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Original article

Religious slaughtering: Implications on pH and temperature of bovine carcasses

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ABSTRACT

The main objective of this study was to investigate the influence of different types of slaughtering on the variations of pH and temperature. Ninety entire male beef cattle, belonging to the Charolais breed, were distributed in three groups based on method of slaughtering. A total of 30 subjects (group K) were slaughtered without stunning, according to the Koscher rite; thirty bovines (group T) were stunned with a captive bolt gun, following the common slaughtering reported by Council Regulation (EC) 1099/2009; other thirty animals (group H) were slaughtered without stunning, based on Halal procedures. The temperature and pH values of carcasses were determined at 3, 6 and 24 h post-mortem, respectively. At 24 h post-mortem of the 30 carcasses evaluated for each group, muscle temperature decreased in all three types of slaughtering. In particular, the average temperature was 4.0, 4.9 and 3.0 °C for traditional, Halal and Koscher slaughtering, respectively. Moreover, at the same time step (24 h post-mortem), muscle pH decreased showing values equal to 5.72, 5.83, 5.81 for traditional, Halal and Koscher slaughtering, respectively. The findings of the present study may be useful to highlight the influence of slaughtering method on pH and, more generally, on meat quality. Additionally, our results show that, in slaughtering carried out without prior stunning, the carcass could be more susceptible to alterations because of the high pH values.

1. Introduction

Slaughter is undoubtedly the most significant process in transformation of animals into meat suitable for human consumption, and this key-phase is regulated by strict regulations on animal welfare, food safety and hygiene, as well as working environment (Bergeaud-Blackler, 2007). The slaughter process is quite complex because of it is characterized by several potential stressor events caused by many factors. Consequently, the aim of stunning is to make animals unconscious during neck cutting and bleeding, without causing pain or distress. In many countries, however, it is a common practice to kill cattle by a religious method without pre-stunning (Önenç and Kaya, 2004). Based on available data, it was demonstrated that almost 26 million of Muslims and 1.1 million of Jews live in the European Union. Thus, the animals slaughtered following religious rituals is elevated in EU (Europe's Growing Muslim Population, 2017).

During cattle slaughtering phases, the stress may be physical, psychological or both. High-quality meat as well as animal welfare in slaughtered animal and economic success are attained by preventing stressing factors (Immonen et al., 2000). Indeed, the correlation between the ante-mortem physiological status and post-mortem muscle metabolism is well established and the influence of slaughter environments on metabolism the muscle and characteristic of meat could be partly explained by stress (Lawrie, 1966).

The physiological stress responses involve the secretion of "stress hormones" such as cortisol and catecholamines (i.e. adrena-

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line and noradrenaline). Adrenaline is known to cover a key-role in determining the quality of meat. In livestock species, certain pres-laughter conditions boost the probability to obtain meat with altered ultimate pH (Terlouw et al., 2021). Available findings reported that the stress during pre-slaughter has effect by reducing pH as well as on meat quality. Moreover, it was demonstrated a negative correlations between the stress level during preslaughter and beef meat physical parameters (Reiche et al., 2019).

Stress throughout the slaughter phase reduces muscle glycogen level and led to produce dark, firm and dry (DFD) meat having also high ultimate pH (Tarrant et al., 1992). In addition, animals under the hours preceding slaughter increased the emotional stress with the result of reduced muscle glycogen leading also to higher ultimate pH (Tarrant et al., 1992). Indeed, muscle glycogen concentration at the time of slaughter is one of the most important factors affecting beef quality. Insufficient glycogen reserves at the time of slaughter result in pH-values above 5.5 and in extreme cases to serious quality problems (Immonen et al., 2000). After the slaughtering phase, biochemical processes proceed, but the blood circulation absence determines the stop of oxygen and glucose delivering to muscles. Therefore, locally deposed glycogen in muscles is utilized as source of energy and anaerobically catabolized. So, products of these processes, in particular H in muscle led to a decline of pH as reported by Robergs et al. (2004).

