PROTEIN SOLUBILITY OF *LONGISSIMUS* FROM STRESS SENSITIVE AND STRESS RESISTANT PIGS

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Background

It is well known that pigs with the porcine stress syndrome (stress sensitive pigs) are more prone to develop pale, soft and exudative (PSE) meat. PSE meat primarily originates from an accelerated rate of *post mortem* (pm) glycolysis. The low pH value early pm, in combination with the still high meat temperature, results in an increased protein denaturation (Bowker et al., 2000). Decreased protein solubility of PSE meat has been reported frequently, but data on the solubility of individual proteins are scarce.

Objectives

To determine the solubility of separate sarcoplasmic and myofibrillar proteins of pork *longissimus* of stress sensitive and stress resistant pigs, by means of semi-quantitative sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Methods

On 3 slaughter days, 18 pigs from a homozygous stress resistant line (17 gilts, 1 boar) and 18 pigs from a stress sensitive line (7 gilts, 3 boars, 5 barrows)(SEGHERSgenetics, Baasrode, Belgium) were slaughtered at the abattoir of our department following low-voltage electrical stunning. In the *longissimus* between the last thoracic and the first lumbar vertebra, pH was measured at 40 min pm and electrical conductivity (PQM: pork quality meter) was measured at 1 h and 24 h pm. At 1 day pm, carcasses were cut and a slice of 3 cm thickness was taken from the middle of the *longissimus lumborum* (LL), and frozen at –18 °C until analysis. CIELAB colour co-ordinates were measured before freezing. The carcass lean meat percentage was determined following a standard dissection procedure and using appropriate regression equations.

Sarcoplasmic and myofibrillar protein solubility was determined after extraction with a low and a higher ionic strength solution. First, 3.00 g of minced meat sample was weighed in a centrifuge tube, and 30 ml of a cold (3°C) solution (150 mM NaCl and 0.01 mM iodo-acetic acid) was added. The sample was homogenised using an ultra-turrax, followed by centrifugation at 2000 g for 10 min. The supernatant was filtered through a folded filter (Schleicher & Schuell 597½) and was considered the soluble sarcoplasmic protein fraction. The pellet was resuspended in another 30 ml of the same solution, centrifuged and the supernatant was decanted. Using the ultra-turrax at the lowest speed, the pellet was resuspended in 45.0 ml of a buffer solution at pH 5.5 (prepared with 0.1 M citric acid, 1 mM EDTA, 0.4 M NaCl and 0.01 mM iodo-acetic acid). The tubes were placed in a water bath at 20 °C for 2 hours, mixed manually every 30 minutes and centrifuged at 5000 g for 20 minutes. The supernatant was carefully decanted and considered the soluble myofibrillar protein fraction. The protein content of both solutions of sarcoplasmic and myofibrillar proteins was determined using the biuret method (Herbert et al., 1971); the sum being the total protein solubility. For protein separation, each solution was diluted twice by adding a buffer at pH 7 containing 2 % SDS, 2 % 2-mercapto-ethanol and 0.40 mg/ml or 0.133 mg/ml BSA for the sarcoplasmic and myofibrillar protein solutions respectively. Proteins were then separated by SDS-PAGE using a 12 % separating gel according to Greaser et al. (1983) and measured semi-quantitatively using BSA as an internal standard as described by Claeys et al. (1995). A load of 5 µl or 15 µl was used to separate the soluble sarcoplasmic or myofibrillar proteins respectively.

Results and discussion

Table 1 shows that the stress sensitive pigs had a significantly higher carcass lean meat %. Significant differences were also found for the parameters known to be indicative of PSE meat: a lower mean pH value at 40 min pm, higher PQM values and a higher mean CIELAB L* value indicating a paler colour for the stress sensitive line. A slightly lower solubility of the sarcoplasmic and a significantly lower solubility of the myofibrillar protein fraction were found for the stress sensitive pigs. The lower overall protein solubility and the differences in meat quality are in line with other reports (Bendall and Wismer-Pedersen, 1962; Boles et al., 1992; De Smet et al., 1993).

