THE EFFECT OF ELECTRICAL STIMULATION ON THE TENDERNESS OF BEEF – AN INVESTIGATION OF PROTEOLYSIS AND MEAT QUALITY

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Background.

Electrical stimulation is used as a tool to avoid cold shortening in muscles exposed to rapid chilling. According to the literature, electrical stimulation is also beneficial to the development of tenderness since it stimulates the enzymatic systems responsible for improving tenderness *(Ducastaing et al., 1985; Dransfield, 1994)*.

Objective.

The aim of this project was to investigate how electrical stimulation affected the rigor development, the degradation of the meat structure and the meat quality of two muscles, *M. longissimus dorsi* and *M. semimembranosus*.

Materials and Methods.

Two muscles, *M. longissimus dorsi* (LD) and *M. semimembranosus* (SM), were excised from six young bulls of the Swedish Lowland breed. Three of them were electrically stimulated 30 minutes p.m., at low voltage (80V, 30 seconds, 15Hz) and the other three were not given any electrical stimulation. The muscles were wrapped in cling film and stored at +8°C. At approximately 5 hours post-mortem, the temperature of the muscles had reached 15°C, and at 24 hours post-mortem the temperature was +8°C. For pre-rigor analyses, a part of the muscle was removed and put in a waterbath programmed with the same temperature gradient that the larger meat samples had been exposed to. At 24 hours post-mortem, the meat was cut into pieces, vacuum packed and stored at +4°C for 3, 7 and 14 days post-mortem.

Warner-Bratzler shear force

The meat samples for the Warner-Bratzler shear force measurements were sliced into 3.5 cm thick pieces, vacuum packed and frozen after ageing. The frozen samples were thawed for 24 hours at +4°C and then cooked in a waterbath for 80 minutes at 74°C, followed by 25 minutes in an ice bath. The 3.5 cm pieces were cut in the fibre direction, each sample having a cross-sectional area of 15×7 mm^2 (1.0 cm^2) and a length of 20 mm. Shear force measurements were performed on an Instron 4301, with a modified cutting device developed by *Bouton and Harris (1978)*. The thickness of the blade was 1.0 mm with a square opening of 26×21 mm^2. A mean value of the ten measurements was used for each piece. Shear force-measurements were performed on samples aged for 3, 7 and 14 days post-mortem.

Extraction of myofibrillar proteins

Ten grams of bovine sample were diluted 1:10 with 0.03 M phosphate buffer pH 7.4 and homogenised in a Stomacher blender for 3 min and finally, centrifuged at 10000g for 20 min at 4°C *(Toldrá et al., 1992)*. The supernatant (sarcoplasmic fraction) was discarded and the pellet was resuspended in 100 ml of 0.03 M phosphate buffer pH 7.4, to ensure maximum extraction of muscle proteinases, and homogenised for 2 min in the stomacher. After centrifuging at 10000g for 20 min at 4°C, the pellet was resuspended in 9 vol of 0.1 M phosphate buffer pH 7.4 containing 0.7 M IK and 0.02% sodium azide, homogenised in the stomacher for 8 min and centrifuged at 10000g for 20 min at 4°C. The supernatant obtained contained the myofibrillar proteins.

Protein concentration

The concentration of protein in the myofibrillar extract was determined in accordance with the method of *Smith et al. (1985)*, using bicinchoninic acid as reagent and bovine serum albumin as standard.

Detection of protein and polypeptide fragments by SDS-PAGE

The procedure was based on the method described by *Toldrá et al. (1992)*, with slight modifications. The myofibrillar extract was mixed in a ratio of 1:1 with 50 mM Tris buffer, pH 6.8, containing 8 M urea, 2 M thiourea, 75 mM dithiothreitol, 3% (w/v) SDS and 0.05% bromophenol blue. This fraction was heated for 4 min at 100°C and used for electrophoresis. The amount of protein injected into the electrophoresis gels was 12 µg in each lane. The molecular mass of the extracted proteins was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 12% polyacrylamide gels. The elution buffer was 50 mM Tris (Trizma base), pH 8.8, 0.384 glycine and 0.1% SDS. Gels were stained with coomassie brilliant blue R-250 (Laemmli, 1970) and unstained overnight. Standard proteins from BioRad were simultaneously run for molecular weight identification.

Results and Discussion.