At slaughter, muscle pH in normal range is ~7.0, but it tends to decrease in meats, and it is also important for its storage life (Odore et al., 2004). Muscle pH has been also reported to play a critical role even in the degradation of muscle proteins during meat storage (Hanzelková et al., 2011). Ultimate pH (pHu, reached 24 h after slaughter) normally drops to about 5.8 and 5.4 and the standard flavor and taste in meat are obtained after an appropriate lowering of the pH within this range. In fact, meat quality is a resultant of the course of temperature and pH in the post-mortem period (Hamoen et al., 2013). Differences post-mortem pH of muscle and the reduction of temperature may explain the changes in meat products in terms of technological and sensory quality (Hwang and Thompson, 2001). Important physical qualitative traits affected by temperature and pH are color, tenderness, and water holding capacity (Guignot et al., 1994). In particular, meat color is the parameter with the greatest discriminating power at the time of purchase, as it is closely associated with the freshness of meat and also with its tenderness. The meat color is essentially given by the final pH value 24 h after slaughter and by the level of myoglobin present in muscle (Dell'Orto et al., 2010). Meat with high pHu results dark, more susceptible to bacterial spoilage and has reduced flavour and shelf life. Moreover, the lower pH, less favorable conditions for growth of dangerous bacteria (Silva et al., 1999). Thus, pHu has been widely used as an indicator of potential meat quality (Jeleníková et al., 2008). Generally, beef meat with pHu above 6.0 denotes a problem in quality, being also negative for human intake, and determines reduced economic returns (Viljoen et al., 2002).

Therefore, the aim of this study was to compare the pH and temperature values in three groups of bovines slaughtered according to three different procedures: conventional slaughtering with stunning, Halal ritual rite and Kosher ritual rite, respectively. In particular, the religious procedures were carried out without stunning of the animals. The parameters of pH and temperature were monitored at 3, 6 and 24 h post-mortem. We aimed to evaluate the influence of time and slaughtering type, as well as their interactions, on beef meat pH and temperature and, consequently, on meat quality. This is a preliminary study that requires further investigation, such as the evaluation of other meat quality parameters as well as of some indicators of animal stress to better evaluate possible correlation between qualitative meat traits and slaughtering procedure.

2. Materials and methods

2.1. Sample collection

The present trial did not require the approval of Ethics Board because of data were attained from regular practices, under supervision of the authors and the official veterinarians. The research was conducted in a slaughterhouse of Apulia region in Southern Italy, on 90 entire male beef cattle (Charolais breed) with an average age of 18 months. The animals selected for the Halal ritual rite had a mean age of 18.2 ± 1.66 months; the animals chosen for the Kosher ritual rite had a mean age of 20.8 ± 1.42 months; finally, the animals selected for the traditional rite had a mean age of 15.6 ± 1.10 months.

The animals were fattened within the same commercial beef farm linked with slaughterhouse. The subjects were equally reared under the same disciplinary of production. A total of 30 individuals (group K) were selected directly on the farm by Rabbis responsible for Shechitàh Committee. The second group of (n = 30; group H) was chosen directly on farm by responsible for Committee Halal. The other animals (n = 30; group T) were selected by the responsible of slaughterhouse in the lairage facilities of the abattoir.

The animals involved in the study were transported and slaughtered in three different days (transportation time of about two hours) and once delivered from truck, they were kept in lairage facility for one hour, having free water access. Although each type of slaughter was conducted with a difference of one week in the three groups, the transport conditions were always the same. The study, in fact, was conducted in November with an almost equal temperature on the various days, the same truck's itinerary and the same driver. Each group was fully slaughtered in a single day by a single operator, different for each group. In particular, the group K was slaughtered by an official person of Jewish faith (shochet) without stunning, according to the Kosher rite; the animals of the group H were processed by a slaughter-man of Islamic faith without stunning, based on Halal procedures; the group T was stunned by a trained operator with a captive bolt gun, according to the traditional slaughtering reported by Council Regulation (EC) 1099/2009 (Council of EU. Directive 2009/1099/EC) on protection of animals at time of killing. Shooting position on the head was at the intersection of imaginary lines drawn between the base of horns and opposite eye. Animals were restrained under full-inversion rotary pen for presenting them in dorsal recumbency for cut. Head was entirely restrained to prevent subjects' movements. Following neck cutting (trachea, esophagus, carotid arteries, and jugular veins), animals were shackled and exsanguinated while hanging. Each carcass was eviscerated and divided before chilling (2 ± 2 °C for 24 h) within 45 min post-mortem.

