# CHANGES IN ENZYMES ASSOCIATED WITH ENERGY METABOLISM IN PRE AND *POST-MORTEM* LONGISSIMUS THORACIS BOVINE MUSCLE ANALYSED BY PROTEOMICS

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## Introduction

Many factors can affect final meat quality (Maltin *et al.*, 2003), of which the rate of early post mortem glycolysis seems to be an important determinant of eating quality (O'Halloran *et al.*, 1997). However, the biochemical mechanisms behind post mortem glycolysis in post mortem muscles are poorly understood. Soluble muscle proteins include many sarcoplasmic and mitochondrial enzymes, which are involved in biochemical metabolic processes in living animals. Hence, to turn muscles into high quality meat, an improved understanding of which metabolic proteins are involved and how the biochemical and energy metabolism is regulated during the early post mortem period, is a prerequisite. Proteomics is an important cornerstone in post-genome sciences and has also been applied in meat science in recent years (Bendixen, 2005; Bouley *et al.*, 2004; Jia *et al.*, 2006; Lametsch *et al.*, 2003). The objective of this study was to investigate the changes in metabolic proteins in bovine muscles between pre and post mortem muscles.

## Materials and Methods

The experiment included 9 NRF (Norwegian Red) dual-purpose young bulls slaughtered (approximately 13 months of age/450 kg live weight) at a performance test station (GENO-Breeding and AI Association) in 2004. The procedure for excision of biopsies had been approved by the Norwegian Animal Research Authority (Immonen *et al.*, 2000). Muscle samples were homogenized in TES buffer (10 mM Tris (pH 7.6), 1 mM EDTA and 0.25 M sucrose). 2-DE and silver staining was performed according to the method described previously (Jia *et al.*, 2006). The protein spots were matched across all 36 gels (2 time points × 9 animals × 2 technical replicates) using Image Master 2D Platinum v. 5.0 (GE Healthcare) and the spot report was imported into Unscrambler version 9.2 (CAMO A/S, Norway) and Matlab version 7.0.4. Significant spots were identified by cross-model validation. Proteins were identified by peptide mass fingerprinting (PMF) and MS/MS of at least 3 protein fragments.

## Results and Discussion

Figure 1A shows a representative 2-DE pattern of the proteins extracted from muscle samples in the pH range between 4 and 7. Most of the soluble proteins were located within the range pH 5 to 7. Image analysis allowed matching of 833 spots across all 36 gels. The PCA score plot of the complete data set of spot intensities from

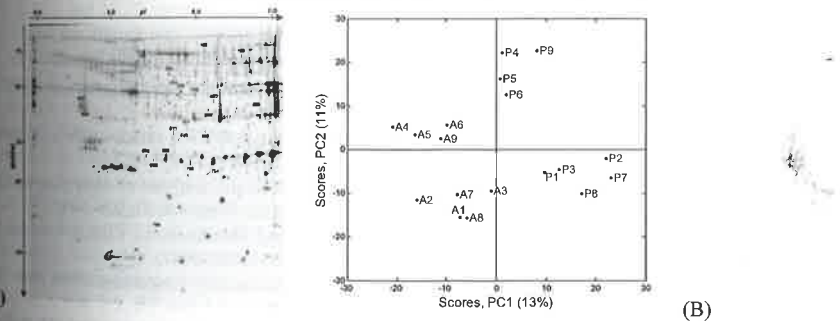


**Figure 1:** A. Silver-stained 2-DE master gel of bovine *longissimus thoracis.* Protein spots that are changed in the post mortem samples are numbered. **B.** Principal component analysis (PCA) score plot of protein spots. A1-A9: biopsies taken from nine animals. P1-P9: Samples taken after slaughter from the same nine animals.

comparative image analyses reveals that two clusters related to pre and post slaughter samples are formed by the first two components (Figure 1B). In the first set of technical replicates 49 protein spots were significantly changed, and in the second technical replicate 56 proteins were significantly changed. The 24 protein spots numbered in Figure 1A were

significantly changed in both data sets and underwent further protein identification. Among the selected 24 protein spots, 18 spots increased and 6 decreased in intensity in the post slaughter samples, respectively.

In this study, several glycolytic enzymes involved in energy metabolism, in addition to 3-hydroxyisobutyrate dehydrogenase and two enzymes in the TCA cycle, increased in intensity after slaughter (Figure 2). The finding that several glycolytic enzymes increased in intensity after slaughter may suggest increased rate of glycolysis, in order to support and maintain the ATP production.

The goal of this study was to investigate the changes in metabolic proteins from live animals to samples taken immediately after slaughter. Several processes such as transportation, lairage, stunning, exsanguination, dehiding and electrical stimulation could contribute to the changes in the protein profile. In this study we did not address these questions, but rather investigated the consequence of all these processing parameters. Further studies are needed to separate the individual effects of the processing steps on the protein profile.


**Figure 2:** Biochemical pathways in energy metabolism that are influenced in bovine *longissimus thoracis* post mortem. The identified metabolic enzymes by MALDI-TOF MS/MS are shown. The arrows show increased enzyme abundance.

## Conclusions

We observed a clear shift in energy metabolism in the muscle post mortem with an increase in enzymes involved in both the glycolytic pathway as well as in the TCA cycle. These findings suggest that an increased aerobic energy metabolism occurs in the first hour after slaughter. The increased aerobic energy metabolism will probably affect the rate of glycolysis in muscles after slaughter and eventually lead to variation in meat quality. However, additional studies including more animals are required to reveal how changes in protein expression in pre and post mortem muscles are related to meat quality traits in cattle.


## Acknowlegements

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