1 **TITLE PAGE**

ARTICLE INFORMATION	Fill in information in each box below
Article Title	**Effect of carcass electrical stimulation and suspension methods on meat quality characteristics of *longissimus lumborum* of young buffalo (*Bubalus bubalis*) bulls**
Running Title (within 10 words)	Effect of carcass stimulation and suspension on buffalo meat quality
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Abstract

Buffalo animals are slaughtered at their early age and carcasses are chilled rapidly which deteriorates its meat quality and decreases the consumer likeliness of buffalo meat. This study investigated the appropriate methods to prevent the quality deterioration of buffalo meat during chilling. Twenty four 18-month-old buffalo bulls were slaughtered, electrically stimulated and suspended either by hip or achilles tendon. After 24 h postmortem, meat quality characteristics were recorded. Results showed that electrical stimulation led to rapid decline of carcass pH compared to non-electrical stimulation method ($p<0.05$). Furthermore, electrically stimulated meat presented lower shear force accompanied with the higher CIE L*, a* and b* values ($p<0.05$). Suspension methods only affect the meat shear values and were lowered in hip suspended samples. It can be concluded that electrical stimulation combined with hip suspension can be adopted to prevent the meat quality deterioration of young buffalo bulls during postmortem storage.

Keywords: buffalo, carcass handling, meat quality, electrical stimulation, suspension methods

1. Introduction

Buffalo is one of the major meat producing animal in south-east Asia (Kandeepan et al., 2013). The compromised feeding, absence of specific meat breed combined with slaughtering of younger buffalo animals produces low muscle:bone ratio of buffalo carcasses in developing countries like Pakistan (Bilal et al., 2006; Purchas et al., 2002). However in commercial abattoirs, buffalo meat is rapidly chilled overnight and deboned at 24 h postmortem. Rapid chilling would benefit the industry by reducing the evaporative loss and growth of spoilage microbes. These benefits to the meat processor consequently, causing meat quality issues like cold toughening that affect the tenderness and color and decreases the consumer likeliness of buffalo meat (Kuffi et al., 2018; Locker et al., 1963). Numerous techniques have been used currently to avoid the development of cold toughening and to improve the meat tenderness. In this study, two methods have been tested to avoid this defect in buffalo.

Electrical stimulation (ES) minimized the detrimental effect of rapid chilling and improved the meat quality. ES causes faster depletion of adenosine-triphosphate (ATP), creatine phosphate (CP) and glycogen contents from postmortem muscles (Simmons et al., 2008). Therefore, ES avoids the cold toughening by accelerating the postmortem glycolysis and pH decline in postmortem muscles

(Simmons et al., 2008). Furthermore, electrical current causes physical disruption of the myolemma that results in release of calcium ions from sarcoplasmic reticulum. Calcium ions then activates the calpain system that lead to proteolytic breakdown of myofibrillar protein, which increases tenderness of the meat (Mota-Rojas et al., 2012). In addition, many studies have shown the role of electrical stimulation in improvement of beef color characteristics (McKenna et al., 2003).

Pelvic suspension (PS) technique also known as tenderstrech is the alternative method to avoid muscle shortening by hanging the carcass from obturator foramen of hip bone (Eikelenboom et al., 1998). Traditionally carcass is hanged using achilles suspension (AS) method. However in this method, vertebral column gets less stretch and become curved that causes shortening of muscles fiber and promotes cold toughening (Torrescano et al., 2003). However in PS method, sarcomere length of the muscles fiber is increased that helps to prevent the cold toughening. Many studies have reported the role of PS method in improvement of tenderness and water-holding capacity of meat (Ahnstrom et al., 2006; Wahlgren et al., 2002). Furthermore, suspension methods had different effect for each muscle type. Ahnstrom et al. (2012) studied the effect of different suspension methods on meat quality of five beef muscles and reported that tenderness of only two (*longissimus dorsi* and *gluteus medius)* muscles was improved by pelvic suspension of bull carcasses. Moreover, pelvic suspension increased the sarcomere lengths of *semimembranosus, longissimus dorsi*, *gluteus medius* and *adductor* muscles.