2.2. Classification of beef carcasses

Mean warm carcass weight for K, H and T groups were 404.3 ± 47.36, 442.3 ± 31.07, and 423.7 ± 25.00 kg, respectively. Each carcass, in the three groups, was similarly chilled. Subjects were similar in conformation and has same fat score. Indeed, no cattle having conformation "S", "E" or "P" and with fat cover "1", "4" or "5" were processed. Classification of conformation and fatness degree were visual assessed by trained and experienced evaluators using the SEUROP system reported in the Commission Delegated Reg. (EU) 2017/1182 (Council of the EU Dir. 2017/1182/EC, 2009). Carcass conformation (S = Superior; E = Excellent; U = Very good; R = Good; O = Fair; P = Poor) was based on the development of carcass profiles, and in particular of the essential parts. The degree of fat cover (from 1 to 5, as low to very high) was based on amount of fat on the outside of carcass and in thorax.

2.3. Determination of temperature and pH

At 3, 6 and 24 h post-mortem, pH and temperature were assessed on the left-half carcass in the *Longissimus dorsi* muscle. To avoid a degree of heterogeneity within the muscle, three measurements were taken for each time point at 1 cm distance and then the means were calculated. Muscle temperature was recorded using a temperature measuring instrument (testo 112, 1-channel) with a robust stainless steel Pt100 food probe IP65, calibratable; fixed cable: 1.2 m; measuring range: −50 to 300 °C; accuracy: class A (Testo SE & Co. KGaA, 79822 Titisee-Neustadt, Deutschland). Muscle pH was recorded by a portable-pH and thermometer with a specific probe (model HI98163, Hanna Instruments Inc., Woonsocket, RI 02895, USA). This meter was supplied with a pH electrode (FC2323) specifically designed for use with meat, introduced in a small cut (about 4 cm in depth) in LD muscle of carcass (at ribs no. 11–12 interface). pH meter was recalibrated every 5 samples. A carcass with pHu (24 h post-mortem) values ≥5.8 were classified as a potential risk of DFD, while meat was judged of normal quality at pH below 5.8.

2.4. Statistical analysis

The values of pH and temperature of the three groups of animals were shown using descriptive statistics. Effect of time and slaughtering type, as well as their interaction on carcass temperature and pH were evaluated. Statistical analysis of data was done (Cohort Stat 2006, version 6.311, Canada). Data were analyzed by repeated measures procedure of ANOVA; when differences were detected, one-way method was done at each time point. Initial tentative statistical analysis had included the beef cattle carcass weight as covariate, but it was not significant in most cases; thus, it was excluded in the final model.

3. Results

Muscle temperature decreased in all three types of slaughtering from 3 to 24 h post-mortem. In particular, the average temperature reduced from 23.1 °C at 3 h, to 16.5 °C at 6 h, to 4.0 °C at 24 h in the group T. Similarly, the average temperatures recorded after 3, 6 and 24 h from the placing of the carcasses in the chilling room were of 25.2, 16.6 and 4.9 °C and of 23.4, 12.9 and 3.0 °C, in the Halal and Kosher ritual rites, respectively (Table 1). In particular, at 24 h post-slaughter, the values of the carcasses slaughtered ranged between 3.35 and 5.40 °C; 3.00 and 6.10 °C; 2.10 and 4.70 °C, in group T, H and K respectively (Fig. 1).

Muscle pH decreased in all three types of slaughtering from 3 to 24 h post-mortem. In fact, the average pH at 3 h post-mortem was of 5.95, 6.06 and 6.04 in the group T, H and K, respectively. At 6 and 24 h post-mortem, the average pH recorded were of 5.84, 5.92 and 5.91, and of 5.72, 5.83 and 5.81, respectively in the traditional slaughtering, in the Halal ritual rite and after the Kosher procedures (Table 1). In particular, at 24 h post-slaughter, the values of the carcasses slaughtered ranged between 5.46 and 6.04; 5.77 and 6.08; 5.49 and 6.09, in group T, H and K respectively (Fig. 2).

