Electron microscopy of contractile bands and quality characteristics in high-voltage electrical stimulation broiler breast meat

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ABSTRACT In this study, 20 broilers were used to examine the effect of high-voltage electrical stimulation (HVES) on meat quality and the microstructure of the pectoralis muscle. After slaughter, carcasses were randomly distributed into 2 treatment groups (n = 10). In the first group, carcasses were electrically stimulated (500 V, 100 Hz) for 60 s. Carcasses in the second group (nonelectrical stimulation) were used as a control. Meat quality was evaluated by the rate of pH, water-holding capacity, cooking loss, color (L*, a*, b*), shear force, and sarcomere length. As a result, HVES increased the rate of muscle pH decline ($P < 0.001$). High-voltage electrical stimulation had no effect on water-holding capacity and cooking loss values. Only L* (lightness) values were improved during the storage time ($P < 0.01$). Tenderness ($P < 0.001$) and sarcomere length ($P < 0.05$) values were significantly increased at 2 and 5 d postmortem. In addition, microstructure examination demonstrated that the stimulated muscles had longer sarcomeres; however, the A-, I-, and Z-bands and the mitochondrial membrane structure were intact in HVES and nonelectrically stimulated carcasses. The results showed that HVES is a useful method for improving the tenderness of broiler breast meat.

Key words: electrical stimulation, microstructure, sarcomere length, tenderness

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INTRODUCTION

Consumer acceptance of meat depends on quality characteristics such as tenderness, color, and palatability attributes, which are influenced by a series of factors, ranging from physical and chemical to histological properties and meat-processing procedures (Chen et al., 2007). Numerous techniques are currently used to improve meat quality postmortem. One of these applications is electrical stimulation (**ES**).

Electrical stimulation involves transmitting an electrical current through the carcasses of freshly slaughtered animals. The nerve and muscle systems react to the current, leading to physical and biochemical changes in the meat (Li et al., 1993). This electrical current accelerates postmortem glycolysis and causes pH decline by depleting the energy reserves in the muscle (Alvarado and Sams, 2000; Castañeda et al., 2005). In ES applications, voltage is the major parameter of the electric current. Because of the installation costs and safety for the operators, low-voltage ES (voltage <100 V) is frequently used in many countries instead of high-voltage ES (**HVES**; voltage >100 V). Researchers have reported that both low-voltage ES and HVES accelerate the development of rigor mortis (Owens and Sams, 1998); however, HVES is more effective for improving the quality of meat (Janz et al., 2001).

In poultry processing, ES has come to be used to reduce the toughness of meat that is deboned before the normal aging period because poultry carcasses enter rigor much more quickly and muscles are attached to the bone structure, which limits the ability of the muscles to contract (Sams, 1990). Some studies have been conducted on the effect of HVES on the quality of poultry carcasses (Thompson et al., 1987; Lyon et al., 1989; Sams et al., 1989; Birkhold et al., 1992), but the effects on the ultrastructure have not been extensively investigated. The aim of the present study was to investigate the effect of HVES on the meat quality and microstructure of the pectoralis muscle of broilers.

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MATERIALS AND METHODS

Birds and Experimental Design

Twenty broilers, 8 wk of age, that were fed and handled under the same management were obtained from a commercial processing facility. The birds were transported 10 km to the abattoir and allowed to rest for approximately 4 h. All birds were then suspended from overhead shackles and electrically stunned. After bleeding for 90 s, carcasses were randomly distributed into 2 treatment groups (n = 10). In the first group (HVES), carcasses were electrically stimulated (500 V, 100 Hz) for 60 s. Carcasses in the second group (NES) were used as a control. After stimulation, the birds were scalded at 56°C for 2 min, picked for 25 s, and mechanically eviscerated. After evisceration, all carcasses were immediately chilled at 0 to 4°C. At 24 h postmortem, the pectoralis muscle was removed from the carcass after measuring the ultimate pH ($\mathbf{pH_u}$) and 4 portions were obtained. The portions were vacuum-packaged in Cryovac barrier bags (Cryovac Sealed Air Corp., Istanbul, Turkey) and stored at 0 to 4°C for up to 7 d postmortem before evaluating the water-holding capacity (**WHC**), cooking loss (**CL**), color [L* (lightness), a* (redness), and b* (yellowness)], shear force (**SF**), sarcomere length (**SL**), and microstructure.

