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RESEARCH ARTICLE

Effects of Electrical Stimulation on Quality and Microstructure of Rapid Chilled Beef Carcasses [1]

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Summary

A total of 15 beef were used to examine the effects of rapid chilling (RC) and electrical stimulation (ES) on meat quality and microstructure of *longissimus dorsi thoracis* (LDT). After slaughter, the right sides of carcasses were randomly assigned ES (500 V, 50 Hz, and 120 s), while the left sides remained as control (NES). Then, carcasses were rapidly chilled (-20±1°C) for 6 h and placed in a conventional chiller (2±1°C). Meat quality was evaluated by water holding capacity (WHC), cooking loss (CL), color (L*, a*, b*), shear force (SF) and sarcomere length (SL). As a result, ES had no impact on WHC, CL and color of LDT from rapid chilled carcasses ($P<0.05$). Tenderness and SL values were significantly increased in stimulated carcasses at 2, 7 and 10 d postmortem ($P<0.001$). Also, microstructure examination of LDT demonstrated that amorphous appearance of the myofibres was found in ES due to the rupture of M-lines and I-bands. The results showed that ES is a useful method for improving tenderness of LDT during storage time and the disadvantageous effect of RC on WHC, CL, and color was equalized.

Keywords: *Meat quality, Sarcomere length, Tenderness, Ultrastructure*

Elektrik Stimülasyonunun Şok Soğutma Uygulanmış Sığır Karkas Kalitesi ve Mikroyapısı Üzerine Etkileri

Özet

Bu çalışmada, şok soğutma (RC) ve elektrik stimülasyonunun (ES), sığır karkaslarından (15 adet) elde edilen *longissimus dorsi thoracis* (LDT) kasının kalitesi ve mikroyapısı üzerine olan etkileri araştırılmıştır. Kesim sonrası, karkaslar ikiye ayrıldıktan sonra sağ yarımlara ES (500 V, 50 Hz, 120 s) uygulaması yapılmıştır. Sol taraf ise araştırmada kontrol grubunu oluşturmuştur (NES). Karkaslar, şok soğutmada (-20±1°C) 6 saat tutulduktan sonra 18 saat soğuk depoda (2±1°C) saklanmıştır. Et kalitesinin belirlenmesi amacıyla LDT kasında su tutma kapasitesi (WHC), pişirme kaybı (CL), renk (L*, a*, b*), gerilme kuvveti (SF) ve sarkomer uzunluğu (SL) değerleri incelenmiştir. Sonuç olarak, ES uygulamasının WHC, CL ve renk değerleri üzerine istatistiksel anlamda önemli bir etkisi görülmemiştir ($P<0.05$). İki, 7 ve 10. günlerde stimüle edilmiş etlerin 2, 7 ve 10. günlerde daha düşük SF ve yüksek SL değerlerine sahip olduğu tespit edilmiştir ($P<0.001$). Ayrıca, M ve I bantlarında kırılmalar nedeniyle LDT kasındaki miyofibrillerin yapısında düzensizlikler belirlenmiştir. Bunlara göre, ES ile sığır etlerinin gevrekliği belirgin şekilde gelişmiştir. Şok soğutmanın WHC, CL ve renk değerleri üzerine olumsuz etkisi giderilmiştir.

Anahtar sözcükler: *Et kalitesi, Sarkomer uzunluğu, Gevreklik, Mikroyapı*

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INTRODUCTION

Variations in meat tenderness are a general concern to the meat industry worldwide [1]. The conditions for the onset and development of rigor have a profound influence on tenderness of meat. First 24 h postmortem, the rate of temperature decline affects the biochemical and structure changes during the conversion of muscle to meat [2]. The efficacy of temperature and pH on tenderization depends on the carcass chilling rate. Today different applications of chilling processes are used to reduce the problems associated with temperature/pH relationships [3].