4. Discussion

Our study compared the measurement of muscle pH and temperature in beef cattle associated with the time post-mortem and the slaughtering method. The results achieved increase our knowledge about the influence of the slaughtering procedures on some physico-chemical parameters of meat and, consequently, on the final product quality. To our knowledge, this is the first study assessing pH and temperature values of beef carcass obtained through three different types of slaughtering, highlighting the effects on meat quality. In future, other well-known parameters for the determination of meat quality and for the assessment of animal stress could be added in order to deeply investigate the differences related to the type of slaughter. In the present research, we evaluated pH and temperature variations in 90 beef cattle based on the method of slaughtering. Our results reported that the presence or absence of stunning are of significant meaning in influencing some meat physico-chemical parameters of the meat. Indeed, following traditional slaughtering with the stunning phase at 24 h post-mortem, the average pH value recorded was equal to 5.72. On the other hand, the average pH values were 5.83 and 5.81 after the Halal and the Kosher ritual rites respectively (Table 1 and Fig. 2).

In the three groups of animals there was a significant effect ($p < 0.0001$) of time on the variations of the temperature values. Indeed, temperature decreased from 3 h to 24 h post-mortem, with intermediate values recorded at 6 h after the entering in the chilling room. These results were expected considering the normal drop in temperature that occurs over time inside the chiller. Regarding the slaughtering type, there was also a significant effect ($p < 0.01$) among the three slaughtering procedures at the same time intervals (Table 1). This variation at the same time point (3, 6 and 24 h post-mortem) was unexpected considering that there was no significant difference in the mean carcass weight of the three groups. We are aware that in animals with high weight, more time is needed to reach a lower core temperature while in smaller animals, heat is quickly released. Several studies (Cadavez et al., 2019; Reiche et al., 2019) have shown that hot carcass weight was inversely correlated with temperature decline rate and heavier carcasses presented higher mean temperatures at the different time points. However, this is not the case of our study as the three groups of animals did not differ significantly. Reiche et al. (2019) reported that during the final stage before slaughter, intensified reactions of stress may have low effect on the temperature of carcass after slaughter; additional processes, not related to the temperature could affect this relation, for example the altered cell

Table 1
Effect of time and slaughtering type and their interaction on carcass temperature and pH of animals slaughtered following traditional, Halal or Kosher procedures ($n = 30$ per type).

		Slaughtering type			Effect		
Item	Time	Traditional	Halal	Kosher	Time	Slaughtering type	Time × Slaughtering type
Temperature°C	3 h	23.1 (0.69)	25.2 (0.85)	23.4 (0.94)	<0.0001	<0.01	<0.001
	6 h	16.5 (0.19)	16.6 (0.23)	12.9 (0.10)			
	24 h	4.0 (0.09)	4.9 (0.12)	3.0 (0.04)			
pH	3 h	5.95 (0.03)	6.06 (0.04)	6.04 (0.05)	<0.01	NS	<0.05
	6 h	5.84 (0.04)	5.92 (0.03)	5.91 (0.03)			
	24 h	5.72 (0.03)	5.83 (0.04)	5.81(0.04)			

[1]NS: not significant. Standard errors in parentheses.