Previous studies examining the electrical stimulation and suspension method were conducted on cattle and lamb animals (Eikelenboom et al., 1998; Kuffi et al., 2018; Simmons et al., 2008; Toohey et al., 2008). However, the effect of electric stimulation combined with suspension method to prevent the meat quality deterioration during rapid chilling of young buffalo bull is not clear in the literature. Therefore, the objective of current study was to investigate the role of electric stimulation combined with suspension method to prevent the detrimental effect of rapid chilling of young buffalo bulls.

2. Materials and methods

2.1 Experimental design and slaughtering

A total of 24 water buffalo (*Bubalus bubalis*) young bulls were selected from Livestock Production and Research Institute Bahadurnagar, Okara, Pakistan, reared under same management conditions and feeding system. Animals were 18 months of age with an average carcass weight of 130 kg (SD = 10). All the animals were transported to the University of Veterinary and Animal Sciences, Lahore, Pakistan under same transportation conditions. Animals were kept in lairage facility for one day to minimize the transportation stress. To ensure that meat was processed hygienically, animals were kept off-feed for 12 h before the slaughtering. After recording the live weight, animals were slaughtered in the morning at University commercial slaughter house facility following the Halal slaughtering guidelines described in Pakistan Halal Standards PS3733.

2.2 Carcass treatments

Electrical stimulation (100 V with 60 Hz) was performed using low voltage electrical stimulator (Model BV-80 Low Voltage Beef Stimulator, Jarvis Products Corporation, Middletown, CT, USA) that was connected to the whole carcass for 30 s within 15 min of exsanguination. Twelve of the 24 selected carcasses were electrically stimulated and tagged while rest of twelve were kept un-stimulated. After that all carcasses were bisected, one side of each carcass was hanged with pelvic suspension method while another side was hanged by achilles suspension method in the walk-in chiller operating at 0-4℃. After overnight chilling, both halves of stimulated or un-stimulated carcasses were transferred into the deboning hall operating at 10-15℃. *Longissimus lumborum* (LL) muscle of every half-carcass was removed between 12^{th} thoracic and last lumbar vertebra at 24 h postmortem. From posterior end of LL muscles, three 2 cm steaks were removed to measure instrumental color. Then three 1 cm (with 50 g of weight) steaks were cut for moisture loss analysis. After that, three 3 cm thick steaks were separated for measurement of cooking loss and tenderness. All the meat quality attributes were measured in triplicate from both sides of stimulated or un-stimulated carcasses. A brief layout of experimental design was shown in supplementary Table S1.

2.3 Meat quality measurement

2.3.1 pH

The pH of the meat sample was measured with pH meter having meat penetrating probe (WTW, pH 3210 SET2, Germany) after calibration with buffers of pH 4.00 and 7.00. The pH was recorded

between 12th thoracic and the first lumbar vertebra at 0 (within 20 min of exsanguination i.e., right after electrical stimulation), 1, 3, 5, 7, 11, and 24 h postmortem.

2.3.2 Color

For color measurement, meat samples were placed in food-grade trays such that the muscle fibers had a perpendicular orientation to the exposed surface. The samples were overwrapped with oxygen-permeable film and displayed in horizontal chiller at 0-4℃ for 1 h of blooming. Then different parameters of color i.e., CIE L*(lightness), a* (redness), b* (yellowness) were recorded using colorimeter (Konica Minolta® CR-410, Osaka, Japan) from three random locations over the samples by avoiding the connective tissue and fat and averaged for statistical analysis. Before measurements, colorimeter was calibrated using the standard white tile CR-A44 at L*= 94.93, a*= -0.13, b*= 2.55 and C= 2.55. The color was measured at 1, 2, 3, 4, 5, 6 and 7 d postmortem.

2.3.3 Cooking loss (%)

For cooking loss, meat samples were weighed using portable weighing scale (SF-400, Yongkang Zhezhong™, Ningbo, China), vacuum packaged (Multivac® Baseline P-100, Geprüfte Scherhert, AGW, Germany) by using bags (SR 150×200, PA/PE 90, Dalziel®, Bellshill, Scotland) and placed in a water bath (WNB45, memmert®, Schwabach, Germany) working at 80℃. Samples were drawn out of the water bath when the core temperature of 72℃ was achieved by following the methods of Ijaz et al. (2020). After this samples were placed at room temperature (20℃) for 45 min and then patted dry with a hand towel and reweighed to calculate the cooking loss. The cooking loss was calculated using the following formula:

$$\text{Cooking loss (\%)} = \frac{(\text{Weight befor cooking - weight after cooking})}{\text{Weight before cooking}} \times 100$$