Carcass chilling is perhaps the most costly procedure in the meat industry [4]. Regarding to the food safety concern and financial advantages, rapid chilling (RC) appears more applicable system; because of producing low level of carcass contamination, extending the shelf life and reducing the evaporative loss of meat [5]. However, application of RC has a risk of producing tough meat with a high shear force caused by cold-induced shortening [6].

Electrical stimulation (ES) is an application that decreases the risk of cold shortening by the depletion of the energy reserves in the muscle [7]. ES involves transmitting an electrical current through the carcasses of freshly slaughtered animals. This electrical current accelerates post-mortem glycolysis and causes pH decline by the depletion of the energy reserves in the muscle [8]. ES has received considerable attention for improving the meat quality; including tenderness, color and palatability attributes [9]. But, there has been little information available in the literature regarding the effects on ultrastructure. The present study was aimed to investigate the effects of rigor temperature individually or combined with ES on the quality and microstructure of beef carcasses.

MATERIAL and METHODS

The research protocol of the current study was approved by the Ethic Committee of the Istanbul University Veterinary Faculty (Approval number: 2008/151).

The 15 beef of same breed (Holstein x Friesian), averaging 2-3 years age and with mean live weight of 540 kg at slaughter were procured from Aytac Feed and Breathing plant. The animals were transported to the slaughterhouse for 1 day before slaughter. After a rest for 22-24 h, beef were slaughtered by Halal method and then carcasses were halved by splitting through the vertebral column within approximately 40 min postmortem. The right sides of carcasses were randomly assigned ES (500 V, 50 Hz, and 120 s), while the left sides remained as control (NES). Then, carcasses were rapidly chilled (-20±1°C; wind velocity, 2 m/s) for 6 h and placed in a conventional chiller (air temperature, 2±1°C; wind velocity, 1 m/s) for 18 h. At 24 h postmortem, *longissimus dorsi thoracis* (LDT) was removed from the carcass and the three portions were obtained. Portions were vacuum packaged in Cryovac barrier bags and stored at 0-4ºC for up to 10 days post-mortem before the evaluating the water holding capacity (WHC), cooking loss (CL), color (L*, a*, b*), shear force (SF) and sarcomere length (SL).

The pH were monitored in the deep portion of LDT at approximately 1, 3, 6, 8, 12, 18 and 24 h postmortem using a portable pH meter (Hanna HI 8314). The pH value was the average of five measurements of different locations within each sample. The pH meter was calibrated inserting the probe into the pH 4.0 and 7.0 buffers.

The percentage of free liquid was evaluated as a measure of WHC by the filter press method [10]. The outline area of the expressible juice and the meat film traced and two areas were measured. CL was calculated from the weight of samples taken before and after cooking. After measurements, the same samples were used for determination of texture parameters.

Meat color was measured using a Color Flex Hunter Lab Color Measurement System (Hunter Associates Laboratory Inc.). Color coordinates values as L* lightness, a* redness, b* yellowness values were recorded. Before each measurement, the apparatus was calibrated using a white, black and reference standard respectively. Color values were obtained considering the average of five readings, performed in different location of the surface [11].

SF of chops was determined by measuring the force required to shear through a cooked sample. Samples were cooked individually in a 100ºC water bath until an internal temperature of 75ºC was reached. The cooked samples were stored in a refrigerator overnight and the pieces were removed parallel to the muscle fiber. The pieces were sheared by a Warner-Bratzler shear attachment mounted on an Instron with a 50 kg load transducer and crosshead speed of 200 mm/min. An average of five sub-samples was accepted to be SF value of that sample.

SL was determined using phase contrast microscopy (Olympus CX41) method [12]. The mean SL of each muscle was determined by measuring 25 myofibrils containing four sarcomeres each.