Meat Quality Measurements: pH Value

The pH was monitored in the deep portion of the pectoralis muscle at approximately 15 min and 24 h (pH_u) postmortem using a portable pH meter (Hanna HI 8314, Med Com, Kartal, Istanbul). The pH value was the average of 5 measurements at different locations within each sample. The pH meter was calibrated by inserting the probe into the pH 4.0 and 7.0 buffers.

WHC and CL

The percentage of free liquid was evaluated as a measure of WHC by the filter press method described by den Hertog-Meischke et al. (1997). The outline area of the expressible juice and the meat film traced, and 2 areas were measured using AUTOCAD (Vatan Computer, Avcilar, Istanbul). Cooking loss was was calculated from the weight of samples taken before and after cooking. After measurements, the same samples were used to determine the texture parameters.

Color

Meat color was measured using a Color Flex Hunter Lab Color Measurement System (Hunter Associates Laboratory Inc., Reston, VA). Color coordinate values were recorded as L, a*, and b* values. Before each measurement, the apparatus was calibrated using a white, black, and reference standard, respectively. Color values were obtained considering the average of 5 readings, performed in different locations on the surface (Hunt et al., 1991).

SF

Shear force of fillets was determined by measuring the force required to shear through a cooked sample. Samples were cooked individually in a 100°C water bath (NB20, Nüve, Findikzade, Turkey) until an internal temperature of 75°C was reached. The cooked samples were cooled at 45 to 50°C for 5 min, and the pieces (2.5 cm thick) 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 at a crosshead speed of 200 mm/min (Mega Danismanlik, Kadiköy, Turkey). The average of 5 subsamples was accepted to be the SF value of that sample.

SL

Sarcomere length was determined using phase contrast microscopy (Olympus CX41, Incekaralar, Uskudar, Turkey) method as described by Cross et al. (1981). The mean SL of each muscle was determined by measuring 25 myofibrils containing 4 sarcomeres each.

Transmission Electron Microscopy

Tissue samples of pectoralis muscles taken at 2, 5, and 7 d in both groups were prefixed in glutaraldehyde-paraformaldehyde (pH 7.4) for 24 h as described by Karnovsky (1965), 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 and propylene oxide, and embedded in Araldite M (Interlab, Hadimkoy, Turkey). The semithin (1 μm) sections from blocks were stained with toluidine blue and cut to a thickness of 300 to 400 Å. These sections were contrast stained as described by Venable and Coggeshall (1965) and were examined under a Zeiss EM 9S-2 transmission electron microscope (Carl Zeiss, Oberkochen, Germany).

Statistical Analysis

The results for pH, WHC, CL, color, SF, and SL parameters from HVES and NES were subjected to ANOVA for repeated measures by using SPSS software (SPSS, 1999). The model used in the statistical analyses included analysis time (d 2, 5, and 7) as a within-subjects factor and groups (HVES and NES) as a between-subjects factor. A contrast test was used to evaluate the significance of the difference. Each result represents the mean of 10 independent determinations.

Table 1. Effect of high-voltage electrical stimulation (HVES) on pH values of broiler breast meat

			Significance		
Muscle pH[1]	HVES (n = 10)	NES[2] (n = 10)	Group	Time	Group × time
pH_{15}	6.21 ± 0.03	6.35 ± 0.03	***	***	***
pH_u	5.82 ± 0.01	5.82 ± 0.01	NS	***	NS

[1]pH_{15} = pH at 15 min postmortem; pH_u = pH at 24 h postmortem (ultimate pH).
[2]NES = nonelectrical stimulation.
***$P < 0.001$; NS, $P > 0.05$.

