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The effect of pre-slaughter electrical stunning on bleeding efficiency, meat quality, histology, and microbial count of several goat muscles

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Abstract

This study was designed to compare the effect of non-electrical stunning and electrical stunning on bleeding efficiency, meat quality characteristics, bacterial count, and histology of longissimus thoraces, semitendinosus, biceps femoris, infraspinatus, semimembranosus, and triceps brachii muscles of goats. Forty goats were randomly divided into two groups: electrical stunning and non-electrical stunning with 20 animals each. Low frequency head-only electrical stunning of 1 Amp for 3 s at a frequency of 50 Hz was used. The slaughter was performed by severing the carotid artery, jugular vein, trachea, and oesophagus. Six muscles were kept in a chiller at 3–4 ºC for 24 h before quality measurements. Samples from the infraspinatus, longissimus thoraces, and semitendinosus muscles were preserved to evaluate histological features. Muscle samples from the non-electrical stunning group had substantially higher blood loss and lower bacterial counts after 72 h across the six muscles compared to the electrical stunning group. No significant differences in meat quality parameters were evident between the two groups. The stained sections of the electrically-stunned muscle samples detected alteration phenomena due to the presence of muscle fibres with split myofibres and myofibres with central rather than peripheral nuclei. Electrical stunning prior to slaughter increased bacterial contamination, decreased blood loss, and altered the position of muscle nuclei.

Keywords: bleeding, central nuclei, characteristics, contamination, peripheral nuclei
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Introduction

Meat quality and safety parameters are highly pertinent for the modern global meat industry as they are directly linked to consumer health and welfare. Substandard meat quality and microbial proliferation are limiting factors that influence quality, safety, and shelf-life of meat products (Bórnez *et al.,* 2009). The chemical transformations that take place during protein denaturation are responsible for several biological

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modifications that affect meat quality and histological characteristics of muscles (Choi *et al.,* 2010). After slaughter without proper storage, meat quickly deteriorates and becomes spoiled and unfit for human consumption due to microbial contamination. Muscle protein structures influence the nutritional values and the quality of meat (Falowo *et al.,* 2014). The slaughtering procedure for meat production is governed by strict regulations related to meat quality, food safety, and hygiene. In this respect, many countries considered animal welfare as a factor to be considered within animal slaughter stunning procedures. Stunning prior to slaughter have been employed in the last 150 years in order to reduce the potential pain and distress that animals undergo during slaughter (Zivotofsky & Strous 2012). Stunning prior to exsanguination is required in many countries around the world for humane reasons (Gregory *et al.,* 2012).

Electrical stunning is accomplished by the passage of a sufficient of electric current through the central nervous system of animal (McNeal *et al.,* 2003; Nakyinsige *et al.,* 2013). Electrical stunning leads to short-term brain dysfunction and unconsciousness until the animal dies as a result of bleeding (Llonch *et al.,* 2015). However, it has been reported that electrical stunning may affect the meat quality due to the amount of blood remaining in the muscle arteries and veins after bleeding (Gregory *et al.,* 2012; Farouk *et al.,* 2014; Aghwan & Regenstein, 2019). Differences in blood loss and slaughter methods (with or without stunning) may contribute to variation in the biochemistry of muscle-to-meat conversion and the expression or abundance of muscle proteome components (Gregory *et al.,* 2012). False aneurysms may be formed as early as 7 s following slaughter without stunning, and on average, they develop within 21 s, which leads to sustained consciousness through failure to bleeding out properly. False aneurysms of the severed arteries are a potential concern in beef cattle as it could extend the duration of brain function prior to death (Bozzo *et al.,* 2020). False aneurysm formation in the carotids and a slow rate of blood loss may extend the period of consciousness during slaughter without stunning.

Bleeding of carcasses to maximize blood loss is considered to be one of the important factors in slaughter procedures to achieve a high-quality product because residual blood in the carcass is a suitable medium for bacterial growth and will lead to a shorter shelf-life and low meat quality parameters (Sabow *et al.,* 2015; Aghwan & Regenstein, 2019). Blood carries oxygen to skeletal muscles for muscle metabolism and heat generation. Differences in blood loss between stunning and non-stunning slaughter methods can influence oxygen deprivation and the onset of anoxia in skeletal muscles and can thus contribute to variations in the biochemistry of muscle-to-meat conversion and the extent of *postmortem* anaerobic glycolysis.

In the *postmortem* muscle proteome, anoxia-induced stress initiates the expression of stress proteins such as hsp 105, hsp 90, hsp 70, and hsp 40, and antioxidant proteins (Jia *et al.,* 2006a, 2006b; 2007; Bjarnadottir *et al*., 2010), which could influence meat quality parameters such as pH and water-holding capacity. The high nutrient content, favourable pH, temperature, relative humidity, and water activity make blood an ideal medium for microbial proliferation. Moreover, muscle makes a very good medium for bacterial growth as well. Thus, effective bleeding efficiency at slaughter is necessary to improve the quality and increase the shelf-life of meat products (Nakyinsige *et al.,* 2014).

The growing popularity and thriving international trade make meat products from stunned animals increasingly available in supermarkets and restaurants globally (Lever & Miele 2012). The objective of the current study was to compare the effect of non-stunning and electrical stunning on bleeding efficiency, meat quality characteristics, bacterial count, and histological morphology of longissimus thoracic (LT), semitendinosus (ST), semimembranosus (SM), biceps femoris (BF), triceps brachii (TB), and infraspinatus (IS) muscles of goats.