Tissue samples of LDT were prefixed in glutaraldehyde-paraformaldehyde (pH 7.4) and subsequently fixed in 1% osmic acid solution for 2 h. After the second fixation, tissue samples were maintained in 1% uranyl acetate for 2 h, dehydrated through an ascending series of graded alcohols, propylene oxide and embedded in Araldite M. The semi-thin (1 µm) from blocks were stained with toluidine blue and 300-400 Angstrom thickness were cut [13]. These sections were contrast stained and were examined under a Carl Zeiss EM 9S-2 transmission electron microscope (Zeiss Oberkochen, Germany) [14].

The results of pH, WHC, CL, color, SF and SL parameters from NES and ES were subjected to analysis of variance for repeated measures of ANOVA [15]. The model used in the statistical analyses included analyze time as a within subject factor and groups as a between subject factor.

RESULTS

Changes of the pH value obtained from rapid chilled carcasses are given in *Table 1*. According to the results, it was found that only mean pH_1 of ES were significantly lower than the NES ($P<0.001$).

The effect of ES on WHC, CL, color parameters, SF and SL are presented in *Table 2*. ES had no impact on WHC, CL and color parameters of LDT from chilled carcasses after vacuum packaging ($P>0.05$). SF values were significantly lower in stimulated carcasses and differences were statistically significant ($P<0.001$). During the storage period, SF decreased for both groups ($P<0.001$). ES influences the length of sarcomeres and longer values has been detected ($P<0.001$).

Electron microscopy showed that the ultrastructure of LDT was changed after ES *(Fig. 1)*. The structural disruption of muscle was detected in stimulated carcasses firstly at 1 h postmortem. Amorphous appearance of the myofibres was found in ES due to the rupture of M-lines (arrows) and Z-bands (arrowheads). In the NES group, the A-bands, I-bands, Z- bands (arrowheads) and the mitochondrial membrane structure were intact and the M-lines (arrows) were visible compared to ES. At 7 d post-mortem, M-lines were invisible; however, Z bands (arrowheads) were discontinuously visible in stimulated sides. On the other hand, bands of myofibrils detected in NES at 7 d were lighter than 2 d. As the ageing time increases, myoflaments of NES and ES were more irregular due to ruptures at the connection sides of myofibrils.

DISCUSSION

The pH values for both ES and NES decreased from 7.02 to 5.61 from 1 to 24 h postmortem ($P<0.001$). Similar results in pH were reported by Li et al.[6] and Zhang et al.[16]. At the end of the chilling process (24 h), no significant

Table 1. *Effect of ES on pH value of rapid chilled carcasses*
Tablo 1. *Şok soğutma uygulanmış karkasların pH değerleri üzerine ES'nin etkisi*

Time of Postmortem	Temperature	NES	ES	Significance		
				Group	Time	Group x Time
1 h	37.17˚C	7.02±0.03^{x}	6.83±0.03^{y}	***	***	***
3 h	24.95˚C	6.48±0.01	6.47±0.01	NS	***	NS
6 h	19.55˚C	6.23±0.01	6.23±0.01	NS	***	NS
12 h	12.60˚C	6.10±0.01	6.09±0.01	NS	***	NS
18 h	9.37˚C	5.80±0.03	5.80±0.03	NS	***	NS
24 h	1.90˚C	5.60±0.01	5.61±0.01	NS	***	NS

NES: *Non-Electrical Stimulated;* ***ES:*** *Electrical Stimulated*
NS: *Not significant (P>0.05)*
x, y : *Means with different letters in a same row are significantly different between stimulation groups (*: P < 0.05; ***: P<0.001)*

Table 2. *Effect of ES on WHC, CL, color, SF and SL of rapid chilled carcasses*
Tablo 2. *Şok soğutma uygulanmış karkasların WHC, CL, renk, SF ve SL değerleri üzerine ES'nin etkisi*