RESULTS AND DISCUSSION

Changes in the pH value obtained from carcasses are given in Table 1. The results indicated that the mean pH of HVES carcasses at 15 minutes was significantly lower than the mean pH of NES carcasses ($P < 0.001$). High-voltage ES accelerates the breakdown of adenosine triphosphate and glycogen and causes a rapid decrease in pH. At 24 h postmortem, no significant differences in pH values ($P > 0.05$) were found between the HVES and NES groups. Similar results for pH were reported by Sams (1990) and Birkhold et al. (1992). The authors suggested that the values for pH_u were in the range of 5.7 to 6.0 for poultry carcasses (Schreurs, 2000; Santos et al., 2004). The results also showed that ES decreased pH faster in poultry than in beef and lamb. Alvarado and Sams (2000) stated that the red fibers were more sensitive to ES treatment.

The effect of HVES on WHC and CL are presented in Table 2. No statistically significant difference was found at 2, 5, or 7 d postmortem ($P > 0.05$), which indicated that HVES had no effect on the WHC and CL of the pectoralis muscle. This was validated by earlier studies (Alvarado and Sams, 2000; Castaneda et al., 2005). The results confirmed that WHC and CL depend on the pH_u value (Honikel, 1999). The pH_u values obtained in HVES and NES were 5.82. However, these results are inconsistent with the studies of Lyon et al. (1989) and Young et al. (2005) in poultry and those of Janz et al. (2001) and den Hertog-Meischke et al. (1997) in beef. Differences may be due to variations in animal species and ES application. Also in this study, we found that CL and WHC values were improved as the storage time increased. Water-holding capacity (more water expelled) decreased with storage in all groups. This can be explained by the temperature effect on postmortem energy metabolism and by the distribution of water in the muscles (Bertram et al., 2001). This was due to the increase in drip loss during storage, which resulted in free water available for expression (Bekhit et al., 2007).

The effect of HVES on color parameters is presented in Table 2. The pectoralis muscles of the HVES-treated sides were significantly brighter than those of the control sides ($P < 0.01$), but HVES application affected neither a* nor b* values ($P > 0.05$). Similar to those was found by Young et al. (2005) and Maki and Froning (1987), Warriss (2000) indicated that rapid muscle acidification by protein denaturation caused greater light reflectance on the meat surface. Bekhit et al. (2007) also reported that an accelerated pH decline at rigor resulted in the denaturation of muscle proteins and greater light reflection. In contrast, Owens and Sams (1998) found no differences between ES and NES. In another study, Sams and Dzuik (1999) found that stimulated samples were darker and redder than control samples.

Shear force values were lower in stimulated carcasses, and differences were statistically significant at 2 and 5 d postmortem ($P < 0.001$; Figure 1). During the storage period, SF decreased for both groups ($P < 0.001$). The results showed that the HVES treatment had a great effect on tenderness. This result was in agreement with those found in previous studies (Thompson et al., 1987; Sams et al., 1989; Birkhold and Sams 1993; Young et al., 2005). Shear force values were found to be significantly positively correlated with age of the birds,

Table 2. Effect of high-voltage electrical stimulation (HVES) on water-holding capacity (WHC), cooking loss (CL), and color parameters of broiler breast meat

	HVES (n = 10)			NES[2] (n = 10)			Significance		
Item[1]	d 2	d 5	d 7	d 2	d 5	d 7	Group	Time	Group × time
WHC (%)	14.02 ± 1.86	9.64 ± 0.58	9.61 ± 2.32	15.25 ± 2.12	10.36 ± 1.27	10.15 ± 3.50	NS	***	NS
CL (%)	23.22 ± 2.24	22.48 ± 3.18	22.31 ± 4.41	24.62 ± 3.16	23.52 ± 2.52	22.41 ± 3.74	NS	***	NS
L*	45.93 ± 1.21	46.71 ± 0.87	48.68 ± 0.96	45.47 ± 1.64	46.21 ± 1.42	48.24 ± 0.75	**	***	NS
a*	6.65 ± 0.14	6.81 ± 0.22	6.52 ± 0.25	6.29 ± 0.21	6.92 ± 0.19	6.52 ± 0.17	NS	***	NS
b*	16.45 ± 0.44	16.51 ± 0.51	16.83 ± 0.62	16.48 ± 0.90	16.73 ± 0.38	16.48 ± 0.72	NS	***	NS