Materials and Methods

The research protocol was approved by the institutional animal care and use committee (IACUC) of the University of Nizwa, Sultanate of Oman (approval no: R052/2019).

Forty, 1-year-old Somalian bucks (12 months old; average body weight of 22.00 ± 1.95 kg), reared under similar management conditions, were purchased from a commercial goat farm. The animals were transported from the farm to the slaughter-house 12 h prior to slaughter. Therefore, the goats had 12 h of lairage time, with access to water, but not feed in order to reduce the gut content. Harvesting of animals with or without electrical stunning was performed at a commercial slaughterhouse in Izki, Sultanate of Oman. Each animal was weighed before slaughter and was randomly assigned to either a non-electrical stunning (NES) group (n = 20), which was slaughtered by neck cutting only after being restrained by the slaughter man and all the vessels in the animals' neck were severed with one cut using a sharp knife; or an

electrical stunning (ES) group (n = 20), which was stunned electrically (Model NDK (BK)-500 with 70V manufactured by CHNT, China). All the stunned animals were unconscious before slaughter. The electrical stunning was used to immobilize the animals followed by severing all the vessels in the animals' neck with one cut similar to the NES group.

The ES group were stunned electrically using the low frequency, head-only method at a constant current of 1.0 A at 50 Hz for 3 s. The voltage was automatically adjusted according to the impedance of the animal's head tissue. The electrical stunning system consist of two frontal, stainless-steel tongs that were placed behind the ears. The animals were properly stunned by following the manufacture's instruction and their hearts did not fibrillate due to the stunning. Similarly, all the animals were individually restrained prior to being stunned by the same person, therefore, they were handled and treated the same. The heart rate of animals was not measured, however, the non-stunned animals moved more roguishly after slaughter than those stunned. The slaughter of both groups was carried out by the same certified slaughter man using an exquisitely sharp knife. The neck cut severed skin, muscle, oesophagus, trachea, carotid arteries, jugular veins, and major nerves without decapitating the head during the process. The average stun-to-stick interval was 10.52 ± 0.25 s. The animals in the two groups were treated using the same procedures such as pre-slaughter handling, slaughter practices, carcass dressing, and hanging position, with the only difference between the two treatments being the stunning procedure (ES vs NES). The current study applied electrical stunning to goats, which is not a permissible practice for animals slaughtered for Muslim's consumption (Farouq *et al*., 2014).

The goats were weighed before slaughter. During exsanguination, blood was collected in a plastic container and weighed. The amount of blood loss was measured using the following formula:

Blood loss (%) = [weight of collected blood/ body live weight before slaughter] × 100

All goats were slaughtered within one hour, with every second animal being allocated to the ES group. After a 2-min bleed-out, carcasses were dressed and eviscerated. Six muscles were removed from the left side of each carcass (LT, ST, BF, IS, SM, and TB) within 20 min of slaughter and kept in labelled, plastic bags inside a cool box (4–5 ℃), while being transported to University of Nizwa's DARIS Center for Scientific Research and Technology Development. All the meat samples for quality evaluation were kept in a chiller at 4–5 ºC for 24 h.

Different tests were used to investigate the various microbiological characteristics of ES and NES meat samples. The total viable count was determined using the standard formula:

TCFU = (N/S×D)

where TCFU, total colony-forming unit; N, number of colonies counted; S, volume transferred to plate; D, dilution).

One millilitre samples were obtained from randomly chosen dilutions and then mixed to ten millilitres with nutrient broth medium. The sample was allowed to grow at 37 ℃ inside the incubator for 24 h. One milliliter of sample was obtained and mixed with 10 ml nutrient agar medium and then incubated at 37 ℃ for 24 h and allowed to grow. For yeast and mould counts, 1 ml was aseptically inoculated on a plate of 10 ml potato dextrose agar. Chloramphenicol was added to inhibit the growth of bacteria. The sample was allowed to grow in favourable conditions of 25 ℃ to 28 ℃ for 48 h. The bacterial counts were presented as colony forming units per gram (cfu/g) (Todar, 2020).

For the coliform test, 10 ml of MacConkey agar medium was used (McCoy, 2015) to grow coliform colonies from 1 ml samples at 37 ℃ for 48 h. Coliform colonies were presented as colony forming units per gram (cfu/g).

For the *Escherichia coli* test, brilliant green bile lactose broth in Durham tubes was prepared to confirm the growth of *E. coli*. With a temperature of 44 ℃ for 48 h, samples in tubes were incubated and allowed to grow. The confirmed positive tubes were sub-cultured using 10 ml broth and incubated at 44.5 ℃ for 24 h. Gas production inside the Durham tubes is an indication of *E. coli* growth (McCoy, G 2015).

For *Salmonella* detection, one gram of ES and one gram of NES meat samples from the middle surface area of each muscle were weighed aseptically and mixed well with 10 ml sterile nutrient broth. Samples were incubated at 37 ℃ for 24 h. One milliliter was transferred aseptically to 10 ml selenite broth. Samples were incubated at 37 ℃ for 24 h. Using 10 ml bismuth sulphite agar plates, a loop full of sample was streaked onto it. Sample was allowed to grow inside the incubator at 37 ℃ for 36 h. To check the growth of *Salmonella*, discrete colonies of black metallic green were observed in the medium. To confirm the

presence of *Salmonella*, the black metallic green colonies were sub-cultured in tubes of triple sugar iron broth. Black colour at the bottom of tubes was considered as evidence of *Salmonella* growth.