2.3.4 Tenderness

The cooked meat samples were cut down into cubes of 1 cm × 1 cm × 6 cm along the direction of muscles fiber using scalpel handle blades. Warner-bratzler shear force (WBSF) values were measured by shearing the cubed under V- Slot blade of Texture Analyzer (TA.XT plus® texture analyzer, Godalming, UK). Before measurement, Texture Analyzer was calibrated with 1 kg weight, at 50 mm distance of return, with 10 mm/s speed of return and an 8 g contact force. The WBSF values were measured in Newton (N/cm^2) as the peak force needed to shear the cubes perpendicular to direction of muscle fibers. WBSF values were taken from at least three cubes and averaged to calculate the tenderness of the samples.

2.3.5 Moisture loss

Meat moisture loss was measured using suspension technique by following the methods of Kim et al. (2015). Samples were weighed and hung in polystyrene bags in display chiller (ALVO, MD-12, Technosight®, Lahore, Pakistan) for 48 h at 4℃. After this samples were blotted dry using a paper towel and reweighed again to measure the moisture loss. The moisture loss was calculated using the following formula:

$$\text{Moisture loss (\%)} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100$$

2.4 Statistical Analysis

Statistical analysis was carried out using Statistical Analysis System (SAS) ver. 9.1 (SAS Institute Inc., Cary, NC, USA). Data were analyzed using MIXED procedure with electrical stimulation, suspension method and their interactions as fixed effects and animal as random effect. The level of significance was calculated using Duncan's Multiple Range test and $p<0.05$ was considered significant. The data were presented as means ± standard error.

3. Results

3.1 Rate of pH decline

The decline of pH of young buffalo carcasses treated with different electric stimulation and suspension methods is shown in Figure 1. Results indicated that ES of buffalo calves exhibited rapid pH decline compared to NS carcasses ($p<0.05$). However, there was no any difference of pH decline between achilles suspension and hip suspension methods ($p>0.05$). Interaction effects of electrical stimulation and suspension methods on rate of pH decline are presented in Figure 2. It showed that rate of pH decline of electrical stimulation combined with achilles suspension (ES+AS) was same with the electrical stimulation combined with hip suspension (ES+HS), however, higher than that of the non-stimulation combined with achilles (NS+AS) and hip suspension (NS+HS) methods ($p<0.05$). Overall, results showed that electrical stimulation had strong effect on rate of pH decline compared to suspension method.

3.2 Shear force values of meat

Meat shear force values of electrical stimulation and suspension methods are shown in Table 1. ES carcasses displayed significantly ($p<0.05$) lower shear force value compared to the NS carcasses. Meat shear force value of HS method were significantly ($p<0.05$) lower as compared to AS method. Shear force showed significant interaction ($p<0.05$) between electrical stimulation and suspension methods and their interactions are further explored. Interestingly, shear force values of electrical stimulation together with hip suspension method (ES+HS) were lowest (33.06), however, non-stimulated along with achilles suspension (NS+AS) produced highest (40.86) shear force values ($p<0.05$).

3.3 Water-holding capacity

Meat cooking and moisture losses of electrical stimulation and suspension methods are shown in Table 1. Results indicated that cooking loss as well as moisture loss were non-significant ($p>0.05$)

between stimulation method and suspension method. Similarly, interactions of stimulation and suspension methods were also non-significant ($p>0.05$).

3.4 Meat color

Meat color parameters of electrical stimulation and suspension methods are shown in Table 1. Results revealed that electrical stimulation significantly ($p<0.05$) increases the color CIE L* (lightness), a* (redness) and b* (yellowness) values as compared to non-stimulated meat. Whereas, color L*, a* and b* values were similar between achilles and hip suspension methods ($p>0.05$). The interactions of electrical stimulation with achilles suspension and hip suspension were non-significant, similarly, interactions of non-electrical stimulation with suspension methods were also similar ($p>0.05$) for all color parameters (L*, a* and b*). However, interactions of electrical stimulation with suspension methods presented significatly ($p<0.05$) higher L*, a* and b* values as compared to the interactions of non-electrical stimulation with the supension methods. It showed that electrical stimulation has substantial effect on meat color than that of the suspension methods. The results of stimulation and suspension methods and their interaction on 2, 3, 4, 5, 6 and 7 d postmortem were non-significant for color CIE L*, a* and b* and presented in supplemetary Table S2.