[1]L* = lightness; a* = redness; b* = yellowess.
[2]NES = nonelectrical stimulation.
**$P < 0.01$; ***$P < 0.001$; NS, $P > 0.05$.

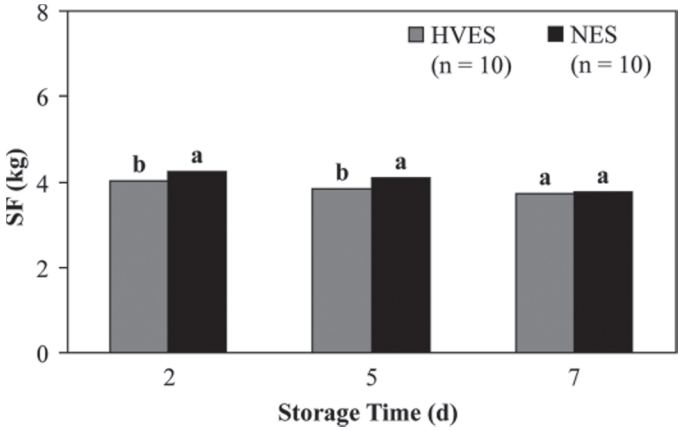

Figure 1. Effect of high-voltage electrical stimulation (HVES) on shear force (SF) values of broiler breast meat. NES = nonelectrical stimulation. Means with different letters (a, b) are significantly different ($P < 0.001$).

BW, breast weight, breast yield, and the diameter and area of myofibers and negatively correlated with myofiber density of the muscle (Chen et al., 2007). Dransfield (1994) reported that the differences in pH might be the cause of the lower SF values in the ES group. Li et al. (2010) showed that pH values at 1 h postmortem were the most useful indicators of tenderness ($P < 0.001$). Our results were supported in this aspect. However, Lowe et al. (2004) found that pH_u was a good predictor of meat tenderness.

The SL values of broiler breast meat are shown in Figure 2. High-voltage electrical stimulation influences the length of sarcomeres, and longer values have been detected at 2 and 5 d postmortem ($P < 0.05$). The average difference in SL between ES and NES was 0.18, 0.09, and 0.01 μm at 2, 5, and 7 d postmortem, respectively. These findings are in general agreement with

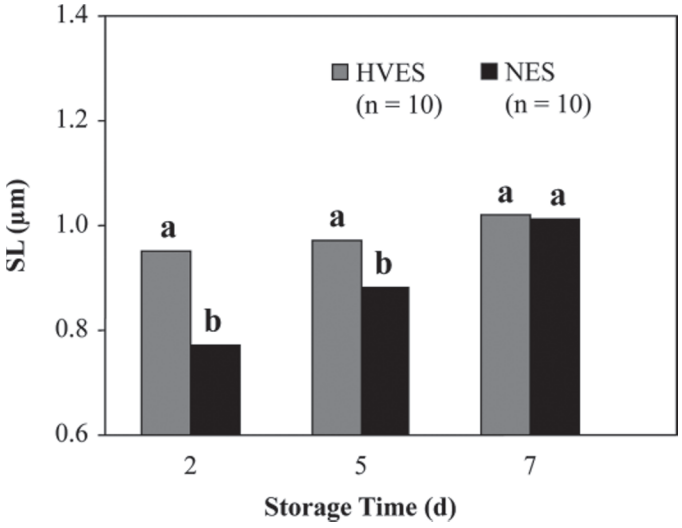

Figure 2. Effect of high-voltage electrical stimulation (HVES) on sarcomere length (SL) values of broiler breast meat. NES = nonelectrical stimulation. Means with different letters (a, b) are significantly different ($P < 0.001$).

