METABOLIC MECHANISM OF COMPENSATORY GROWTH IN PIGS

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Background

It has been known for decades that compensatory growth occurs in pigs following a period of feed restriction. Recent studies have shown that compensatory growth also results in more tender meat (Kristensen et al. 2002). However, it is still unclear why compensatory growth would lead to an increase in meat tenderness. It has been hypothesized that the improved tenderness is a result of increased post mortem protein degradation as a consequence of elevated protein turnover during compensatory growth.

Objectives

The aim of the present study was to investigate changes in the proteome of the muscle due to compensatory growth in pigs by analyzing muscle samples taken at slaughter and 48 hours after slaughter.

Materials and methods

Sixteen female pigs were divided into two groups, that either had free access to the feed (control) or were fed restricted from d 28 to d 80, after which had free access to the feed (compensatory). The pigs were slaughtered at d 140. Meat tenderness was analyzed by a sensory panel. Muscle samples from longissimus dorsi were taken at slaughter and 48 hours after slaughter. The muscle samples were homogenized in 0.1M Tris pH 8.0 added protease inhibitor (complete, Roche). The samples were centrifuged 20min, 25000xg, 4ºC and the supernatant was used for two-dimensional gel electrophoresis (2DE). Eleven cm, pH 4-7 IPG strips and 8-16% gel (Criterion, Bio-Rad) were used for the first and second dimension, respectively. The proteins were visualized with silver staining. ImageMaster 2D Platinum software v5 was used for image analysis and the 2D gels were analyzed in two groups, one containing the samples taken at slaughter and the other the samples taken 48 hours after slaughter. ANOVA (SAS) was used to analyze effect of compensatory growth. The proteins of interest were identified with the use of a MALDI-TOF-TOF instrument (4700 Proteomics analyzer, Applied Biosystems).

Results and discussion

The sensory analysis showed that compensatory growth resulted in a significant ($p<0.05$) increase in meat tenderness compared to meat from control pigs.

At slaughter, eight different proteins were found affected by compensatory growth of the pigs (see fig. 1). The stress proteins HSP70 and HSP27 that both are believed to participate in the organization and protection of the myofibrils (Liu and Steinacker, 2001) and may also have an important role in stabilization of the myofibrils post mortem. Therefore, it can be speculated that these proteins also have an impact on meat tenderness. Changes of proteins Enolase 3 and Glycerol-3-phosphate dehydrogenase, both part of the glycolytic pathway, were also observed. These changes are probably a consequence of regulation in the energy metabolism as the pigs showing compensatory growth have a higher protein turnover compared to control pigs. Changes in the *in vivo* glycolytic metabolism may also lead to changes in the post mortem metabolism. It is well established that the post mortem metabolism has an effect on meat tenderness and the relation between compensatory growth and meat tenderness could be a consequence of changes in the glycolysis. The relation of aldehyde dehydrogenase E2, aldehyde dehydrogenase E3 and biphosphoglycerate mutase to compensatory growth is unclear.