Meat quality assessments included ultimate pH, expressed juice, cooking loss, shear force, sarcomere length, myofibrillar fragmentation index, and colour, which were determined following the procedures described by Kadim *et al.* (2003). Duplicates of 1.5–2 g of each muscle sample were crushed and homogenized at 20–22 °C (using an Ultra Turrax T25 homogenizer) for 30 min in 10 ml deionized water in the presence of 5 mM sodium iodoacetate to inhibit further glycolysis (Kadim *et al.,* 2003). The ultimate pH (24-h *postmortem*) was measured using a Metrohm pH meter (Model No. 744: Toledo: Seven Easy, UK) with a glass electrode. The pH meter was calibrated each time before testing.

Triplicates of 25 mm-thick slices were cut from each muscle. The slices were weighed (W1), placed in plastic bags, then cooked by immersing in a water bath at 70 °C for 90 min and the fluid was drained-out (Kadim *et al.,* 2003). The cooked meat samples were kept in a chiller overnight (3–4 °C). They were carefully dried with tissue to remove excess surface moisture and re-weighed (W2) to determine cooking losses. The cooking loss percentage was estimated using the following formula:

cooking loss (%) = (W1 - W2)/W1 × 100

For the assessment of tenderness, after cooling, 12 cores (13 mm × 13 mm square cross-sections) were cut parallel to the orientation of the muscle fibres from the centre of each slice, using two scalpel blades at a fixed distance (13 mm) (Kadim *et al.,* 2003). Cores were prepared to ensure that shears were made across the fibres. Each core was then sheared perpendicularly to the fibres in two places using a texture analyser machine (Stable Micro Systems, Texture Analyzer, Model TA.XT.Plus, UK). Calibration of the device was done at 5 kg weight and a blade speed of 10 mm/s. The device measures the maximum force (kg) that is required to cut across the muscle fibres and the average sheer force of the blocks used for the analysis.

Sarcomere length using laser diffraction was determined using the procedure described by Cross *et al*. (1980/1981). Briefly, the apparatus consisted of a helium–neon laser (wavelength of 632–638 nm) that was mounted on an optics bench with a specimen-holding device and a screen. Individual fibres from the muscle bundle, which were removed from the centre of each muscle strip were teased and placed on a microscope slide with a drop of solution (0.25M KCl, 0.29M boric acid, and 5mM EDTA in 2.5% glutaraldehyde). The slide was then placed horizontally in the path of a vertically-orientated laser beam to give an array of diffraction bands. These bands were perpendicular to the long axis of the fibres. Sarcomere lengths were calculated using the following formula:

Sarcomere length (µm) = $632.8 \times 10^{-3} \times D \times N/(-\sim TD)^2 + 1$ (Cross *et al.* (1980/1981).

Twenty-five fibres were measured from each individual on each sample piece.

Expressed juice determinations of the water-holding capacity were based on measuring water liberated when pressure was applied to the muscle tissue. Expressed juice was assessed using the filter paper method, as the total wetted area less the meat area (cm^2) relative to the weight of the sample (g) (Hamm 1986). A cube of 500 ± 20 mg of meat from the inside of the LT sample was placed on a filter paper (Whatman No. 1, 11.0 cm in diameter) between Perspex plates. The plates were screwed together tightly for exactly 5 min. The wet and meat areas were measured with a planimeter. Duplicate measurements of expressed juice were made for each sample. Expressed juice values were calculated based on the following formula:

Expressed juice (cm^2 /g) = (Wet area (cm^2) - meat area (cm^2)/meat weight (g)

Myofibrillar fragmentation index was measured by using a modification of the method by Johnson *et al.* (1990). This basically measures the proportion of muscle fragments that pass through a 231-µm screen after the sample had been subjected to a standard homogenization treatment. A 5 g (+0.5 g) sample of diced (6 mm^3 pieces) sample was added to 50 ml of cold physiological saline (85% NaCl) plus five drops of antifoam A emulsion (Sigma Chemical) in a 50-ml graduated cylinder and homogenized at 1/4 speed using an 18-mm diameter shaft on an Ultra-Turrax homogenizer for 30-second periods separated by a 30-second rest period. The homogenate was poured into a pre-weighed filter (231 × 231 µm holes). The filter typically ceased dripping after 2–3 h, at which time they were dried at 26–28 °C in an incubator for 40 h before being re-weighed.

Meat colour values were determined using a Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Japan). Prior to use, the colorimeter was calibrated against black and white tiles. Approximately 60 min after exposing the fresh surface (blooming), CIE L*, a*, and b* light reflectance coordinates of the muscle surface was measured at room temperature (20 ± 2 °C). The meter was calibrated using a Minolta calibration plate (L* = 97.59, a* = 5.00, b* = +6.76). The L* value relates to lightness; the a* value to red–green hue where a positive value relates to the red intensity; and the b* value to the yellow–blue where a positive value relates to yellow. Triplicate readings for each sample were recorded for *L** (lightness), *a** (redness), and *b** (yellowness) values.

