

## Project Summary

### **Effect of Low Voltage Electrical Stimulation on Changes in Proteome of Bovine Longissimus Muscle during Postmortem Aging**

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## Background

Protein degradation and denaturation during postmortem storage of beef are known to influence beef quality, especially tenderness of beef. The early postmortem environment in muscle can have a significant effect on the processes that dictate fresh meat quality. It is known that the rate and extent of pH decline influences protein denaturation as well as enzyme function. Small changes in enzyme activity or protein solubility have the capacity to alter the rate and extent of changes in muscle and meat ultrastructure.

For many years it was understood that protein changes were not significant until after several days of postmortem storage. However, it is likely that this observation is due to the sensitivity of methods used. New approaches to determine protein modifications (solubility, denaturation, degradation) have provided insight in the response - at the molecular and cellular level - to changes in the early postmortem environment. These techniques have been focused on directed experiments focused on the activity of singular enzymes, inhibitors and substrates. The development of technologies to monitor changes in the entire proteome provides the means for a global inquiry of the extent to which the early postmortem environment causes changes in protein profile. Alteration in the postmortem environment by early postmortem electrical stimulation of beef carcasses is known to increase protein degradation and the rate of tenderization during postmortem storage. In previous experiments, researchers have documented the early postmortem electrical stimulation results in a more rapid pH decline, more rapid  $\mu$ -calpain autolysis and more rapid tenderization in bovine Top Loin (*Longissimus dorsi*) steaks.

## Objective

The objective of this study was to use Two Dimensional Difference In Gel Electrophoresis (2D-DIGE) to determine the effect of postmortem electrical stimulation (ES) on changes in insoluble and soluble-protein fractions of bovine *Longissimus dorsi* muscle (LD) to help identify proteins that might be used as markers of tenderness.

## Methodology

Eight market-weight beef steers were slaughtered at Iowa State University. Each carcass was split and one half was electrically stimulated (**ES**; 100V, 60Hz for 1 min) within 90 min (average 81 min) of exsanguinations and the other half carcass was not-stimulated (**NES**) and was used as a control. At 24 hours postmortem, LD muscles (n=8, respectively) from each side of each carcass were removed. Steaks were cut perpendicular to the long axis of the muscle and individually vacuum packaged. Steaks were aged at 4°C for a total aging time of 1 or 9 days postmortem. Because the LD is commonly used as a whole-muscle retail cut and because it is known to respond to electrical stimulation, the LD was used as the reference sample and served as an internal control for electrical stimulation for each side of each carcass. Decline of pH, as well as  $\mu$ -calpain autolysis were determined. Sensory tenderness also was determined.

The soluble and insoluble protein fractions were extracted and subjected to 2D DIGE to determine what proteins were differentially expressed. Proteins that were different between the electrically stimulated and non-stimulated samples were identified.

## **Findings**

Application of ES resulted in lower LD pH at 3.5, 5.5 and 8.5 hours. No treatment differences were detected in ultimate pH. Application of ES resulted in greater autolysis of  $\mu$ -calpain and greater sensory tenderness scores in LD steaks evaluated 1 day postmortem.

ES reduced the abundance of several enzymes in the soluble protein fraction of samples evaluated at days 1 and 9 (Table 1) in experiments that utilized both pH 3-10 pH gradient strips as well as pH 4-7 pH gradient strips. Spots with homology to creatine kinase, annexin and pyruvate dehydrogenase were more soluble in response to ES.

Fewer differences were detected in the experiments designed to investigate the effect of ES on the insoluble fraction (Table 2). The corresponding loss of several enzymes (fructose biphosphate aldolase, glyceraldehyde 3 -P dehydrogenase) in the soluble fraction resulted in an increase in those proteins in the insoluble fraction. ES resulted in a greater abundance of myosin light chain 2, tropomyosin beta chain and tropomyosin alpha chain in the insoluble fraction of steaks aged 9 days.

## **Implications**

The proteins that were identified as being different between the ES and non-ES treated samples are candidates for markers to be used to identify and classify products that differ in tenderness. This could lead to rapid, minimally or non- invasive methods to help predict tenderness.

Table 1. Effect of ES on abundance of specific proteins in the soluble fraction of LD steaks at 1 and 9 d postmortem<sup>a</sup>

| Day     | Identified proteins                      | fold change <sup>b</sup> |
|---------|------------------------------------------|--------------------------|
| pH 3-10 |                                          |                          |
| 1       | Fructose-bisphosphate aldolase A         | +1.12                    |
| 1       | Glyceraldehyde-3-phosphate dehydrogenase | +1.13                    |
| 1       | Phosphoglycerate kinase 1                | +1.08                    |
| 1       | Pyruvate kinase isozyme M1               | +1.10                    |
| 9       | Glyceraldehyde-3-phosphate dehydrogenase | +1.20                    |
| 9       | Creatine kinase M-type                   | -1.08                    |
| pH 4-7  |                                          |                          |
| 1       | Glycerol-3 phosphate dehydrogenase       | +1.20                    |
| 1       | Pyruvate dehydrogenase                   | -1.19                    |
| 1       | Annexin                                  | -1.44                    |
| 9       | Glyceraldehyde-3-phosphate dehydrogenase | +1.30                    |

<sup>a</sup> All differences reported are significant at the  $P < 0.05$  level.

<sup>b</sup> + is more abundant in NES than in ES and – less abundant in NES than in ES.

In this pilot study with 8 carcasses, differences in abundance of some of the key substrates to calpains (desmin, troponin-T) were not detected. However the treatment did demonstrate that some proteins are less soluble in response to ES and a more rapid pH decline.

In the current experiment, ES did result in a more rapid pH decline and more autolysis of  $\mu$ -calpain. A slight improvement in sensory tenderness –detected at day 1 – was not maintained in steaks aged 9 days.

Table 2. Effect of ES on abundance of specific proteins in the insoluble fraction of LD steaks at 1 and 9 d postmortem<sup>a</sup>

| Day | Identified proteins                      | fold change <sup>b</sup> |
|-----|------------------------------------------|--------------------------|
|     | pH 3-10                                  |                          |
| 9   | Glyceraldehyde-3-phosphate dehydrogenase | -1.16                    |
| 9   | Fructose-bisphosphate aldolase A         | -1.14                    |
| 9   | Fructose-bisphosphate aldolase A         | -1.18                    |
|     | pH 4-7                                   |                          |
| 1   | Myosin regulatory Light Chain 2          | -1.13                    |
| 1   | Tropomyosin Beta Chain                   | -1.14                    |
| 1   | Tropomyosin Alpha Chain                  | -1.17                    |

<sup>a</sup> All differences reported are significant at the P<0.05 level.

<sup>b</sup> (-) less abundant in NES than in ES.

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