Parameter	Stimulation Group		Day			Significance		
	NES	ES	Day II	Day VII	Day X	Group	Time	Group x Time
WHC (%)	15.85±0.41	15.97±0.41	13.48±0.36^{c}	15.85±0.46^{b}	18.39±0.41^{a}	NS	***	NS
CL (%)	41.38±0.67	41.24±0.67	41.78±0.86^{ab}	42.19±0.56^{a}	39.96±0.41^{b}	NS	*	NS
L*	33.21±0.78	33.21±0.78	32.06±0.04^{b}	34.15±0.10^{a}	33.41±0.08^{ab}	NS	***	NS
a*	15.89±0.12	15.68±0.12	13.02±0.20^{c}	16.05±0.10^{b}	18.30±0.11^{a}	NS	***	NS
b*	15.83±0.09	15.84±0.09	12.36±0.10^{c}	16.51±0.10^{b}	18.64±0.14^{a}	NS	***	NS
SF (kg)	4.68±0.11^{x}	4.26±0.11^{y}	5.17±0.19^{a}	4.40±0.07^{b}	3.84±0.06^{c}	***	***	***
SL (µm)	1.35±0.01^{y}	1.41±0.01^{x}	1.32±0.01^{c}	1.39±0.01^{ab}	1.43±0.01^{a}	***	***	***

NES: *Non-Electrical Stimulated;* ***ES:*** *Electrical Stimulated;* ***WHC:*** *Water Holding Capacity;* ***CL:*** *Cooking Loss;* ***SF:*** *Shear Force;* ***SL:*** *Sarcomere Length*
NS: *Not significant (P>0.05)*
a, b, c, : *Means with different letters in a same row are significantly different between day values, (*: P<0.05; ***: P<0.001)*
x, y : *Means with different letters in a same row are significantly different between stimulation groups*

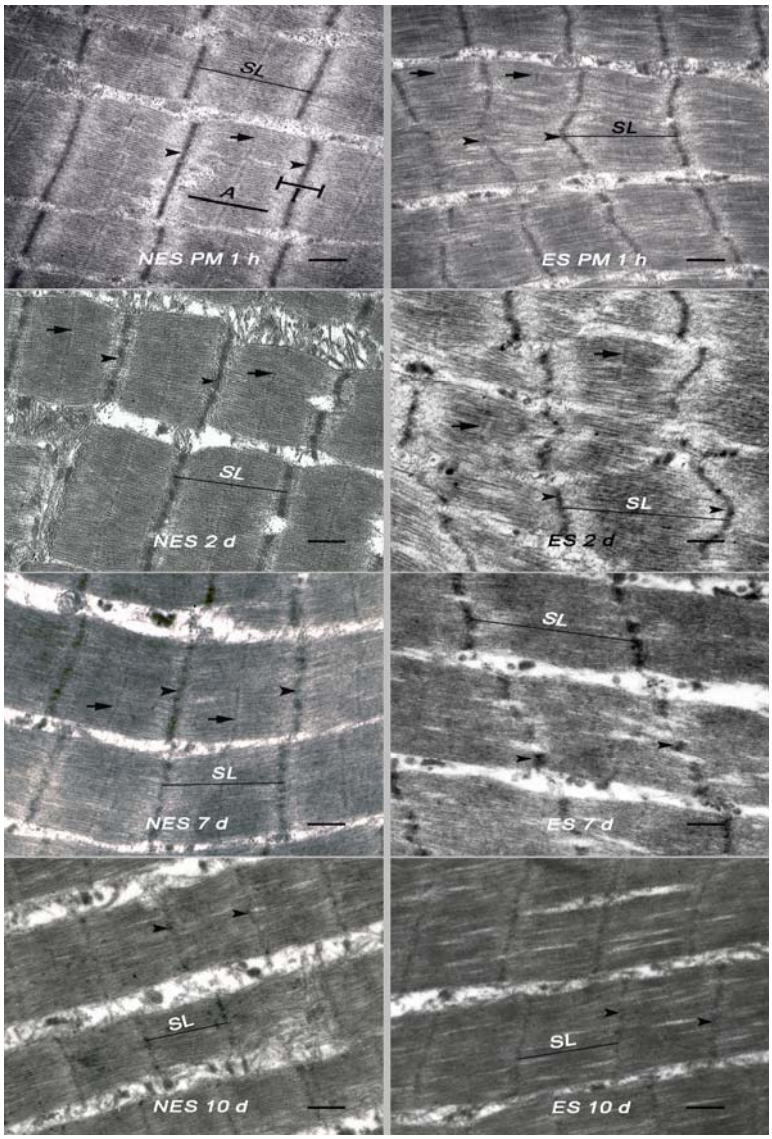

Fig 1. Electron microscopic appearance of LDT myofibrils. A: A band, SL: sarcomer length, Z band: arrowheads, M-line: arrows. Bar: 0.3 µm