For light microscope investigation, a small bundle (parallel to the muscle fibres) of tissue was dissected under the stereomicroscope, immersed in fresh 10% formaldehyde solution, then processed by a tissue processing machine (140 Biomass). In this process, tissues were dehydrated by 70% and 90% ethanol for 1 h each and three changes of 100% ethanol were made for 1 h, 2 h, and 2½ h, respectively. Tissues were then placed in three changes of xylene for 1 h, 1 h, and 2 h, respectively, then infiltrated in three changes of melted paraffin wax at 60 °C for 1 h each. Tissues then embedded in pure paraffin wax by using the Embedding Center Machine (AEC 380). Paraffin blocks were prepared using standard histology procedures (Al-Amri *et al*., 2020). Finally, paraffin blocks were sectioned into 4-µm thin layers by using a semi-auto microtome. Sections were left in a floating-out path in order to stretch and were placed into a microscopic glass, then transferred into a preheated oven at 60 °C overnight to ensure the adhering of the sections onto the slide. Sections were stained with haematoxylin and eosin. Finally, slides were dehydrated with gradually increased EtOH concentrations of 70%, 95%, and 100% for 2 min, then cleared in two changes of xylene for 5 min each. Slides were mounted with D.P.X and analysed under a digital light microscope (Al-Amri *et al.,* 2020).

The effect of slaughter methods on blood loss, microbial counts, and meat quality parameters were analysed using the GLM procedure for analysis of variance (SAS, 2007). Statistical Analysis Software (SAS) package (Version 9.2) software was used, in which the parameters were fitted as dependent variables and the slaughter methods were fitted as fixed effects. Differences between means were assessed using the least significant difference procedure at a significance value of $P < 0.05$.

Results and Discussion

The percentage of blood loss (with small standard deviation; SD) for goats slaughtered without electrical stunning (4.19%) was higher ($P < 0.05$) (Figure 1) than for those subjected to electrical stunning (3.25%). The main factors influencing blood loss are wound size, blood vessels cut, cardiac arrest, muscle contractions, bleeding time, and hanging of carcass (Gregory, 2005). The NES method ensures the cutting of the major vein carrying blood to the brain hence facilitating maximum bleeding (Schreurs, 1999). Variation in blood loss between slaughter without stunning and pre-slaughter electrical stunning could be due to the response of the individual goat to stunning procedures. Neck cutting in livestock while the heart is still pumping should result in 75–85% of the total blood being lost in the first 60 s (Blackmore & Newhook, 1976). Moreover, the time period for bleeding is much quicker in small ruminants with 50% being lost after 14 s and 90% after 56 s (Anil *et al*., 2004). The present results agree with the findings of Kirton *et al*. (1980; 1981) and Sabow *et al*. (2016), who reported that small ruminants subjected to pre-slaughter electrical stunning had a lower blood loss following slaughter compared to those with no electrical stunning. The substantially lower bleeding loss in electrically-stunned goats might be due to the incidence of ventricular fibrillation and stoppage of the heart at the time of applying stunning (Vogel *et al*., 2011). In contrast, Anil *et al.* (2004) and Khalid *et al.* (2015) reported similar blood loss in sheep subjected to pre-slaughter electrical stunning and those without stunning. Velarde *et al.* (2003) observed that the amount of blood loss in lambs subjected to electrical stunning prior to bleeding was slightly higher than those without electrical stunning. The contradictory results of the current study with other studies, might be due to different animal species used, different voltage of stunning, duration of stunning, and different procedures to measure blood loss.

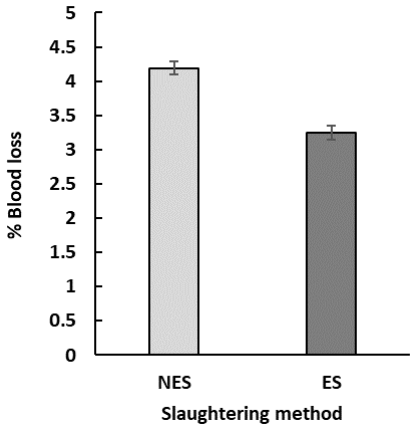

Figure 1 Blood loss as a % ± standard deviation (SD) of live weight in Somalian goats slaughtered without electrical stunning (NES) and with electrical stunning (ES) with a significant difference (P <0.05) between the slaughter methods

The impacts of slaughter method on bacterial count of goat muscles during 72-h *postmortem* refrigerated storage (3–4 ºC) are presented in Table 1. Bacterial growth in meat products is affected by health status of the animal during slaughter, the hygiene of the slaughter-house and its equipment, and storage conditions (Koutsoumanis & Sofos 2004). At 72 h *postmortem*, meat samples from *LT, ST, SM, BF, TB*, and *IS* muscles in goats from the ES group had higher (P <0.05) total aerobic counts of *E. coli* and *Salmonella* than those from NES group; this may be attributed to the low blood loss as a result of the method of slaughter. A positive correlation between residual blood in meat and microbial growth was reported by Nakyinsige *et al*. (2012). Therefore, an effective bleed-out is essential for low microbial counts in meat. The high microbial count for both methods of slaughter might be as a result of residual blood in the carcass, which serves as nutrient for microbial growth. This may also be due to cross-contamination during slaughtering procedures, dissection of muscles, and preparation of sub-samples. Microbial counts frequently have a major role in the spoilage of meat, but it is often low in freshly slaughtered meat, increasing as spoilage progresses (Rahman *et al.,* 2005). The meat preparation processes allow successions in dominating species and the development of high numbers of contaminating *E. coli* and *Salmonella*. Primary sources of these species are most probably the workers and their equipment, and a consequence of the intensive handling of the meat samples during this investigation to ensure the precise muscles were removed and that accurate sub-samples were prepared to minimize experimental error. A decrease in the blood pressure of animals after slaughter affects the total drainage of blood from the numerous capillaries in the muscle so that some blood is retained within the carcass (Alvarado *et al*., 2007; Lerner 2009; Nakyinsige *et al*., 2014).The present results were supported by findings of Sabow et al. (2015), Sabow *et al.* (2016) in goats, Nakyinsige *et al.* (2014) in rabbits, and Addeen *et al.* (2014) and Ali *et al.* (2011) in broiler chickens, who found substantially lower counts of bacteria in meat obtained from livestock slaughtered without electrical stunning. These results suggest that effective bleeding is essential for low microbial counts in goat meat.