The pH-decline during rigor in LD muscles is shown in Fig. 1a as mean values for the three animals in each group. In the LD muscles, there were significant differences at all times, except at 24 hours. The experimental data points were fitted to a non-linear decaying model, $pH(t) = pH_\infty + (pH_0+pH_\infty)\cdot\exp(-t/\tau)$, where pH_∞ is the ultimate pH, pH_0 the pH for t=0 and τ is a characteristic time constant. The time constant was 5.38 hours for the NES and 1.99 hours for the ES animals. Figure 1b shows the pH-fall in SM muscles. Most striking was the fact that electrical stimulation did not seem to have any effect on the SM muscles, both pH-courses having almost identical time constants (4.88 and 4.43 hours respectively).

The results of the Warner-Bratzler shear force measurements are presented in Fig. 2, for both LD and SM muscles. For the LD muscles the electrically stimulated muscles showed lower shear resistance in comparison to non-electrically stimulated ones at 3, 7 and 14 days post-mortem, but the differences were not significant ($p>0.05$). For SM, on the other hand, the differences in shear resistance were smaller than for LD, and the electrically stimulated SM muscles obtained higher shear resistance than the NES SM muscles. The fact that electrically stimulated SM muscles received higher shear force than non-electrically stimulated muscles is remarkable but similar results have been seen by *Hertzman et al. (1993)*.

Considering the SDS-PAGE analysis, the 30 kDa fragment appeared earlier on in the LD muscles, compared to the SM muscles, which was to be expected since LD has a higher enzymatic activity. In the LD muscles, the 30 kDa fragment appeared at 24h in ES and at 3-7 days in NES while in the SM muscles it appeared at 3 days in ES and at 7 days in NES. It was also noted that the appearance of the 30 kDa fragment was earlier in the animals which had undergone electrical stimulation for both muscles, supporting the hypothesis that electrical stimulation is beneficial to the enzymatic activity. However, for the SM muscle, this is an interesting result since, according to the pH-falls in Fig. 1b, there seemed to be no effect of electrical stimulation on the SM muscles. The Warner-Bratzler shear force measurements indicated that the electrically stimulated SM muscles were tougher than the non-electrically stimulated ones.

Conclusions.

Electrical stimulation was beneficial to LD muscles with regard to pH-fall during rigor devolopment, Warner-Bratzler shear force and proteolytic activity (earlier appearance of 30 kDa fragment). For the SM muscle, on the other hand, electrical stimulation did not affect the pH-fall. The Warner-Bratzler shear force was higher for electrically stimulated muscles, but the proteolytic activity seemed to be affected in a positive way, since the 30 kDa fragment appeared earlier on for the electrically stimulated SM muscles.

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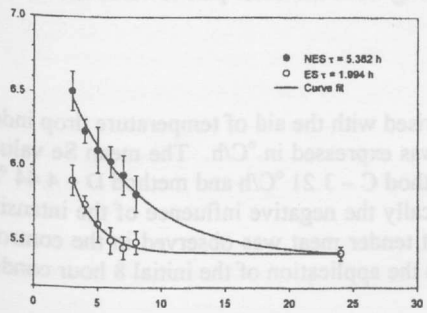
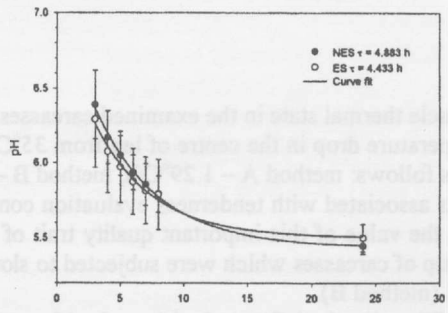


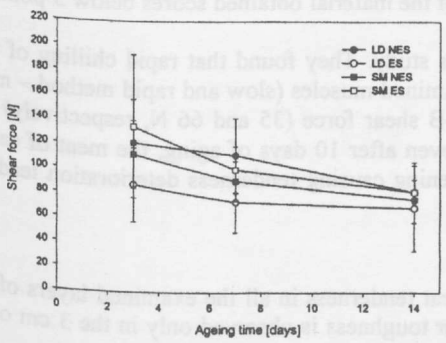
1a.



1b.

Figure 1a. The pH-fall in non-electrically stimulated LD muscles (filled symbols) and electrically stimulated ones (open symbols). The characteristic time constant, τ, was 5.38 and 1.99 hours for the respective treatment.

Figure 1b. The pH-fall in non-electrically stimulated SM muscles (filled symbols) and electrically stimulated ones (open symbols). The characteristic time constant, τ, was 4.88 and 4.43 hours for the respective treatment.



2.

Figure 2. Warner-Bratzler shear force as a function of ageing time for both LD (●,○) and SM (■,□) muscles. Filled symbols denote non-electrically stimulated animals and open symbols electrically stimulated ones.