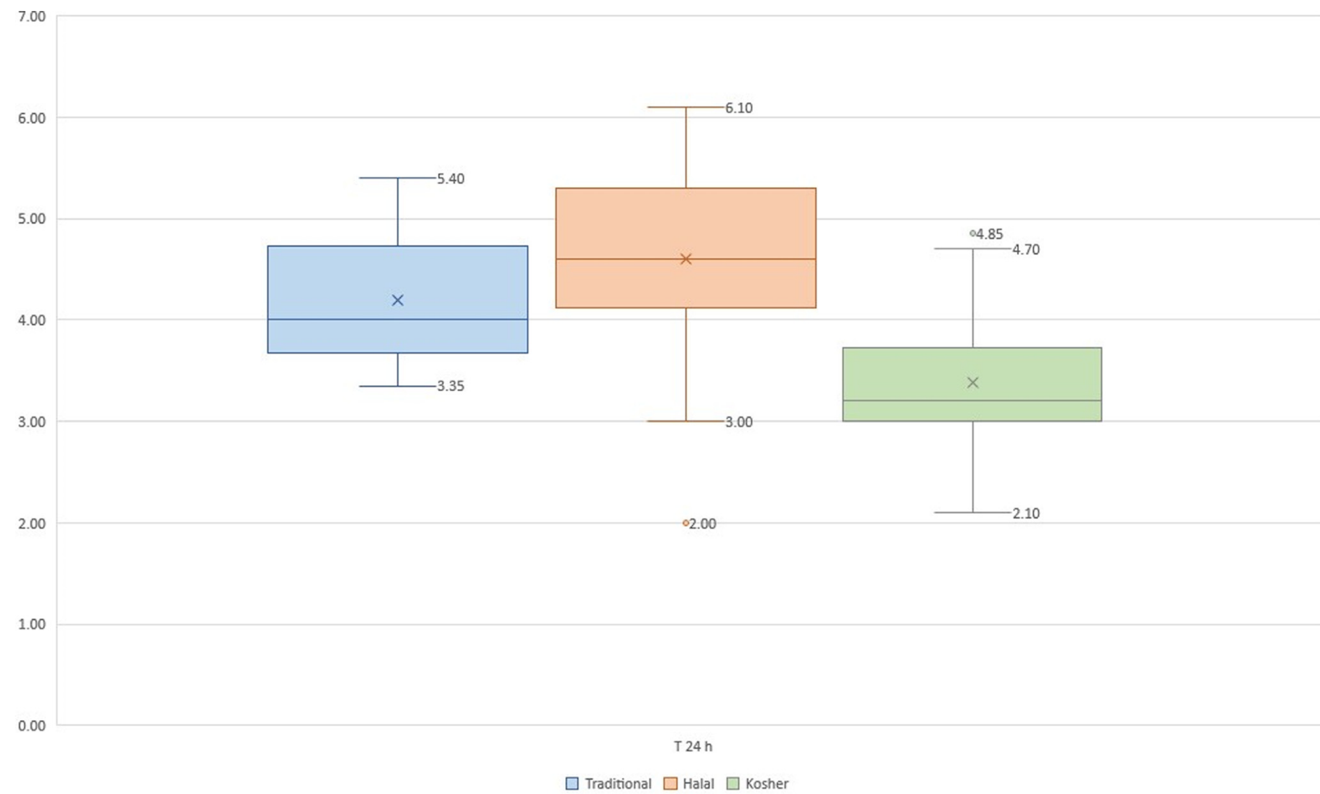

Fig. 1. Distribution of temperature values at 24 h post-slaughter in the three groups of animals.