Table 1 The population of total aerobic counts (log_{10} CFU/g) of *Escherichia coli* and *Salmonella* bacteria at 72-h *postmortem* in longissimus thoracis, semitendinosus, biceps femoris, infraspinatus, semimembranosus, and triceps brachii muscles in goats slaughtered without (NES) and with electrical stunning (ES)

Muscle	*E. Coli*				*Salmonella*			
	Slaughter methods				Slaughter methods			
	NES	ES	*P*-value	Sig.[1]	NES	ES	*P*-value	Sig.[1]
Longissimus thoracis	3.39±0.35	5.79±0.59	0.002	*	3.19±0.21	5.38±0.44	0.009	*
Semitendinosus	4.43±0.41	6.43±0.65	0.009	*	3.43±0.34	5.98±0.59	0.008	*
Semimembranosus	5.61±0.54	8.02±0.79	0.008	*	4.62±0.33	5.63±0.62	0.004	*
Biceps femoris	3.42±0.29	5.22±0.45	0.004	*	5.03±0.47	7.43±0.68	0.006	*
Triceps brachii	5.63±0.61	7.41±0.66	0.002	*	1.84±0.20	4.24±0.32	0.001	*
Infraspinatus	3.19±0.22	5.13±0.51	0.004	*	3.60±0.38	5.44±0.55	0.004	*

[1] Significant; * P <0.05

The effect of slaughtering method on quality parameters measurements for ST, SM, BF, LT, TB and IS muscles is presented in Table 2. The ultimate pH is an important factor which influences the physical and chemical traits of muscle at the time of slaughter and following the development of rigor mortis (Macanga *et al*., 2011; Mortimer *et al*., 2014). Slaughtering method had no significant effect on ultimate muscle pH value at 24-h *postmortem* across six muscles. The current results are consistent with the results of Sabow *et al*. (2017) in goats, Onenc & Kaya (2004) in cattle, and Velarde *et al.* (2003) and Vergara *et al*. (2005) in lambs, who reported non-significant differences in ultimate pH in non-stunned and electrically-stunned animals. Ultimate pH is considered a major factor affecting the meat quality characteristics, which is related to the rate of glycogen breakdown (Watanabe *et al*., 1996).

Expressed juice (water holding capacity) is the ability of meat to preserve an essential quantity of both inherent and supplemented water and is a quality attribute for the industry and also for the consumer (Prevolnik *et al*., 2010; Modzelewska-Kapituła *et al*., 2015). The results of expressed juice and cooking loss percentage in *ST, SM, BF, LT, TB,* and *IS* muscles from goats subjected to electrical stunning or without stunning are presented in Table 2. The results showed that slaughter methods had no significant effect on expressed juice and cooking loss percentage at 24-h *postmortem*. According to Nakyinsige *et al*. (2014), expressed juice and cooking losses are affected by muscle pH and temperature. In the present study, the similarity in the ultimate pH across muscles and the slaughter methods might have been responsible for the absence of difference in expressed juice and cooking loss values between the slaughter methods. The present results are in agreement with those of Sabow *et al*. (2017) in goats and Onenc & Kaya (2004) in cattle, who reported that animals subjected to pre-slaughter electrical stunning had similar expressed juice as those slaughtered without stunning. Most investigators reported that meat quality parameters of livestock slaughtered without electrical stunning were comparable to those stunned (Gregory *et al*., 2008).

Conventional methods of electrical stunning are associated with adverse effects on the "spiritual quality" of meat quality characteristics (Farouk *et al.,* 2014). In contrast, Onenc & Kaya (2004), Linares *et al*. (2007), Agbeniga *et al*. (2013), and Nakyinsige *et al*. (2014) found a higher cooking loss in meat from electrically-stunned livestock compared to those slaughtered without stunning. An increase in plasma catecholamines is associated with the electrical stunning technique during slaughter and has a significant influence on proteolysis since it reduces the space of muscle fibres and consequently decreases drip loss (Linares *et al*., 2007). The implication of the slightly increased cooking loss means a reduction in the size of the meat portion with a possible reduction in quality due to the loss of valuable protein and flavour compounds.

Tenderness is one of the most important parameters of meat quality characteristics that influences consumers' eating satisfaction (Hildrum *et al*., 2009). The shear force values of *ST, SM, BF, LT*, *TB,* and *IS* muscles from goats subjected to electrical stunning prior to slaughter and without stunning are shown in Table 2. Slaughter method had no significant effect on shear force values of selected muscles. In agreement with the present results, Sabow *et al*. (2017), Buyukunal & Nazli (2007), and Linares *et al*. (2007) reported that shear force values of meat from goats and lambs subjected to head-only electrical stunning

and those slaughtered without stunning were similar. The possible mechanism through which electrical stunning did not improve tenderness may be the low frequency and voltage used.