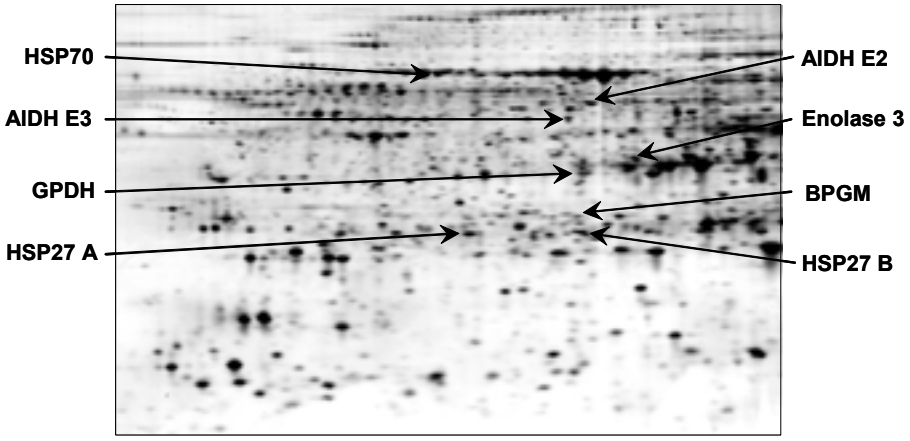

Figure 1. Effect of compensatory growth at slaughter. The arrows show the identified protein changes between pigs showing compensatory growth and kontrol. Heat AlDH E2; Aldehyde dehydrogenase E2. AlDH E3; Aldehyde dehydrogenase E3. GPDH; Glycerol-3-phosphate dehydrogenase. BPGM; 2,3 biphosphoglycerate mutase.

Figure 2A shows that the proteins found to be affected by compensatory growth at slaughter all have higher intensity when the pigs were fed ad libitum. It was presumed that the intensity of some effected spots would be higher in muscle samples from pigs that were fed compensatory as a consequence of the elevated protein turnover. The intensities of the proteins do not necessary reflect the activity of the identified enzyme, as the protein spots could also be an inactive isoform. Furthermore, only the sarcoplasmic protein fraction was used in the present study. Another explanation could be that mainly the amount of myofibrillar proteins increases during compensatory growth, and as a result, the relative amount sarcoplasmic proteins will decrease. However, compensatory growth did not affect the protein concentration of the sarcoplasmic protein extracted from the muscle, and the analysis of the sarcoplasmic protein fractions with 1D gel electrophoresis showed no effect on the relatively small amount of actin and myosin. Finally, the same intensity pattern was not observed in the samples taken 48 hours after slaughter (Fig. 2B).

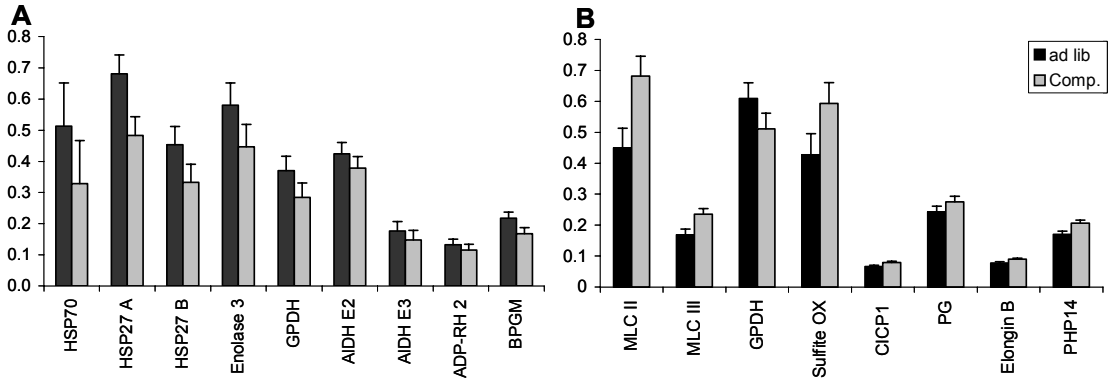

Figure 2. Intensity at slaughter (A) and 48 hours post mortem (B) of proteins that were found to be affected by compensatory growth. AlDH E2; Aldehyde dehydrogenase E2. AlDH E3; Aldehyde dehydrogenase E3. GPDH; Glycerol-3-phosphate dehydrogenase. BPGM; 2,3 biphosphoglycerate mutase. ClCP 1; Chloride intracellular channel 1. Sulfite OX; Sulfite oxidase. MLC II; Myosin light chain II. PHP14; Phosphohistidine phosphatase 14. MLC III; Myosin light chain III.