4. Discussion

Present study explained that electrically stimulated carcasses showed rapid pH decline as compared to non-stimulated carcasses, as a result of this cold shortening of the young buffalo meat can be avoided (Davey et al., 1976). These results were similar with the findings of Cross (1979) and Honikel et al. (1983). This may be due to the fact electrical stimulation causes faster depletion of ATP, CP and glycogen from muscles by accelerating the postmortem glycolysis which leads to the rapid pH decline in postmortem muscles fibers (Simmons et al., 2008). When the carcass is

electrically stimulated, ATP level is depleted, which is required for the contraction of muscle structure so severe contraction of muscle or cold shortening is avoided, as a result of this tenderness of meat is enhanced (Dutson et al., 1980). On other the hand, rate of pH fall of achilles and hip suspension methods were same. Ahnstrom et al. (2012) and Hou et al. (2014) explained the same results in their study that suspension methods did not affect the pH value.

WBSF represents the tenderness of meat, higher the WBSF values lower will be th tenderness of meat. Electrical stimulation enhances the tenderness of meat by significantly reducing the shear force values. Similar findings were also found by Aalhus et al. (1994) and Simmons et al. (2008), they noted the lower shear force value of electrically stimulated compared to non-stimulated carcasses. Geesink et al. (2006) explained that electrical stimulation enhances the tenderness of meat by accelerating the postmortem proteolysis. The acceleration in postmortem proteolysis is primarly due to the increased activity of μ- and m-calpain. Electrical stimulation increases intracellular calcium level, which is required for initiating the proteolytic activity of calpain system, especially μ-calpain. Therefore, electrical stimulation enhances the tenderness of meat by accelerating the degradation of myofibrillar and cytoskeleton structure (titin, nebulin and desmin), which are responsible for structural integrity of myofibril lattice (Soria & Corva, 2004). Furthermore, electrical stimulation increases the physical disruption of cells and helps the release of lysosomal proteases like proteolytic cathepsins and calpains into the cytosol, which again favor the enhancement of meat tenderness (Dutson et al., 1980). Additionaly, electrical stimulation leads to rapid pH decline and helps to prevent the determinental effect of cold toughening. On the other hand, hip suspension significantly lowers the shear force value of the carcasses. These findings were also reported in the literature (Bayraktaroglu & Kahraman, 2011; Wahlgren et al., 2002). Ahnstrom et al. (2012) explained in his study that hip suspension improves the tenderness of meat

about 15-40%. Stretching during hip suspension method results in the reduction of adhesion between myofilaments and decrease connective tissue strength, so shear force value is decreased (Liu et al., 2016).

Electrical stimulation did not show any effect on cooking and moisture losses. These observations were also found in previous studies (Derbyshire et al., 2007; Strydom et al., 2005). Electrical stimulation induced fast pH decline and earlier activation of proteolytic enzymes in postmortem muscles. The fast pH decline accelerated the reduction in net negative ions and lactate ions (CH_3CHOO^-) act as anionic chaotrope that would weaken the interaction between proteins and water molecules (Fujita et al., 2007; Li et al., 2011). Moreover, the establishment of actomyosin bond during rigor development could decrease the space between myofilaments (Offer et al., 1992). All these processes could favor the decrease of water-holding capacity in the postmortem muscles. In contrast, early activation of proteolytic enzymes could degrade the myofibrillar proteins that could help to increase the space between myofilaments to hold the water in myofibres (Huff-Lonergan et al., 2005). As a result, the overall effect of electrical stimulation on water-holding capacity of buffalo meat remained negligible. Similarly, hip suspension method had no effect on cooking and moisture losses that was supported by Ahnstrom et al. (2012) and Strydom et al. (2005). Derbyshire et al. (2007) explained that suspension methods do not affect the meat losses, because the rate of pH fall and proteolysis remained the same in hip and achilles suspension methods.

