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Pig *Longissimus lumborum* proteome: Part I. Effects of genetic background, rearing environment and gender

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ABSTRACT

A 2 × 2 × 2 factorial experiment on *Longissimus lumborum* of 24 pigs found that rearing environment (indoors or outdoors), breed of sire (Duroc or Large White), and gender (female or castrated male) influenced 22, 10, and 88 proteins of the soluble fraction, respectively, containing 220 matched spots in total. Some proteins were influenced by more than one main effect. Outdoor rearing resulted in lower levels of enzymes of the glycolytic pathway suggesting a more oxidative metabolism. Breed of sire slightly altered the balance of enzymes of the glycolytic pathway. Gender had profound effects. In particular, different enzyme levels suggest a more lipid oriented energy metabolism, and a higher extractability of myofibrillar proteins suggest altered control of the contractile apparatus, in castrated males. Differences in extractability did not explain the profound gender effects. Glycogen content, ultimate pH, drip and thawing losses showed main or interactive effects of the three treatment factors.

1. Introduction

Technological and sensory pork quality depends on genetic and rearing background of the pigs used (Bee, Guex, & Herzog, 2004; Enfält, Lundstrom, Hansson, Lundeheim, & Nystrom, 1997; Terlouw & Rybarczyk, 2008). Oxidative and proteolytic processes in the *post-mortem* muscle determine to a large extent meat quality (Bendall, 1973; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004; Taylor et al., 1995). Proteins are central in these reactions as enzymes, but also as targets of oxidative and proteolytic activities. It is likely that variations in protein levels mediate the effects of genetic and rearing background on meat quality. The objective of our work was to use proteomics to increase our understanding of the role of certain proteins or of the biochemical cellular events they mirror, in animal and environment-related influences on meat quality. Proteomic studies screen a chosen fraction of the cellular protein content at a given moment, generally containing several hundred proteins. Use of this technique has proven useful to identify proteins that play a central role in meat quality. For example, they allowed to identification of proteins whose levels differed according to pork colour, drip loss or shear force (Hwang et al., 2004; Laville et al., 2007; Sayd et al., 2006). Such results may help to understand cellular mechanisms underlying variation in meat quality.

The present paper reports the effects of genetic background, rearing environment and gender on protein levels in longissimus muscle samples obtained immediately after slaughter. The evaluation of relationships between protein levels and longissimus meat quality parameters will be reported in a separate paper (Kwasiborski et al., accepted for publication). The study uses the soluble cellular fraction that contains enzymes and regulators of protein expression.

2. Materials and methods

2.1. Animals, housing and slaughter

Ten Large White × Landrace sows were inseminated with mixed semen from PIC Duroc and Large White sires. From about 100 piglets used for a larger study on behaviour, physiology and meat quality (Terlouw, Berne, & Astruc, submitted for publication), 48 have been selected, based on parenthood and weight. Pigs were born on the site in an outdoor farrowing system. They were weaned at 4 weeks and kept indoors until fattening at 2 months

Abbreviations: αParv, parvalbumin alpha; PPP, pyridoxal phosphate phosphatase; HSP72, heat shock protein 72 kDa; GDI2, guanosine diphosphate dissociation inhibitor 2; ACYI, aminoacylase I; ALDH, aldehyde dehydrogenase; PKM1, pyruvate kinase isoform M1; β-ENO3, β-enolase 3; cG3PDH, cytosolic glycerol-3-phosphate dehydrogenase; CGI-29, CGI-29 protein; PRDX6, peroxiredoxin 6; TPI, triosephosphate isomerase; PGAM1, phosphoglycerate mutase 1; MB, myoglobin; COF 2, cofilin 2.

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of age. Half of each genetic type was fattened indoors in four 2.2 × 2.9 m pens with slatted floors in a heated and ventilated animal room of 4 × 8.8 m. The other half was fattened outdoors on 850 m^2 parcels with little grass. Each parcel contained a 7.5 m^2 hut, a trough, a bowl drinker and a mud hole with water, as a bathing opportunity. There were six pigs (three females, three castrated males) for a single breed of sire per pen or parcel. Pigs were put into the fattening system in April and slaughtered in October. Outdoor pigs were fed *ad libitum*, indoor pigs were slightly restricted in order to standardise slaughter weight at similar ages. Liquefied food was distributed at 0600 h and 1800 h.

Pigs were slaughtered at about 8 months of age in a small local abattoir after a fasting period of 24 h. They were stunned on the floor with manually operated low voltage tongs. Slaughter took place on two different days, at a 1-week interval, balanced for housing system and breed of sire. Five minutes after bleeding, a 5 g sample of the *Longissumus lumborum* (LL) was excised and immediately frozen in liquid nitrogen. Upon arrival at the laboratory, the cups were stored at −80 °C until use for the proteomic study. Muscle temperatures of the LL were measured at 15 and 45 min after bleeding. Samples were also taken 45 min after bleeding to measure pH and for later assay of lactate and glycogen contents. pH was measured on the spot (glass electrode: Inlab 427, Mettler Toledo, Greifensee, Switzerland, connected to a portable pH meter: Schött-Geräte, Germany) after immediate homogenisation of the sample in 18 ml of 5 mM iodoacetate.

Twenty-four hours after slaughter, a 1-g sample was taken from each muscle for subsequent lactate and glycogen assays. Temperature, pH, redness (a^*), yellowness (b^*) and lightness (L^*) were recorded directly on the muscle. Colour was measured using a Minolta Chromameter (CR-300, Minolta Corp., Osaka, Japan) equipped with a 0° viewing angle and using illuminant C.