Sarcomere length is a useful indicator of muscle contraction (Li *et al*., 2012). The longer the sarcomere length, the more susceptible to postmortem proteolysis (Weaver *et al.,* 2008). The sarcomere length in six muscles was not affected by the slaughtering method (Table 2). These results indicate that with or without electrical stunning, methods of slaughter reduced the overlap of actin and myosin at the same rate. The current results contradict the findings by Kim *et al*. (2013) but concur with those of Sabow *et al*. (2015) and Sabow *et al.* (2017), who found that the sarcomere length in goat muscle was not affected by slaughter method.

The effects of slaughtering method on colour coordinates (*L*, a*,* and *b**) of ST, SM, BF, LT, TB, and IS muscles in goats were not statistically significant (Table 2). Similarly, Sabow *et al*. (2017) in goats, Vergara & Gallego (2000), Velarde *et al.* (2003) in lambs, and Onenc & Kaya (2004) in cattle found no differences between not-electrically stunned and stunned livestock for L*, a*, and b* colour values. The slight increase in the values of a* and b* caused a pink coloration, which is likely a result of improper bleeding. This result is in line with the observations of many researchers (Barbut & Mittal, 1993).

Myofibril fragmentation index (MFI) is an indicator of the degradation of the structure of the myofibrillar proteins during rigor mortis (Kandeepan *et al*., 2009; Li *et al*., 2012). Effects of slaughtering methods on the MFI of six muscles are presented in Table 2. At 24-h *postmortem*, muscle samples from the NES group exhibited numerical but not significantly higher MFI values than those from ES group. This explains why muscle samples from the NES group had a lower numerical shear force compared to muscle samples from the ES treatment. A negative correlation between MFI and shear force value has been reported by Hou *et al.* (2014). The numerically high MFI in electrically stunned goat muscles might be due to the pH decline caused by electrical stunning, which can be attributed to the proteolytic changes in myofibrillar and sarcoplasmic portions occurring earlier in muscles having rapid reduction in pH (Sierra Fernandez-Suarez *et al*., 2012; Sabow *et al*., 2017). The findings concur with Rees *et al*. (2003), Sabow *et al*. (2015), and Sabow *et al.* (2017), who found that stunning technique in pork and goats did not influence MFI values post-rigor.

The goat skeletal muscle fibre or myofibre is a syncytium as it possesses hundreds of nuclei within the cell membrane. In a fully matured myofibre, most nuclei are positioned and spaced regularly at the periphery, just below the sarcolemma (Folker & Baylies 2013). LT, ST, and IS muscle sections were stained with haematoxylin and eosin, which stains the cytoplasm pink and the nuclei dark purple (Figures 2–4). Haematoxylin and eosin staining provides general information on the morphology of the analysed muscle tissue, sufficient to detect any morphological alterations. The stained sections of non-electrical stunning methods showed muscle fibres with similar patterns, arranged in bundles, peripherally-located nuclei, and with uniform and unfragmented sarcoplasm. The sarcoplasm contained all cytoplasmic organelles, but consisted mostly of myofibrils that, occupying most of the available space, push the numerous nuclei to the periphery of the muscle fibre (Greising *et al.,* 2012). The intercellular space is occupied by reticular endomysium connective tissue. The two types of striations were clearly visible. The longitudinal striations represent myofibrils that are arranged in parallel with each other. Alignment of myofibrils results in transverse banding patterns as alternating dark and light bands of myofilaments; the light bands are bisected by Z-discs. The dark bands are also bisected by a clear H-zone and the centre of the H-zone is occupied by the M-disc. The contractile unit of skeletal muscle is the sarcomere, extending from one Z-disc to its neighbouring Z-disc.

Table 2 Effect of slaughter methods without electrical stunning (NES) and with electrical stunning (ES) on meat quality characteristics of six goat muscles

	Quality characteristic[1]								
	pH	CL %	EJ	SF (kg)	SL (µm)	MFI	Colour		
Muscle							*L**	*a**	*b**
Semitendinosus									
NES	5.66	28.7	33.8	5.51	1.79	79.3	27.8	11.8	2.49
ES	5.59	29.5	33.5	5.42	1.81	83.1	26.3	12.4	2.88
SEM[2]	0.05	2.25	2.01	0.06	0.07	1.15	0.98	0.85	0.56
Significant[3]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Semimembranosus									
NES	5.65	27.5	33.8	5.59	1.80	78.2	27.9	11.8	2.36
ES	5.58	27.8	33.5	5.49	1.83	79.4	26.4	12.4	2.98
SEM[2]	0.04	2.26	2.56	0.55	0.08	1.08	0.89	0.83	0.59
Significant[3]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Biceps femoris									
NES	5.64	28.9	36.3	5.50	1.78	77.9	27.8	12.2	2.62
ES	5.57	29.5	34.6	5.41	1.80	78.5	26.6	12.9	2.88
SEM[2]	0.03	2.45	2.98	0.64	0.05	1.31	0.07	0.79	0.48
Significant[3]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Longissimus thoracic									
NES	5.63	28.3	33.2	5.59	1.82	80.4	28.8	11.8	2.53
ES	5.57	29.4	32.7	5.45	1.85	81.2	27.6	12.3	2.77
SEM[2]	0.05	2.23	1.98	0.56	0.06	1.21	0.07	0.65	0.39
Significant[3]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Triceps brachii									
NES	5.63	27.1	32.2	5.36	1.80	79.8	28.3	11.5	2.39
ES	5.58	27.9	31.3	5.24	1.83	79.9	27.4	12.0	2.80
SEM[2]	0.04	2.19	1.56	0.55	005	1.17	0.086	0.98	0.56
Significant[3]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Infraspinatus									
NES	5.65	27.0	31.5	4.84	1.80	79.6	28.6	11.3	2.46
ES	5.55	27.2	30.9	4.65	1.83	80.2	27.8	11.9	2.93
SEM[2]	0.05	2.13	1.84	0.64	0.04	1.09	0.091	0.78	0.57
Significant[3]	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]Quality parameters: CL%: cooking loss%, EJ: expressed juice (cm^2 /g), SF: shear-force (kg), SL: sarcomere length (µm), Colour: L*, lightness, a*: redness, b*: yellowness. MFI: myofibril fragmentation index (%)

