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EFFECTS OF LOCAL ELECTRICAL STIMULATION ON TENDERIZATION OF BEEF

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INTRODUCTION

Early post-mortem electrical stimulation and improvement of the meat quality has been studied during the last 25 years. By using high voltage carcass stimulation, the increased rate of glycolysis and earlier onset of rigor mortis was shown to reduce the development of cold shortening and thaw rigor.

A number of authors (Bouton *et al.*, 1980; Fabiansson and Buchter, 1984; Eikelenboom *et al.*, 1985) have demonstrated a similar effect on pH fall with low voltage electrical stimulation (LVES). The resulting effects of LVES on tenderness is mainly due to the prevention of cold shortening, but other mechanisms, i.e., disruption of fibres or accelerated ageing by endogenous proteases, is still a matter of discussion. While most evidence seems to endorse a beneficial effect of LVES on tenderness some studies show no major tenderization improvements (Jeremiah *et al.*, 1985; Crouse *et al.*, 1985; Unruh *et al.*, 1986; Hawrysh and Wolfe, 1987; Pommier *et al.*, 1987). These workers found toughening either to be increased or not affected by LVES at respectively slow and rapid cooling conditioning.

A method of local electrical stimulation (LES) in a muscle was developed by Braathen (1984). By using the so-called DFD-eliminator it was possible to sort out carcasses on the slaughter line which are DFD. This selection may be done with pH-measuring in the loin within one hour after slaughtering (Braathen, 1990).

In further studies Hald (1991) and Hald and Klastrup (1992) approved that LES could be used for detecting DFD carcasses on the slaughter line.

The purpose of this experiment was to study the effect of carcass stimulation (CS) respectively at 9 and 80 volts and LES in the loin, on some physical and sensory properties of loins from young bulls.

MATERIALS AND METHODS

Stimulation parameters

Thirty-four carcasses of young bulls of Black Pied Danish cattle were randomly designated to one of three treatment groups. One group of 12 carcasses was non-electrically stimulated (NES) and the two others were low voltage electrically stimulated (LVES) within two to five 5 minutes after stunning. CS80: 10 carcasses were stimulated using the Swedish MIT-AB equipment (80V, 14Hz, impulse duration 5ms; 20-30mA) (Fabiansson and Buchter, 1984). CS9: 12 carcasses were stimulated via a rectal-probe (Hawrysh and Wolfe, 1987) and with a clamp connected to the throat (sticking cut) as the negative pole. Carcasses were stimulated with 9V (14Hz, impulse duration 7ms, 5-9mA) for two minutes (Paleari *et al.*, 1991). Local electrical stimulation (LES) was carried out with the DFD-eliminator (Braathens type SC-300 special) delivering 78V (20Hz, impulse duration 7ms; 30-50mA) for three minutes (Braathen, 1984,

1990). Electrodes were placed in *m.longissimus dorsi* (LD) between the 1st-2nd lumbar vertebra and the 6th-7th rib vertebra. The LES treatment was performed on all right sides immediately after dehiding, approximately 35 minutes post-mortem.

Sampling procedure

After conventional slaughter procedure, carcasses were held at 10 to 12°C for two hours before final chilling at 3±1°C, 1m/s. Twenty-four hours post-mortem the LD was removed from each side, 8th-13th rib and cut into subsamples. From the 8th-11th rib, two subsamples for shear force measurement were vacuum packed and randomly assigned to two or 14 days of storage at 4°C. Three cuts, 11th-13th rib, were used for further measurements as described below. All samples, except that used for drip loss and colour determination, were stored at -24°C from the end of the conditioning period until further measurements could be made approximately eight to 12 weeks.

Measurement

pH was measured at 1, 3, 6 and 22 hours after slaughter on the LD between the 10th and 11th rib at a depth of 4cm using a Knick Portamess 655 pH-meter and a direct insertion probe electrode (Ingold LOT 406 3). The temperature in the LD was measured one hour post-mortem with a portable Technoterm 1500 thermometer inserted into the LD centre. Drip loss was measured on a slice, 25mm, suspended in a plastic pouch and stored at 2 to 4°C for 24 hours. Drip loss is calculated as the percentage of weight loss of original weight. Steaks for colour measurements, 25mm, were packed in a foodtainer using high oxygen permeable film. The samples were kept at 4°C for 80 minutes and, after removing the film, the colour was measured by recording the Hunter L-, a- and b-value using a Datacolor-Dataflash 2000. Four measurements per sample were taken for calculating mean values. For shear force, frozen samples (aged two or 14 days) were thawed at 4°C for about 16 hours. Four blocks of each sample were cut parallel to the muscle fibres (20x20x60mm) and cooked in tubes containing 0,9%NaCl, 80°C for 25 minutes. Peak shear force values were measured using a Warner-Bratzler (WB) shear blade fixed to an Instron Universal Testing Machine Model 4301 and expressed as N/cm². Twelve measurements per sample were taken for calculating mean values. Sarcomere length was measured using a laser diffraction technique developed at the Danish Meat Research Institute. Fifty scans per sample were taken for calculating mean values. For myofibrillar fragmentation (MFI), frozen samples (aged two and 14 days) were thawed at 4°C for about 16 hours. Myofibrils were isolated as described by Moller *et al.* (1973). The absorbency of the diluted myofibril suspension, 0.4mg protein/ml suspension was measured at 540nm. Measurement of Troponin-T and the proteins in the 30-33kDa region after SDS gel electrophoresis of the myofibrils was carried out as outlined by Penny and Fergusson-Pryce (1979). Electrophoresis was run on 4% acrylamide stacking gel and 10% acrylamide separation gel using a vertical system (LKB 2001-001). Two gels from each sample within a treatment were scanned on a LKB 2202 laser densitometer and protein concentrations in the peak areas of Troponin-T and the 30-33kDa were expressed as µg BSA equivalents/mg myofibrillar protein. For Cathepsin B+L, two hours post-mortem samples of LD (5g) were pressed between two stainless steel plates under reproducible conditions. The meat juice obtained and squeezed tissue were placed in tubes and frozen in liquid nitrogen. The samples were transferred to -60°C (for no more than eight weeks) until fluorescence assay of Cathepsin B+L was carried out as outlined by Ostdal *et al.* (1992). Activities are expressed as the percentage of free activity = soluble activity (meat juice) in percentage of total (meat juice + squeezed tissue) activity. Analysis of variance was used to test the effects of level of carcass stimulation (CS), LES and ageing on the various parameters by using SAS-programs (GLM-programs).