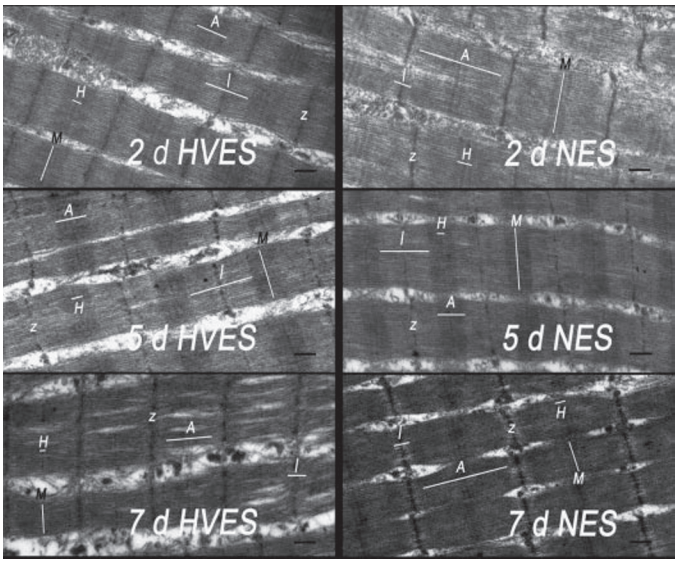

Figure 3. Electron microscopy of pectoralis myofibrils. HVES = high-voltage electrical stimulation; NES = nonelectrical stimulation; H = H-band; M = M-line; A = A-band; I = I-band; Z = Z-band. Bar: 0.2 μm.

the results of Birkhold and Sams (1993), Owens and Sams (1998), and Sams and Dzuik (1999). Hwang et al. (2003) indicated that SL is a significant determinant of tenderness. Alvarado and Sams (2000) observed that ES might have reduced sarcomere shortening because of early deboning, whereas the increased fragmentation might have contributed to some additional tenderness. Furthermore, SL increased with storage. The increased length might be caused by a combination of increased proteolysis and low pH-induced swelling (Bruce and Ball, 1990; Olsson et al., 1994).

Electron microscopy showed that the ultrastructure of the pectoralis muscle was changed after HVES (Figure 3). In this study, the stimulated muscles had longer sarcomeres; however, the A-, I-, and Z-bands and the mitochondrial membrane structure were intact in the HVES and NES groups. The differences in SL could be attributed to the contraction of the muscle. These findings are consistent with the studies of Birkhold et al. (1992) and Birkhold and Sams (1993). The authors observed that the lower pH of the stimulated muscles might have inhibited myofibrillar fragmentation by accelerating the normal decline in intracellular pH, the activity of calcium, and the reduction of neutral protease. Sams et al. (1991) postulated that muscle tensioning caused a wavy appearance and that some of the wrinkling might be the result of passive contraction. In contrast, Thompson et al. (1987) and Lyon et al. (1989) reported that banding patterns were determined to be damaged in stimulated sides. The rate of degradation of myofibrils has also been suggested to cause the improvement in tenderness. The microstructure images also revealed that the myofilaments of HVES and NES were more irregular because of ruptures at the connection sites of the myofibrils as the aging time increased.

In conclusion, HVES increased the rate of muscle pH decline. High-voltage electrical stimulation had no effect on WHC and CL values. Only L* values were improved during the storage time. Tenderness ($P < 0.001$) and SL ($P < 0.05$) values were significantly increased at 2 and 5 d postmortem. In addition, microstructure examination demonstrated that the stimulated muscles had longer sarcomeres. The results show that HVES is a useful method for improving the tenderness of broiler breast meat.

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