Electrical stimulation increased the color L*, a* and b* values of the meat as compared to non-stimulated meat. Similar findings were also reported by Li et al. (2011) and Toohey et al. (2008). Nazli et al. (2010) revealed that electrical stimulation leads to rapid acidification and denaturation of myofibrillar proteins, both result in more reflectance of light from the meat surface, which

increased the color lightness (L*) of meat. Higher rate of postmortem proteolysis in electrically stimulated meat lead to weakening of ultra-structure of myofibers that adversely affect the actomyosin bond and allows the oxygen to penetrate deeper into the muscles, which produced a thick layer of oxymyoglobin and increased the color redness (a*) value (Toohey et al., 2008). Conversely, meat color was not affected by the suspension methods. Color is primarily depends upon rate of pH decline and protein degradation, which remained same between hip and achilles suspension methods (Bayraktaroglu & Kahraman, 2011). In the current study, electrical stimulation and suspension methods did not affect the color parameters during 2 to 7 days of postmortem storage of buffalo meat. Li et al. (2011) explored the effect of low-voltage ES on color stability of bovine muscles and reported that ES increased the color a* values at 24 h postmortem but it did not affect the color stability, which is in agreement with the current study. On the other hand, Hou et al. (2014) studied the impact of suspension methods and ageing time on meat quality of beef. They reported that color L*, a* and b* values at 1 day were similar with 7 day postmortem and suspension methods did not show any significant effect on color stability during first 7 days of postmortem storage.

5. Conclusions

The results of this study showed that electrical stimulation increased the rate of pH decline, improved the tenderness and color of buffalo meat. Furthermore, hip suspension had no impact on pH, water-holding capacity and color of meat, however, it increased the tenderness. It is recommended that the local meat industry should adopt such post-slaughter technologies i.e., electrical stimulation in combination with hip suspension to improve the meat quality and to prevent the detrimental effects of postmortem chilling of young buffalo bulls.

Conflict interest

The authors declare no potential conflict of interest.

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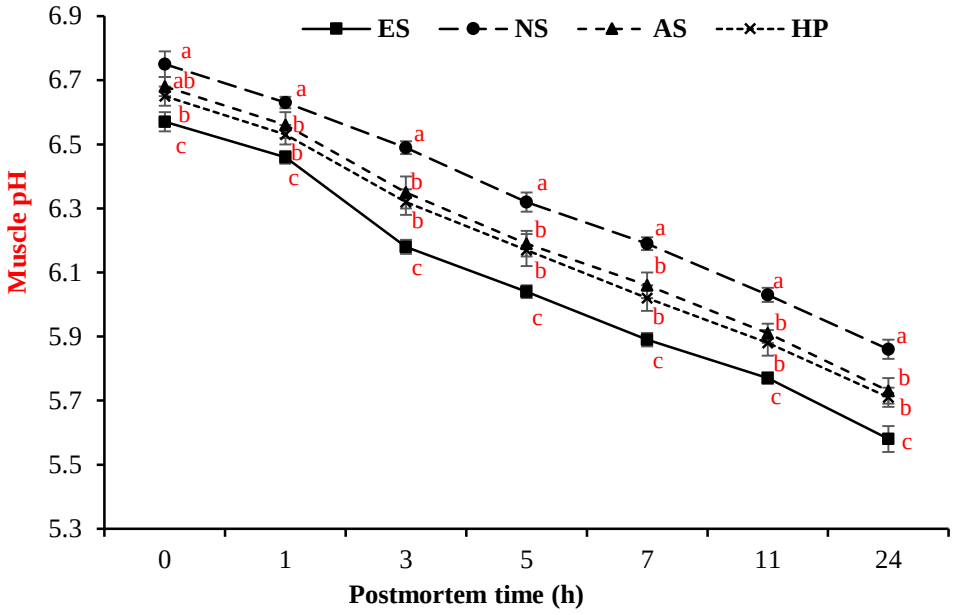
Figure legends

Fig. 1. Effects of carcass electrical stimulation (ES: electrically stimulated; NS: non-stimulated) and suspension methods (AS: achilles suspension; HS: hip suspension) on rate of pH decline of *longissimus lumborum* of young buffalo bulls during different postmortem time. a-c: different superscripts are indicating significant difference ($p<0.05$) between ES and NS. Values are expressed as means ± standard error.

Fig. 2. Interaction effects of electrical stimulation and suspension methods on rate of pH decline of *longissimus lumborum* of young buffalo bulls during different postmortem time. ESAS: electrically stimulated + achilles suspension; ESHS: electrically stimulated + hip suspension; NSAS: non-stimulated + achilles suspension; NS/HS: non-Stimulated + hip suspension. a-b: different superscripts are indicate significant difference ($p<0.05$) between treatments. Values are expressed as means ± standard error.