[2]SEM: standard error of mean

[3]significant: NS; not-significant, * P <0.01

Lipid droplets appeared in some images, depending on the location. Cross-sectional examination of muscle bundles (fasciae) showed intact fascicles with varying diameters (Images are not presented). Muscle fasciae are surrounded by collagenous connective tissue called the epimysium. Each fascicle is composed of numerous muscle fibres of varying diameters and surrounded by the collagenous connective tissue, the perimysium. The peripherally-located nuclei of the three selected muscles appeared as dark-purple shapes (Figures 2–4). However, the stained section of the electrically-stunned samples from the longissimus thoracis, semitendinosus, and infraspinatus muscles were altered due to the presence of split muscle fibres and muscle fibres with central nuclei rather than peripheral nuclei. This alteration may be due to the fact

that electrical stunning caused an increase in oxidative stress with the production of reactive oxygen species (ROS), which often causes cellular alterations such as muscle atrophy (Steinbacher & Eckl, 2015) characterized by split or fragmented myofibres, and myofibres with central nuclei (Greaves, 2007) or increased production of heat shock proteins (HSPs) in order to protect against subsequent periods of stress damage and to facilitate a rapid recovery when damage occurs (Brioche *et al*., 2016; Jackson 2009). According to Trovato *et al.* (2016), any physical activity induces significant structural and metabolic changes in skeletal muscle. In relation to the stress, different molecular pathways are activated causing muscle hypertrophy and its adaptation (Lamon *et al*., 2014). The findings of the present study indicated that electrical stunning could exert changes in the myofibrillar matrix of *postmortem* goat muscle by splitting of myofibres, and myofibres with central nuclei (Figures 2–4: image B).

In the histopathological analysis of skeletal muscle, different qualitative parameters such as shape and type of the muscle fiber, and number and location of nuclei are considered fundamental for the detection of morphological alterations. However, further studies are needed to support the present results using cross-sectional images. The effect of electrical stunning on satellite cell-specific marker proteins and more details of muscle structure using electronic microscopes are also needed.

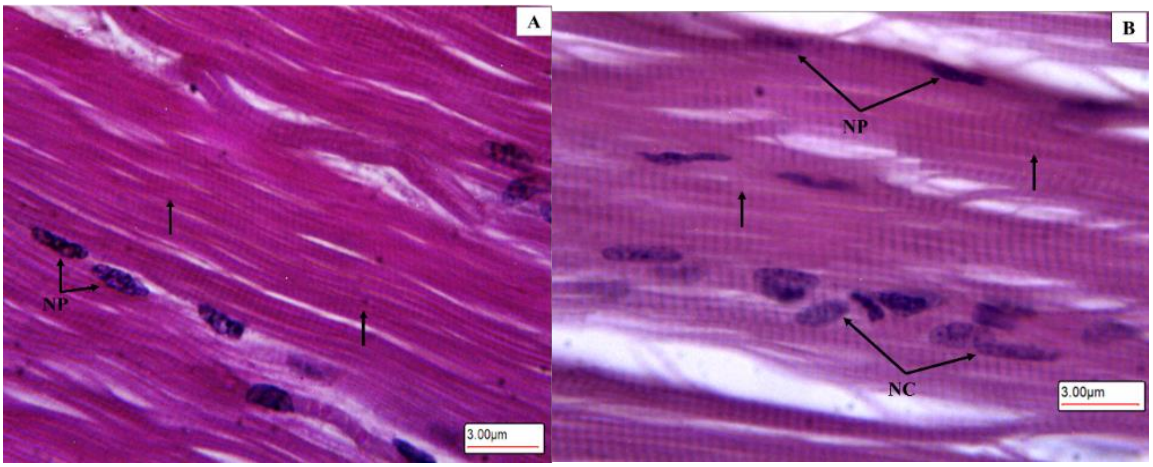

Figure 2 Image (A) higher magnification of non-electrical stunning (A) of longissimus thoracis muscle stained with haematoxylin and eosin (H&E) showing skeletal muscle with uniform muscle fibres (arrow); their nuclei (NP) are peripherally located. Mag 1000×
Image (B) electrical stunning of *longissimus thoracis* muscle showing long muscle fibres with some nuclei located on the periphery (Np) and others in the centre of the muscle fiber (NC). In both images, the striated (arrow) muscle fibres consist of contractile fibrils made up of actin and myosin filaments. Mag. 1000×