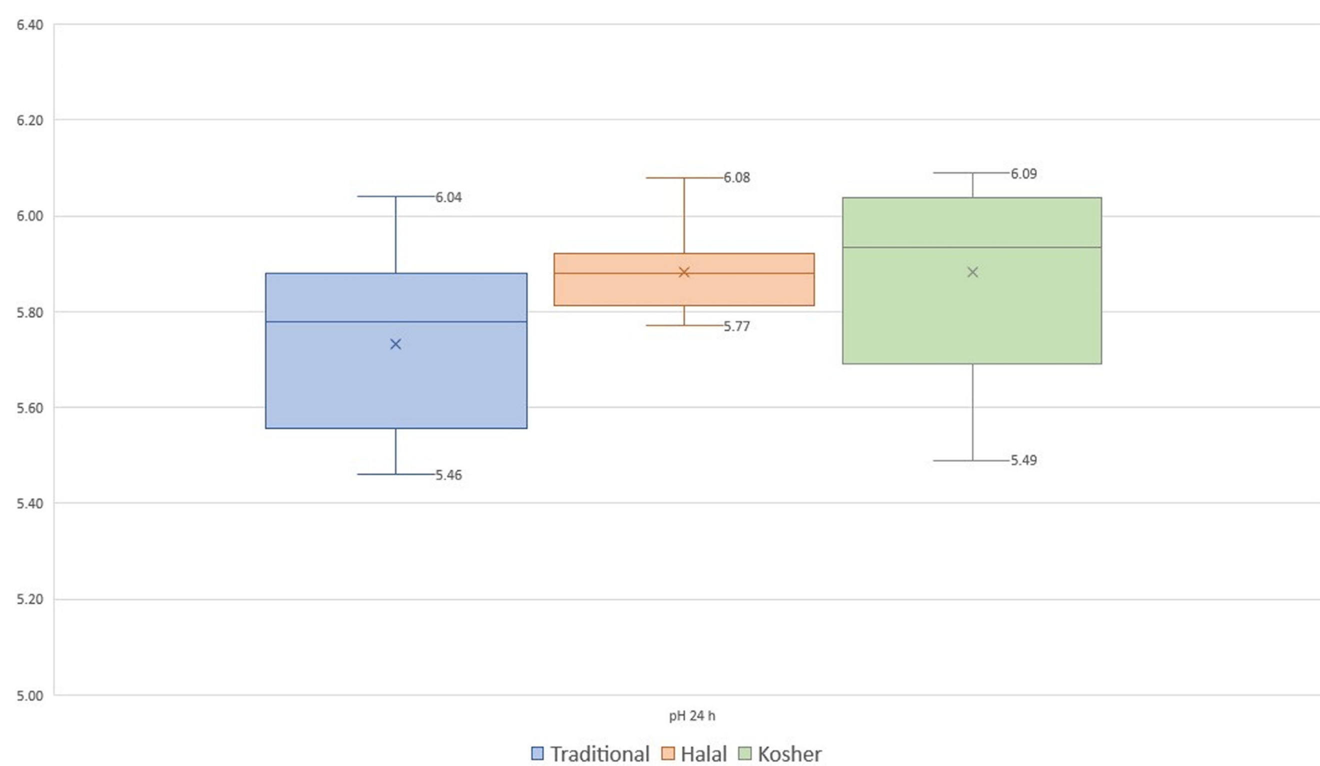

Fig. 2. Distribution of pH values at 24 h post-slaughter in the three groups of animals.

metabolism by catecholamines. In future research we should focus more on the temperature variation in animals with similar weight and that before slaughter were bred and transported in the same way, without situations of additional stress, to understand if there is a correlation between the type of slaughter and the temperature lowering.

Regarding pH values, the slaughtering type was not significant in the variation of this chemical parameter. Indeed, there was no significance in the context of the same slaughtering procedure during the three steps as well as no significance at the same time point among the three groups of animals. On the other hand, there were a significant effect of time ($p < 0.01$) and between time and slaughtering type ($p < 0.05$) on the pH averages (Table 1). Several studies showed that pH24 is affected by many factor as carcass conformation (Cadavez et al., 2019), gender, fat cover and the interaction among factors (Mach et al., 2008). It is clear that all these variables could not have influenced the results of our study because the selected animals were all entire male, showed a similar conformation and degree of fat cover, had a very narrow age range and the difference in the hot carcass weight was not significant. Moreover, all the three groups of bovines were maintained in lairage facility of abattoir for the same time (one hour), until the slaughter. Consequently, the only variable that may have influenced the pH values was the slaughtering procedure. It is therefore evident that the variable linked to the slaughtering type is a key-factor in determining pH_{24} level. Intensified movement and emotive before slaughter may led to reduction of glycogen storage, with the consequence of pH reduction and increased pH_u as reported by Tarrant et al. (1992) and more recently by Terlouw et al. (2021). Nevertheless, this situation did not occur in our study because the animals had undergone the same handling and were treated in the same way before slaughter, without any additional stress for any of the three groups of animals.

In contrast, in a study conducted by Bourguet et al. (2010), pH reduction and pH_u were not affected by conditions of slaughter. Indeed, an added stress treatment did not reduce glycogen in muscle and, thus, did not have an effect on pH variations. Reiche et al. (2019) also showed that pH_u was not affected by method of slaughter. In particular, the lack of effect of slaughter conditions on pH_u was relared to the high glycolytic capacities during slaughter. On the other hand, stress at slaughter can determine higher pH_u or more fast pH decline in post-mortem. Bourguet et al. (2010) assessed in cattle that increased stress was a rapid decline of pH. A lower initial post-mortem pH assessed in Halal slaughtered animals suggested that some aspects of this procedures led to more stress than the conventional procedures (Bourguet et al., 2011). However, in that study the authors highlighted a reduced muscles temperature of halal when compared to conventional processed animals and this result disagree to our findings, where animals slaughtered with the Halal ritual rite showed the highest temperature.