Muscle samples of longissimus dorsi taken 48 hours post mortem were also analyzed (Fig. 3) as post mortem protein changes have a great influence on the tenderness of meat. A hypothesis of the influence of compensatory growth on meat tenderness has been that it was a consequence of increased post mortem protein degradation. Therefore, it was presumed that the intensity of some protein fragments would be affected by compensatory growth. However, the proteins that were found affected by compensatory growth

at 48 hours post mortem were all full-length. Hence, the results from the present study could not confirm the hypothesis. It was found that the intensity of both Myosin light chain (MLC) II and III were affected by compensatory growth 48 hours after slaughter. MLC have an important role in the structure of the muscle cell, and it has previously been reported that MLC II is related to tenderness (Lametsch et al. 2003). However, it is not clear if the impact MLC has on tenderness is a consequence of post mortem proteolyses or modification (Lametsch et al. 2003). Recently, it was reported that MLC is dephosphorylated *post mortem* and that the dephosphorylation is related to the *post mortem* metabolism (Morzel, M. et al. 2004). The mitochondrial protein sulfite oxidase and the two nuclear proteins elongin B and chloride intracellular channel 1 increase in intensity 48 hours after slaughter as an effect of compensatory growth. An explanation of this observation could be that the number of mitochondria and satellite cells is increased as a consequence of the increase in protein turnover, and that the proteins from these two organelles leaked into the sarcoplasma *post mortem*. The effect of compensatory growth at 48 hours after slaughter on protein gamma and phosphohistidine phosphatase 14 is unclear.

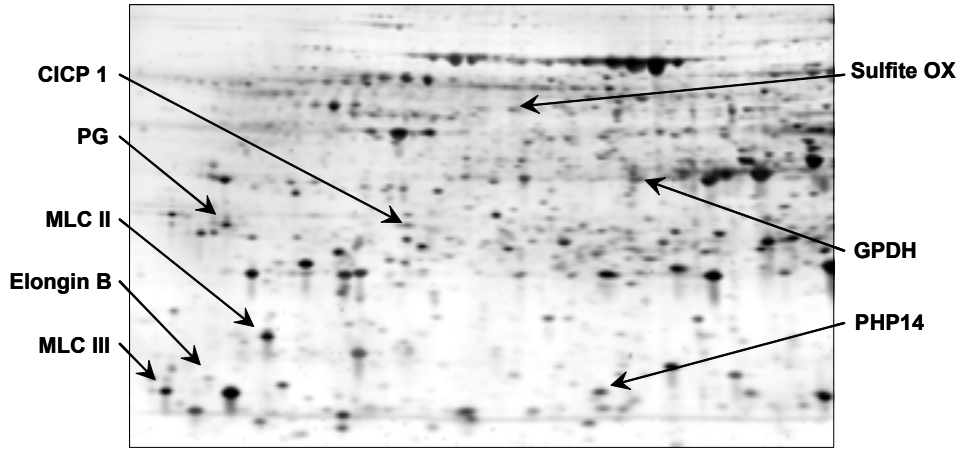

Figure 3. Effect of compensatory growth 48 hours after slaughter. The arrows show the identified protein changes between pigs showing compensatory growth and kontrol. ClCP 1; Chloride intracellular channel 1. Sulfite OX; Sulfite oxidase. GPDH; Glycerol-3-phosphate dehydrogenase. MLC II; Myosin light chain II. PHP14; Phosphohistidine phosphatase 14. MLC III; Myosin light chain III.

Conclusions

The results of the present study could not confirm the hypothesis that the impact of compensatory growth on meat tenderness is a consequence of increased post mortem protein degradation. Compensatory growth affected the at-slaughter level of the two stress protein HSP70 and HSP27. At 48 hours post mortem the protein isoforms myosin light chain II and III showed increased intensity in the sarcoplasmic fraction. It can be speculated that these changes could have an effect on the meat texture. Further studies are needed to clear this and reveal the precise effect of compensatory growth on the activity of the identified protein.

References

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