343 **Fig. 1.**



344 **Fig. 2.**

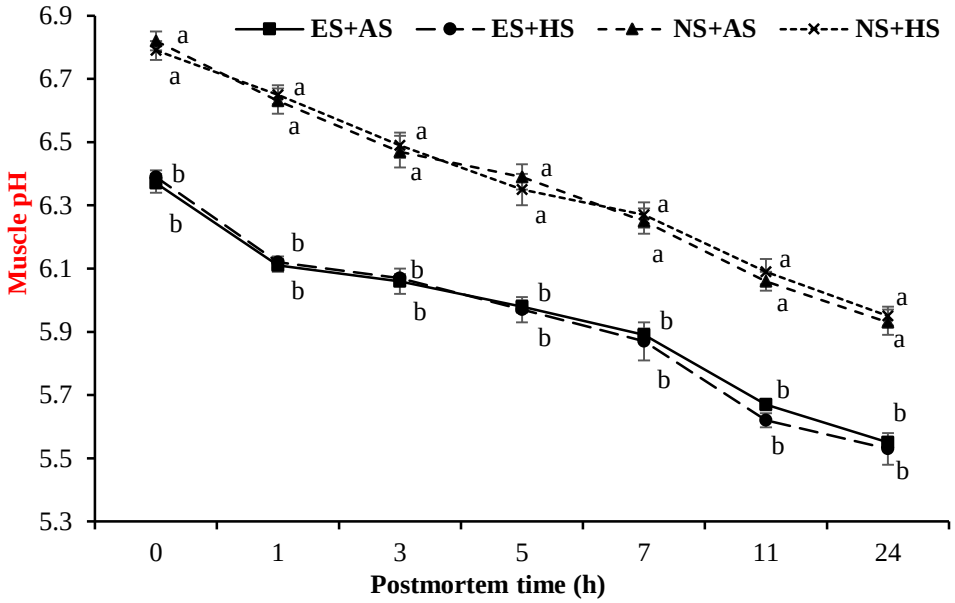


Table 1. Main and interaction effects of electrical stimulation and different suspension methods of carcasses on meat shear force, cooking loss, moisture loss and color parameters (CIE L*, a* and b*) of *longissimus lumborum* of young buffalo bulls at 24 h postmortem.

Parameters	Main effect				Interaction effect			
	Stimulation method		Suspension method		ES		NS	
	ES	NS	AS	HS	AS	HS	AS	HS
Shear force (N/cm^2)	34.65^{b}± 0.34	39.46^{a}± 0.30	38.55^{a}±0.9	35.56^{b}±0.52	36.24^{c}± 0.14	33.06^{d}± 0.06	40.86^{a}± 0.11	38.05^{b}± 0.09
Cooking loss (%)	29.02±0.15	29.07± 0.14	29.24±0.11	28.85±0.16	29.27±0.14	28.77±0.24	29.20± 0.18	28.93± 0.20
Moisture loss (%)	4.33±0.30	4.37±0.25	4.44±0.27	4.27±0.27	4.18±0.39	4.49±0.46	4.69±0.39	4.05±0.20
L* (lightness)	51.32 ^{a}±0.12	47.75^{b}± 0.15	49.45±0.40	49.62±0.39	51.24^{a}± 0.18	51.40^{a}± 0.17	47.66^{b}± 0.22	47.87^{b}± 0.20
a* (redness)	20.33^{a}± 0.08	17.67^{b}± 0.06	18.96±0.29	19.04±0.29	20.28^{a}± 0.12	20.38^{a}± 0.09	17.63^{b}± 0.08	17.70^{b}± 0.10
b* (yellowness)	10.09^{a}± 0.08	7.53^{b}±0.05	8.85±0.27	8.77±0.28	10.12^{a}± 0.10	10.07^{a}± 0.14	7.59^{b}±0.06	7.47^{b}±0.09

a-d: different alphabets as superscripts within a row indicate significant difference ($p<0.05$) between treatments. ES: electrically stimulated; NS: non-stimulated; AS: achilles suspension; HS: hip suspension. Values are expressed as means ± standard error.

