PE1.23 Postmortem changes in phosphorylation of metabolic enzymes in relation to the RN- genotype. 195.00

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***Abstract*—the aim of the presented work was to investigate postmortem changes of the phosphrylation level of the phosphorproteins in the sarcoplasmic fraction of the longisismus dorsi muscle from pig, with and without the RN- gene. The results revealed that several of the protein in the sarcoplasmic fraction are phophorylated and 11 of the 19 phosphorproteins were found to change significantly in the level of phosphorylation postmortem ($p<0.05$). When the postmortem changes of the phosphorproteins are compared between the two genotypes it is revealed that two phosphorproteins are significantly different in the level of phosphorylation. The major part of the proteins in the sarcoplasmic fraction are enzymes that are involved in the energy metabolism. Protein phosphorylation is a key factor in controlling these metabolic enzymes and information about the changes in phosphorylation after slaughter is essential for a better understanding of the postmortem metabolism and the influence of genotypes such as the RN genotype.**

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***Index Terms*—Postmortem metabolism, glycolysis, protein phosphorylation, RN- genotype.**

I.INTRODUCTION

he termination of the blood circulation at death Tinitiates a complex series of changes in muscular tissue. Initially the oxygen supply stops which results in change of energy metabolism from aerobic oxidative metabolism to anaerobic glycolytic metabolism. The ATP turnover is still high in the postmortem muscle as many enzymes such as the non-contractile ATP-ase of myosin, are still using ATP (1). This results in accumulation of lactic acid in the muscle post mortem and the pH drops from about 7.2 to around 5.3-5.7, depending on genotype, muscle type, feeding, stress, slaughter process and species (2). As the breakdown of ATP exceeds its synthesis by glycolysis, less ATP is available and the formation of actomyosin bonds shortens sarcomeres and increases muscle tension, signaling the onset of rigor mortis.

Because of the stoppage of circulation of blood, glucose has to be generated though breakdown of glycogen involving glycogen debranching enzyme, glycogen phosphorylase and phosphoglucomutase where glycogen phosphorylase is the rate limiting enzyme (3). In the glycolysis the rate limiting enzymes are hexokinase, phosphofructosekinase (PFK-1) and pyruvate kinase catalyzing the essential irreversible reactions. Protein phosphorylation is one of the major factors controlling these rate limiting enzymes.

In pigs a dominant mutation (the RN^- allele) has been identified in the gene coding for adenosine monophosphate-activated protein kinase (AMPK), causing a marked increase in the muscle glycogen content (4). It is well established that the RN^- allele leads to an extended pH decline resulting in a decrease in the WHC (3). Furthermore, it has been revealed that carriers of the RN^- allele have a higher basal AMPK activity and diminished AMP dependence. However, the precise mechanism behind the extended pH is still not clear. Some studies show no difference in the rate of the pH decline in muscles of pig carrying the RN^- allele compared with wild-type pigs (5), where as other show a faster pH decline in pigs carrying the RN^- allele (6).

The regulation of the glycolysis is well established and several studies have investigated the postmortem metabolism in muscles, however, the cause of the variation in the rate of the pH decline is still unclear and more holistic studies characterizing both glycolytic enzymes and metabolites are needed.

II. MATERIALS AND METHODS

Eight clinically healthy pigs (Yorkshire/Swedish Landrace x Hampshire), four carriers and four non-carriers of the RN^- allele shown by DNA-analysis

on the blood (Milan et al., 2000) were studied. The pigs were housed in pens (4 pigs/pen) with straw as bedding. They were fed a commercial finisher diet ad lib and had free access to water. The pigs were killed with a captive bolt and exsanguinated when they weighed 67 ± 13 kg. Samples of LD were collected at different time points postmortem (pm), immediately frozen in liquid nitrogen and stored at -80ºC until analysis. The first sample was taken within 22 minutes pm and then samples were taken at 30 min, 1, 2, and 48 hours pm.

One gram of frozen muscle sample was homogenized in 6 ml of 0.05M KCl, 5mM Iodoacetamide, Complete (Roche) and PhosStop (Roche) using a Ultra-Turrax T25 equipped with a S25N-18 G dispersing element (Ika Labortechnik, Staufen, Germany). The pH was determined in the suspension and neutralized by adding 1ml of 0.7M Tris (pH 8.3) and 70mM DTT. The sample was centrifuged 20min at 15.000xg (4ºC). The supernatant was analyzed using SDS-PAGE according to the manufacture (4-12% Bis-Tris NuPage gels, 15wells, with Mes running buffer, Invitrogen, Denmark). Staining of phosphorproteins were made with Pro-Q Diamond (Invitrogen, Denmark) followed by a total protein staining made with Sypro Ruby (Invitrogen, Denmark). The staining was made according to the manufacture. Detection of Pro-Q Diamond and Sypro Ruby staining were made using a Typhoon scanner (GE-Healthcare, Denmark). Image analysis was made using Phoretix 1D, V2003.2 (Nonlinear Dynamics, Denmark).

III.RESULTS AND DISCUSSION

The staining of the phosphorylated protein with Pro-Q Diamond showed that several of the protein in the sarcoplasmic fraction are phophorylated (see Figure 1).