Ritual slaughters were reported to produce meat with high pH after extended storage time (Zuckerman and Mannheim, 2001). Önenç and Kaya (2004) showed that the animals stunned with percussive captive bolt had a lower pH24 than the animals not stunned and the animals electrical stunned. A study conducted in a slaughterhouse comparing, at different time intervals, the pH values of animals slaughtered after stunning and with kosher rite, demonstrated the tendency of the latter to reach lower acidity values, at the expense of an optimal shelf life (Giuntini, 2017). Another more recent study confirmed that, in religious slaughters, the pH value remained quite high even at 24 h post-mortem. Furthermore, the animals slaughtered without prior stunning showed evident signs of suffering (i.e., movements) even after neck cutting (Pérez-Linares et al., 2013).

The effects of the acute autonomic stress response on post-mortem muscle pH and meat quality are well known, as reported by Knowles (1999). Acute preslaughter stress influences not only muscles color, water-holding capacity and firmness, but also decreases tenderness of meat (Viljoen et al., 2002). Watanabe et al. (1996) revealed that post-mortem meat tenderness was significantly related to epinephrine-induced variations in pH_u. Meat tenderness was significantly associated to higher glycogen concentrations before slaughter, indicating a reduced level of stress at abattoir (Reiche et al., 2019).

A value of pH_{24} above 6.0 could result in dark meat (namely DFD) that is more vulnerable to bacteria and less storable (Gardner et al., 2001). This type of meat become less acceptable to the consumer because of the increased tenderness variation and poor palatability (Dell'Orto et al., 2010; Viljoen et al., 2002), intensive growth of bacteria, and often slime formation (Apple et al., 2005). Indeed, the characteristics of meat having significant impact on consumers' taste are juiciness, tenderness, as well as flavor of cooked meat (Regenstein et al., 2003).

Conversely, other studies (Purchas, 1990) showed that meat with high pH_u is often, but not always, more tender. Moreover, the correlation between muscle pH and meat tenderness was curvilinear, and meat toughness tended to be greatest when final muscle pH ranged 5.28–6.21 (Watanabe et al., 1996). Although in these studies dark cutting meat was more tender and juicier, consumers would not choose it due to the dark color (Warner, 1989). Nevertheless, it was suggested by Warner et al. (2007) that acute stress during preslaughter could decrease meat tenderness, also when pH is unaffected.

Changes in stress-related behavioral and physiological status at slaughter seem to better justify differences between individuals regarding the metabolism indicators of muscle in post-mortem phase, and this was previously found in many species such as pigs, lambs and cows (Terlouw et al., 2021). Several studies showed that hormone levels and electroencephalogram (ECG) trends are indicators of the high level of suffering of the animals subjected to the religious rites (Bozzo et al., 2018). Indeed, without stunning, a quantity of animals take more time to reduce consciousness because, when subjects are slaughtered by following halal or shechita methods, their carotids are predisposed to false aneurysms (Gregory et al., 2010). This can lead to postpone onset of unconsciousness and nociceptive neuronal signals can achieve in brain (Gregory et al., 2010).

Therefore, we can hypothesize that the higher pH24 in the animals slaughtered with the ritual rites is linked to a slower bleeding which prolonged the animal's state of consciousness, resulting in more struggling, faster metabolism, and increased glycogen consumption. It is clear that a lack glycogen at slaughter may determine a pH below 5.5, and also to significant reduction of meat quality. However, all the animals in our study were treated equally in pre-slaughter period and had similar weight and conformation. Consequently, we assume that there was no difference in the glycogen stores of the animals slaughtered without stunning, rather that glycogen consumption was higher during bleeding.

5. Conclusion

Although the susceptibility of animals to some degree of stress is unavoidable in abattoir, the aim should be to slaughter animals with traditional, Kosher or Halal techniques minimizing the stress and obtaining correct pH values. The findings of the study may be useful to highlight the impact of slaughtering methods on pH and, more generally, on meat characteristics. Our results assessed that the pre-stunning in bovine species decreased the risk of meat with high pH_u, probably because of these animals were exposed to lower stressful conditions and, consequently, lower glycogen consumption. Furthermore, the detection of a pH_{24} value not ideal for the normal biochemical conversion of muscle into meat indicates that, in slaughtering carried out without prior stunning, the carcasses could be more susceptible to alterations. However, these earliest findings need to be validated by further investigations, utilizing more samples and assessing supplementary meat quality indicators.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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