Table S1. A brief layout of experimental design showing the use of electric stimulation and carcass suspension methods on young buffalo bulls carcasses.

Animal Species	Use of Electric Stimulation	Carcass cutting	Carcass Suspension Methods
Buffalo bulls n=24	Electric stimulation (whole carcass) n=12	Cut into two halves	Hip suspension method (one half of the carcass)
			Achilles suspension (another half of the carcass)
	No electric Stimulation (whole carcass) n=12	Cut into two halves	Hip suspension method (one half of the carcass)
			Achilles suspension (another half of the carcass)

Table S2. Main and interaction effects of electrical stimulation and different suspension methods meat color CIE L*, a* and b* of *longissimus lumborum* of young buffalo bulls at different postmortem times.

Postmortem days	Main effect				Interaction effect			
	Stimulation method		Suspension method		ES		NS	
	ES	NS	AS	HS	AS	HS	AS	HS
				L*				
Day 2	47.3 ±0.13	47.18 ±0.14	47.28 ±0.13	47.22 ±0.14	47.42±0.21	47.24±0.17	47.15 ±0.17	47.20 ±0.23
Day 3	46.58±0.19	46.37±0.23	46.42±0.18	46.53 ±0.23	46.55 ±0.29	46.62±0.25	46.30±0.23	46.45±0.40
Day 4	45.82±0.15	45.77 ±0.16	45.81 ±0.15	45.79 ±0.17	45.75 ±0.21	45.9 ±0.23	45.86 ±0.22	45.67±0.25
Day 5	45.46±0.08	45.06±0.14	45.25 ±0.13	45.28 ±0.11	45.39±0.10	45.53±0.11	45.11±0.23	45.02±0.17
Day 6	44.7 ±0.17	44.59 ±0.15	44.66 ±0.16	44.65 ±0.16	44.69±0.26	44.74±0.23	44.62±0.21	44.56±0.22
Day 7	43.7 ±0.16	43.48 ±0.18	43.63 ±0.16	43.59 ±0.18	43.84±0.18	43.64±0.26	43.42±0.25	43.55±0.26
				a*				
Day 2	15.89±0.05	15.76±0.04	15.84±0.05	15.81±0.05	15.92±0.07	15.86±0.08	15.76±0.06	15.76±0.07
Day 3	14.83±0.07	14.82±0.05	14.79±0.06	14.86±0.07	14.76±0.05	14.91±0.13	14.82±0.10	14.81±0.05
Day 4	13.67±0.04	13.74±0.05	13.71±0.03	13.71±0.05	13.71±0.04	13.63±0.06	13.70±0.05	13.78±0.09
Day 5	13.19±0.06	13.28±0.07	13.22±0.05	13.24±0.08	13.13±0.04	13.24±0.11	13.31±0.09	13.24±0.11
Day 6	12.64±0.05	12.74±0.03	12.67±0.05	12.72±0.03	12.60±0.08	12.67±0.05	12.73±0.04	12.76±0.05
Day 7	11.33±0.14	11.34±0.09	11.32±0.11	11.35±0.13	11.25±0.18	11.41±0.22	11.38±0.12	11.29±0.13
				b*				
Day 2	6.90±0.12	6.68±0.12	6.79±0.13	6.79±0.11	6.92±0.16	6.88±0.18	6.65±0.20	6.71±0.13
Day 3	6.44±0.02	6.40±0.07	6.42±0.06	6.42±0.04	6.49±0.04	6.40±0.03	6.34±0.12	6.45±0.08
Day 4	6.28±0.02	6.36±0.04	6.29±0.04	6.35±0.03	6.25±0.03	6.32±0.03	6.33±0.06	6.39±0.06
Day 5	5.74±0.10	5.58±0.10	5.66±0.10	5.56±0.10	5.76±0.14	5.73±0.16	5.56±0.14	5.60±0.14
Day 6	4.81±0.02	4.80±0.04	4.84±0.03	4.77±0.03	4.85±0.03	4.76±0.03	4.82±0.06	4.77±0.05
Day 7	4.15±0.05	4.19±0.09	4.21±0.07	4.13±0.07	4.14±0.07	4.16±0.06	4.28±0.12	4.10±0.13

ES: electrically stimulated; NS: non-stimulated; AS: achilles suspension; HS: hip suspension. Values are expressed as means ± standard error.