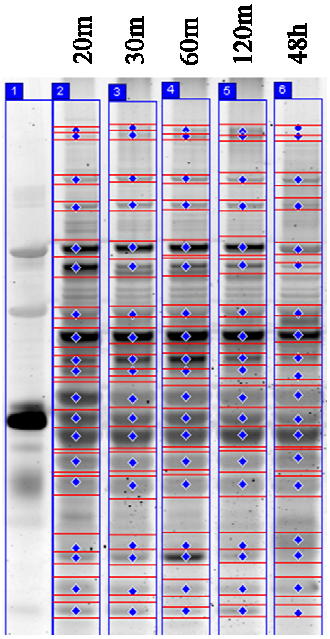

Figure 1. SDS-PAGE gel stained with Pro-Q Diamond. Samples from one pig differnt postmortem time points. Nineteen bands were analysed.

Nineteen bands were selected and quantified and the average postmortem changes of these phosphorproteins are illustrated in Figure 2. Eleven of the 19 phosphorproteins are changing significantly in intensity postmortem ($p<0.05$).

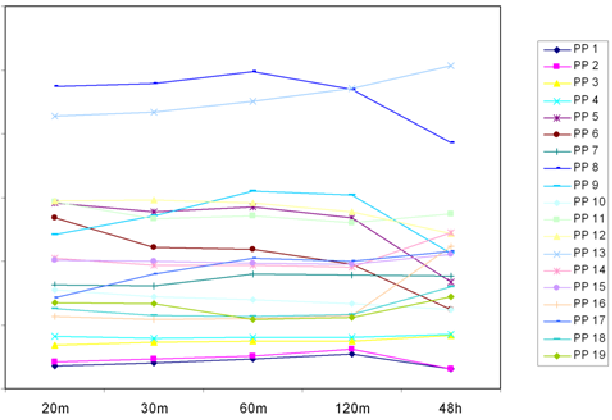

Figure 2. The average postmortem changes of nineteen phosphor proteins in the sacoplasmic fraction of eight animals.

When the postmortem changes of the phosphorproteins are compared between the two genotypes it is revealed that phosphorprotein 2 and 7 are significant different (see figure 3). Phosphorprotein 2 is shown to have a lower and phosphorprotein 7 a higher level of phosphorylation

in pigs carrying the RN^- allele.

In the sarcoplasmic fraction the majority of the proteins are enzymes that are involved in the energy metabolism. Protein phosphorylation is a key factor in controlling these metabolic enzymes and information about the changes in phosphorylation after slaughter is essential for a better understanding of the postmortem metabolism and the influence of genotypes such as the RN genotype.

In future studies the phosphorprotein will be identified with mass spectrometry.

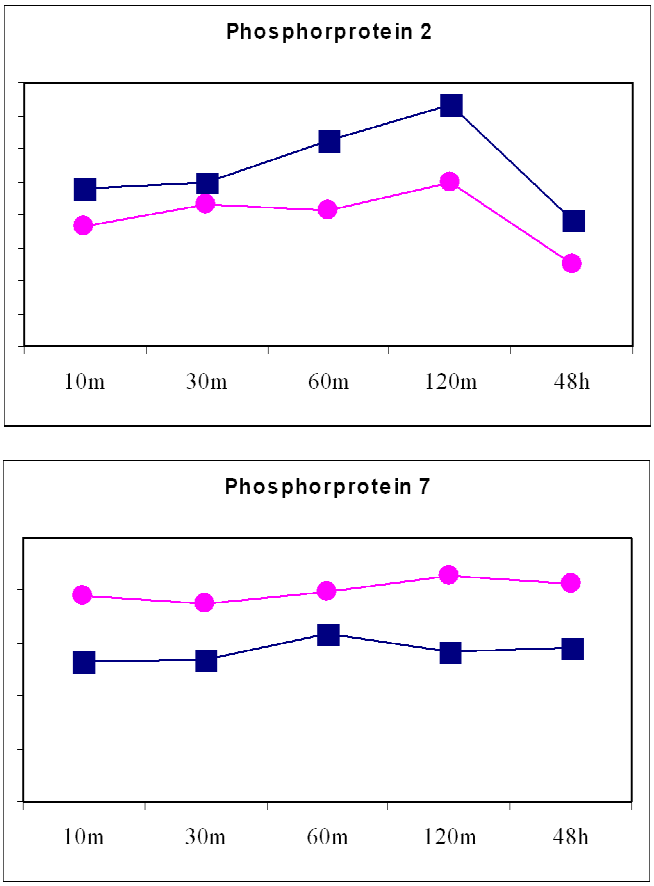

Figure 3. Significant differences in phosphorprotein 2 and 7 in relation to genotype. Four animals of each genotype. ■ Wildtype, ● RN^-

IV CONCLUSION

The presented results demonstrate that several of the proteins in the sarcoplasmic fraction are phosphorylated and that the degree of phophorylation are changing postmortem. It was also revealed that the proteinphosphorylation was affected by the RN^- genotype.

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