To evaluate drip losses, a 2-cm-thick slice of the LL was cut at the level of the last rib 24 h after slaughter, suspended in a plastic bag and left at 4 °C (Honikel, 1998). The amount of water lost was measured 72 h (drip loss d3) and 120 h later (drip loss d5) and expressed as a percentage of the initial sample weight. To evaluate thawing losses, a 7-cm-thick sample was subsequently cut from the LL, weighed, vacuum packed, and frozen after ageing (4 days after slaughter). About 4 weeks later, samples were thawed overnight at +4 °C, unpacked, blotted and weighed. Thawing losses were expressed as the percentage of the initial sample weight. Samples were subsequently cut to a standardised size (5 × 8 × 4 cm), weighed and vacuum packed. They were then heated in a water bath at 70 °C for 50 min and cooled in a +4 °C water bath. They were then unpacked and weighed after removal of liquid and precipitated protein. Cooking losses were expressed as the percentage of the initial weight of the standardised-cut sample.

2.2. Glycogen and lactic acid assays

About 200 mg of lyophilised muscle was ground and suspended in 10 ml of perchloric acid 0.5 M. After hydrolysis of the glycogen by amyloglucosidase (Bergmeyer, 1974), glucose content of the homogenate was determined. Lactic acid was determined on the supernatant after centrifugation (20 min at 2500*g*) of the perchloric homogenate following Bergmeyer (1974) procedure. Glycolytic potential (GP), the sum of compounds likely to produce lactic acid *post-mortem*, was calculated using the formula proposed by Monin and Sellier (1985). Concentrations are expressed as μmol lactate equivalents/g of fresh tissue.

Only 24 pigs were used for the present study. Pigs were selected according to their glycolytic potential and ultimate pH ($r = -0.60$, $p < 0.0001$). Males and females were represented with different symbols in a scatter plot and the three highest and three lowest values were selected for each genetic type and each rearing system, while balancing for gender. Thus, if for a given genetic type and rearing system, two higher and one lower values were selected for castrated male pigs and one higher and two lower values were selected for female pigs, the reverse was done for the other genetic type and rearing system (Fig. 1).

2.3. Sarcoplasmic protein extraction, two-dimensional electrophoresis and image analysis

Protein extraction, electrophoresis and image analysis were carried out according to the procedure described by Sayd et al. (2006). Frozen muscle was homogenised at 4 °C using a glass bead agitator (Retsch, Haan, Germany) in a buffer made of 40 mM Tris–HCl (pH 8.8), 2 mM EDTA, and a protease inhibitor cocktail for mammalian tissues, containing AEBSF, Aprotinin, Leupeptin, Bestatin, Pepstatin A and E-64 (Sigma–Aldrich, Saint Louis, Missouri, USA). This buffer was chosen for its weak ionic strength, limiting extraction of the actomyosin complex (Lametsch et al., 2006). Due to the proportion of protein content it represents, actomyosin, had it been extracted, would mask a large number of the proteins of interest for this study (Sayd et al., 2006). The homogenate was centrifuged (4 °C, 10 min, 10,000*g*) and the supernatant, referred to as the soluble fraction, was stored at −80 °C. The protein content was determined using the Bradford method (Bradford, 1976).

For electrophoresis, 900 μg of soluble proteins suspended in a urea 7 M, thio-urea 2 M and CHAPS 2% buffer were loaded onto immobilised pH gradient strips of 17 cm (pH 5–8, Biorad, Hercules, USA). Isoelectric focusing was performed using a Protean IEF cell system (Biorad). Gels were passively rehydrated for 16 h before application of rapid voltage ramping to reach a total of 85 kV h. For the second dimension, proteins were separated on 12% sodium

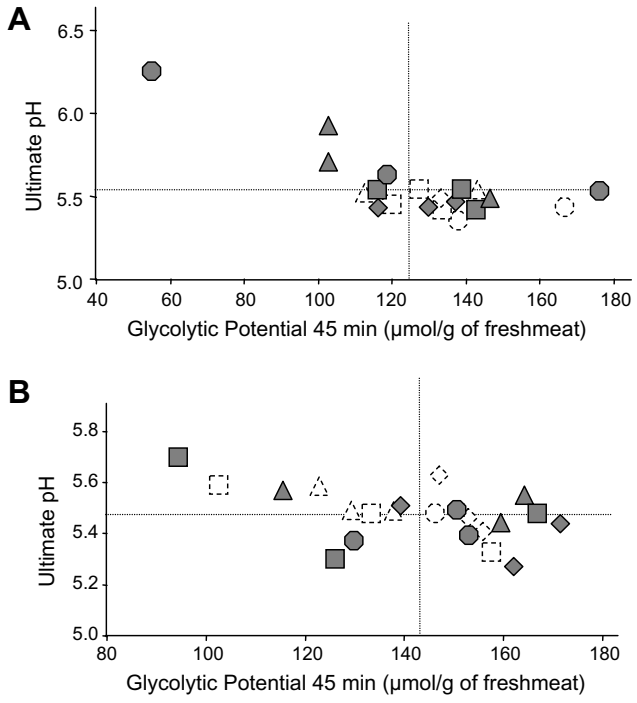

Fig. 1. Scatter plot showing glycolytic potential and ultimate pH for indoor/Duroc-sired (triangles), indoor/Large White sired (diamonds), outdoor/Duroc-sired (squares) and outdoor/Large White (rounds) for (A) castrated males and (B) females of the original experiment (Terlouw et al., submitted for publication). Grey and white symbols indicate selected and non-selected samples, respectively, for the present experiment. Horizontal and vertical lines indicate average values of the ultimate pH and glycolytic potential calculated over the 48 pigs that finished the original experiment.