Şekil 1. LDT miyofibrillerinin elektron mikroskobik görünümü. A: A bandı, SL: sarkomer uzunluğu, Z: okbaşları, M-çizgisi: oklar. Bar: 0.3 µm

differences were found between ES and NES groups about pH values (P>0.05). In this study, carcass temperature at 1 h was 37.17°C and muscle temperature showed the fastest drop at 3 h. Hannula and Pudanne [17] stated that the pH and temperature at the onset of rigor mortis could be important for beef tenderness. The temperature was above 16°C in carcasses, when muscle pH less than 6.20 is reached. It was implied that application of RC in this study had decreased the risk of cold shortening.

WHC and CL depend on the ultimate pH value [18]. The ultimate pH values obtained in NES and ES were 5.60 and 5.61, respectively (P>0.05). Theoretically, ES decrease WHC by increasing CL due to denaturation of hydrophilic proteins [9,10]. Similar to those was found by Li et al.[6] in beef carcasses. Also in this study, it was found that CL (P<0.05) and WHC values (P<0.001) were improved as the storage time increased. It can be explained by the temperature effect on postmortem energy metabolism and distribution of water in the muscles [19].

Initial color parameters were not affected by ES. This is in support to work of Zhang et al.[16] in pork carcasses. The similar color values belong to NES and ES could be due to the same rate of pH decline at rigor. In contrary, Aalhus et al.[4] and Janz et al.[9] reported that combination of blast chilling and ES treatments resulted in samples significantly lighter than controls. The differences can be related the muscle chilling temperature which influences the glycolytic rate and stimulation voltage. RC has been reported to decrease L* values [20], whereas ES is known to improve meat color [8]. The present study showed that the disadvantageous effect of RC on color was equalized by ES.

pH value at 1 h postmortem were the most useful indicator for beef tenderness [6]. Our results were supported by this aspect. However, Lowe et al.[21] found that the ultimate pH was a good predictor for meat tenderness. In another study by Aalhus et al.[4], the greatest SF values were observed when blast chilling was applied. On the other hand, Zhang et al.[16] reported that the combination

of ES and RC has no significant effect on tenderness. Differences may be due to variations in animal species and ES application.

The SF values have been found to correlate negatively to SL. This is validated by earlier studies [22,23]. In contrary, Savell et al.[24] reported that LDT of stimulated beef carcasses had similar SL to the NES. White et al.[25] and Olsson et al.[26] found that ES had no significant effect on SL. Applying ES to the whole carcass does not affect all muscles equally. Furthermore, SL increased with storage. The increased length may be caused by a combination of increased proteolysis and low pH induced swelling.

The microstructure images revealed that SL was determined to be damaged in ES during storage. The rate of degradation of myofibrils has also been suggested to cause the improvement in tenderness [27]. These alterations could be attributed to the proteolysis. The results were similar to the findings obtained by Bruce and Ball [21] and Luo et al.[28].

The results of this study showed that ES had no impact on WHC, CL and color of LDT from rapid chilled carcasses. Tenderness and SL values were significantly increased in stimulated carcasses. Also, microstructure examination of LDT demonstrated that amorphous appearance of the myofibres was found in ES due to the rupture of M-lines and I-bands.

In conclusion, ES is a useful method for improving tenderness of LDT during storage time. Also the disadvantageous effect of RC on WHC, CL, and color was equalized by ES.

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