RESULTS AND DISCUSSION

pH

Carcass stimulation did not effect pH or the rate of pH-decline, which is in contract to earlier studies (Fabiansson and Buchter, 1985; Eikelenboom *et al.*, 1985). However, pH-values from controls appeared very low, at about 6.0 one hour post-mortem. An ultimate pH of approx 5.50 was found in all groups six hours post-mortem. LES decreased pH-value

(P<0.001) at 1, 3 and 6 hours post-mortem while ultimate pH was unaffected (Figure 1).

Temperature

In the LD muscle, 10∘C was obtained at eight to 10 hours post-mortem so no cold shortening is expected in this experiment. Stimulated sides showed a significantly (P<0.01) higher temperature, respectively 34.5∘C and 34.9∘C for CS9 and CS80, as compared to control carcasses of 34.0∘C. LES did not cause additional temperature increase.

Drip loss

Neither carcass stimulation (CS9 and CS80) not LES affected drip loss. This is in accordance with Fabiansson and Buchter (1984) and Bouton *et al.* (1980), but Eikelenboom *et al.* (1985) reported a higher drip loss of approx. 1% after LVES.

Colour

Significant effect of carcass stimulation on L-value was observed, i.e., an increased L-value for the stimulated carcasses (CS9 and CS80; see Table 1), while no effect of LES on L-value was found. Both CS (see Table 1) and LES resulted in higher a-values (P<0.001), respectively 17.11 for +LES and 16.65 for non-LES, while increased b-values were only found by CS (P<0.001),(see Table 1). From this experiment, a brighter and more red meat colour is obtained by electrical stimulation, as in agreement to Paleari *et al.* (1991) and Fabiansson and Buchter (1984).

Shear force

No effect of CS stimulation was found, while LES significantly (P<0.001) reduced WB values. However, as LES and ageing interaction is significant (P=0.015) mean values of both are shown in Figure 2. Ageing significantly reduced WB values for all groups, although the ageing effect on non-LES sides is greatest. In contrast to previous results (Bouton *et al.*, 1980; Fabiansson and Buchter, 1984; Eikelenboom *et al.*, 1985; Ring and Taylor, 1981), this experiment did not show significant effects of LVES on tenderness.
The accelerated tenderisation found for LES, i.e., shear force values at about 20% less compared to non-LES already two days post-mortem, may be influenced by an earlier release of cathepsins due to lower pH-values as obtained after LES (Dutson, 1983).

Sarcomere length

No significant effects of carcass stimulation on sarcomere length were found, while LES caused significant (P<0.01) change in sarcomere length, from 1.76μm (-LES) to 1.81 μm (+LES). The correlation between sarcomere length and shear force value showed no correlation for +LES sides r=0,004 (n=34), but for -LES sides, r=0,39 (n=34). This trend is in accordance with earlier work (Moller and Vestergaard, 1987; Smulders *et al.*, 1990) in which the correlation between sarcomere length and shear force values appeared lower by decreased early post-mortem pH-values.

MFI and SDS-PAGE

On a reduced material selected by focusing on LES, two groups with five carcasses in each group were selected according to high or low effect of LES. No effect of LES on MFI was found; however ageing resulted in an increased myofibrillar fragmentation, i.e., MFI increased from 0.71 at two days to 0.81 at 14 days. A correlation between MFI

and shear force value of 0.59 was found, which is somewhat lower than earlier observed (Møller *et al.*, 1973). The well-established ageing effect of Troponin-T (TnT) degradation followed by the appearance of the 30-33kDa degradation bands was observed, while no additional effect of LES appeared, probably due to a large variation between animals.

Cathepsin B+L

The fluorescence assay for measurement of Cathepsin B+L only showed an effect of carcass stimulation (LVES) (see Figure 3). The significantly ($P<0.001$) higher percentage of free activity after LVES treatment can not be explained by a pH effect from the present results, but the higher temperature for the stimulated carcasses may have contributed to a more rapid release of Cathepsin B+L.

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Table 1. Hunter values after low voltage electrical stimulation.

Treatment	NES	CS9	CS80
L-value	32.86^{a}	34.56^{c}	33.56^{b}
a-value	16.41^{a}	16.73^{b}	17.68^{c}
b-value	7.80^{a}	8.43^{b}	8.60^{b}

Figures with different superscripts differ significantly ($P<0.05$). n=24 per mean for NES (non-electrically stimulated) and CS9 (stimulated with 9V). n=20 per mean for CS80 (stimulated with 80V).