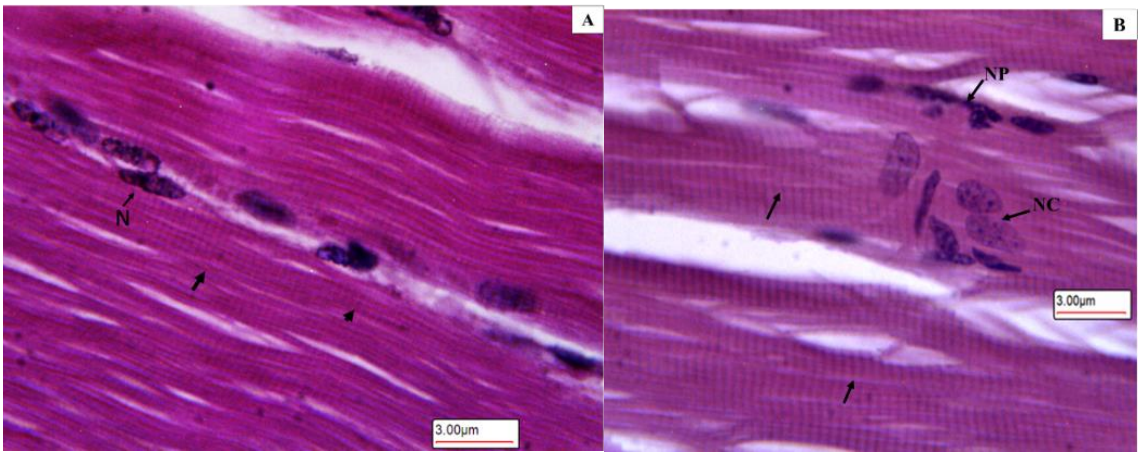

Figure 3 Image (A) higher magnification of non-electrical stunning (A) of the *semitendinosus* muscle stained with haematoxylin and eosin (H&E), showing skeletal muscle with uniform muscle fibres (arrow), their nuclei (NP) are peripherally-located. Mag. 1000×
Image (B) electrical stunning of *semitendinosus* muscle showing long muscle fibres with some nuclei located on the periphery (Np) and others in the centre of the muscle fiber (NC). In both images, the striated (arrow) muscle fibres consist of contractile fibrils made up of actin and myosin filaments. Mag. 1000×

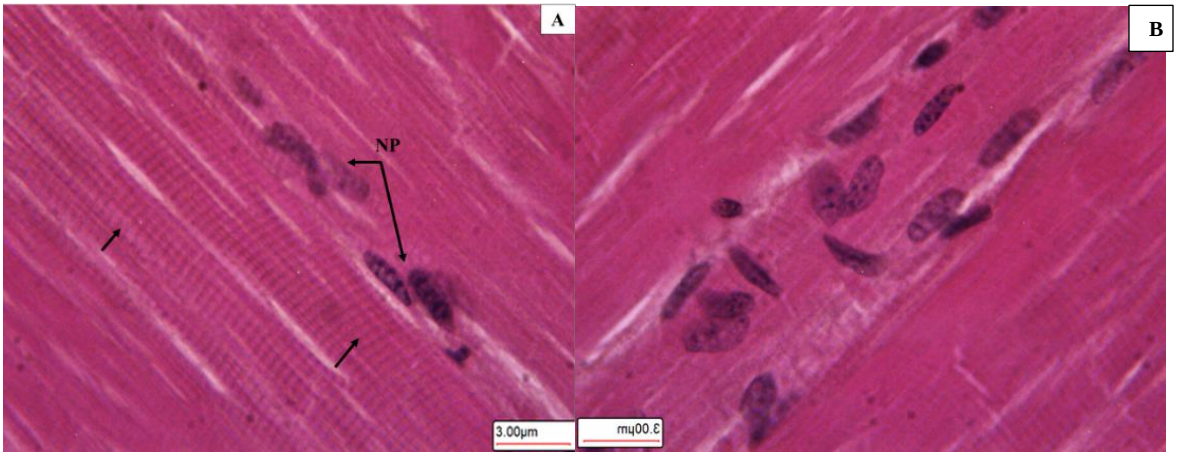

Figure 4 4 Image (A) Higher magnification of non-electrical stunning (A) of the *infraspinatus* muscle stained with haematoxylin and eosin (H&E), showing skeletal muscle with uniform muscle fibres (arrow), their nuclei (NP) are peripherally-located. Mag. 1000×
Image (B) electrical stunning of the *infraspinatus* muscle showing long muscle fibres with some nuclei located on the periphery (Np) and others in the centre of the muscle fibre (NC). In both images, the striated (arrow) muscle fibres consist of contractile fibrils made up of actin and myosin filaments. Mag. 1000×

Conclusions

The present study indicates that blood loss from goats subjected to slaughter without stunning is substantially lower than that from electrically stunned prior to slaughter. At 72 h *postmortem*, meat samples from LT, ST, SM, BF, TB, and IS muscles from electrically stunned goats had higher ($P < 0.05$) total aerobic counts of *E. coli* and *Salmonella* than those from non-electrically stunned animals. No significant differences in meat quality characteristics were detected between the two slaughter methods. The stained sections of the electrically-stunned muscle samples indicated alteration phenomena as some muscle fibres had split myofibers with central nuclei rather than peripheral nuclei. This study revealed that electrical stunning prior

to slaughter increased bacterial contamination, decreased blood loss, and altered the position of muscle nuclei.

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Conflict of Interest Declaration

All authors declare no conflict of interest for this article.

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