the lowering of blood pressure is recorded by the baroreceptors of the main blood vessels, causing the heart rate to increase in order to raise the blood pressure [32]. Consequently, it seems reasonable that lambs electrically stunned should lose blood more rapidly in the initial phases compared to non-stunned animals. For this reason, stunning accelerates the loss of consciousness, with benefits for animal welfare [33]. Indeed, a previous study [34] showed that stress levels immediately after exsanguination were much higher in the Halal-slaughtered animals than in those slaughtered after traditional stunning. However, within 2 min from neck cutting, the lambs'

bleeding may be considered complete [35,36], while the application or not of stunning does not affect the total blood loss of the animals [11].

Regarding the timing to evaluate the permanence or return of CR and RB in lambs, there are conflicting data in the literature. Newhook and Blackmore [37] stated that, after an effective neck cut, sheep generally lose consciousness (EEG assessment) within 2–7 s, and cortical brain death occurs by approximately 14 s [38]. The DIALREL project [18] reported that most sheep and goats lose consciousness within 2 s to 20 s after ventral neck cutting, but sheep can show signs of recovery for longer times in exceptional cases. According to Velarde et al. [16], spontaneous breathing returned at about 29 s post-stun, whereas the corneal reflex returned at about 38 s. On the other hand, Rodríguez et al. [39] showed that rhythmic breathing disappeared at an average time of 44 (± 4.2) s after neck cutting of lambs without stunning, ranging between 30 s and 60 s. Conversely, the corneal reflex disappeared at 116 (± 11.01) s, ranging between 80 and 160 s after neck cutting.

Our results revealed the presence of animal consciousness also after 90 s post-cut, and this prolonged consciousness, in line with the findings reported by Rodríguez et al. [39], may be attributable to inefficient bleeding when lambs are slaughtered without head restraint. Indeed, in the slaughterhouse of our study, the lambs were restrained only at the level of the limbs in both types of slaughtering, without a specific head restraint. Animals not mechanically restrained after the neck cut present a slower bleeding process and, accordingly, prolonged unnecessary suffering [15]. Grandin [40] also reported that during slaughter without pre-stunning, complete immobilization of the head is necessary to minimize pain and distress. Consequently, animals slaughtered without stunning should be individually and mechanically restrained [15], and a sharp knife should be used to ensure an accurate cut of the throat [1].

In our study, the onset of reflexes was not influenced by the operators in either type of slaughtering. Indeed, in the case of religious slaughtering, the neck cuts were not affected by the manual skills of different persons because they were always performed by the same operator. On the other hand, during traditional slaughtering, the electrical stunning was always successful, without differences among the animals. Indeed, in case of mis-stunning, the operator is alerted by a flashing red light and an alarm, while, during these slaughters, there was always a green light to indicate the correct procedure. Moreover, between one lamb and the next, the cleanliness of the stun electric gripper was also evaluated to ensure that no wool residues slowed the current conduction. Furthermore, as established in the health control plan of the slaughterhouse, after neck cutting and in both types of slaughtering, the operators kept the animal's head raised for about 10 s to check the formation of false aneurysms due to an improper neck cut. In this study, the cut of the blood vessels was always correct because no false aneurysms were detected.

Lambs presenting CR and/or RB are not considered suitable by the Halal certification bodies of the slaughterhouse because the killing of an animal through stunning is not accepted as Halal in this slaughterhouse. The DIALREL project [18] reports that in the event of inefficient bleeding or prolonged consciousness during repeated checks after neck cutting, animals should be stunned with an appropriate method as soon as possible, even if the religious authorities declare the animal unsuitable for consumption. According to the DIALREL, this corrective action should be done within 45 s post-cut for cattle and within 30 s post-cut for small ruminants and poultry. Therefore, considering the results of our study 30 s post-cut, there were 26 lambs presenting one of the two reflexes in group A and 66 lambs in group B. This could lead to a much higher percentage of rejected carcasses in group B (55%), owing to the corrective action (captive bolt stun) not accepted by the Halal certification bodies of the slaughterhouse. On the other hand, waiting for 90 s post-cut, the percentage of rejected carcasses would be much lower (13.33%) because the involved animals of group B were only 16. Consequently, it would be advisable to wait a longer period of time for the assessment of the animal's state of consciousness before intervening with stunning and excluding the carcass from the Halal market.

5. Conclusions

The findings of the present study show that the pre-stunning of the lambs reduced the animal's state of consciousness and, consequently, reduced suffering, pain, and distress. Indeed, the permanence of the reflexes in Halal slaughter could be reduced by introducing a reversible stunning method to make the animal temporarily unconscious. However, these preliminary results need to be confirmed by future research, correlating the permanence of the signs of consciousness with a higher level of animal stress by testing endocrine parameters, heart rate, or hematological and biochemical analytes. Our results may be useful to propose the development of slaughter methods not in conflict with Islamic principles and to recommend the assessment of the animal's state of consciousness in wider time intervals.

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