Figure 1 shows an example of the separation patterns for the two protein fractions. It should be mentioned that a much larger proportion of the sarcoplasmic proteins (± 80 %) is extracted compared to the myofibrillar proteins (< 10 %). The identification of the different protein bands was done by comparison with patterns found in literature (Savage et al., 1990; Claeys et al., 1995), leaving some unknown bands. Several sarcoplasmic proteins (bands 6-11, 13-15, 19, 21-23 and 28) also appeared in the myofibrillar fraction (see further), accounting for approximately 30 % of the total amount of extracted protein in the myofibrillar fraction. The semi-quantitative data for the individual proteins obtained from the SDS-PAGE patterns are presented in Figure 2. The concentration of all proteins in the sarcoplasmic fraction, except the unknown protein band 20, was higher for the group of stress resistant pigs compared to the stress sensitive group, with significant differences for phosphorylase b/phosphorylase b kinase, phosphofructokinase, phosphoglucose isomerase, creatin phosphokinase, phosphoglycerate mutase, myokinase and protein band 25 (unknown) and band 26 (presumably troponin I). Earlier reports mentioned a decreased solubility of a sarcoplasmic protein with a molecular weight of about 95 kDa, most probably phosphorylase b (band 6, Figure 1), and a protein migrating just below actin with a molecular weight of about 41 kDa, likely corresponding to creatin phosphokinase (band 13, Figure 1), but also of phosphoglycerate kinase (co-migrating with creatin phosphokinase) and lactate dehydrogenase (band 19, Figure 1) (Fisher et al., 1978; Boles et al., 1992). Of the proteins quantified in the soluble myofibrillar fraction (extracted with 0.4 M NaCl), only bands 16 and 17 (respectively assigned as troponin T and β-tropomyosin) and band 20 showed a slightly, but not significantly, higher concentration for the stress resistant compared to the stress sensitive group. All other proteins in this fraction seemed to be less extractable from the meat samples of the stress sensitive pigs. Hence, most proteins in both fractions showed the same tendency. Borchert et al. (1969) noticed no differences in starch gel electrophoresis patterns between PSE and 'normal' meat samples, although the total sarcoplasmic protein solubility was lower in the PSE samples in their study like in ours.

The finding that several sarcoplasmic proteins appeared in the myofibrillar fraction suggests that denaturation of sarcoplasmic proteins occurred. This could be interpreted as a confirmation of the hypothesis of Bendall and Wismer-Pedersen (1962) that the decreased solubility of myofibrillar proteins in PSE meat is a result of the precipitation of denatured sarcoplasmic proteins onto myofibrils, as was suggested by Boles et al. (1992). However, the amount and the proportion of sarcoplasmic proteins in the myofibrillar fraction was not higher in the stress-sensitive group. As stated by Bowker et al. (2000), the appearance of sarcoplasmic proteins in the myofibrillar fraction is no proof of their precipitation onto myofibrils, but may just reflect their denaturation and insolubility.

Conclusions

A reduced extractability of a sarcoplasmic and a myofibrillar protein fraction in *longissimus lumborum* of stress sensitive compared to stress resistant pigs was found. The reduction was similar for most separate proteins.

Pertinent literature

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Table 1. Carcass and meat quality characteristics of stress resistant and stress sensitive pigs in the present study

	Stress resistant (n=18)	Stress sensitive (n=18)	p
Live weight (kg)	103	99	0.202
Lean meat %	61.7	64.3	0.009
pH 40 min pm	6.08	5.78	0.000
PQM 1 hour pm	4.4	7.8	0.003
PQM 24 hours pm	8.2	9.4	0.050
CIELAB L* value	55.4	58.1	0.032
CIELAB a* value	7.6	7.5	0.827
Solubility sarcoplasmic proteins[1]	67.5	61.7	0.068
Solubility myofibrillar proteins[1]	25.3	22.2	0.002

1: expressed as mg protein / g fresh meat

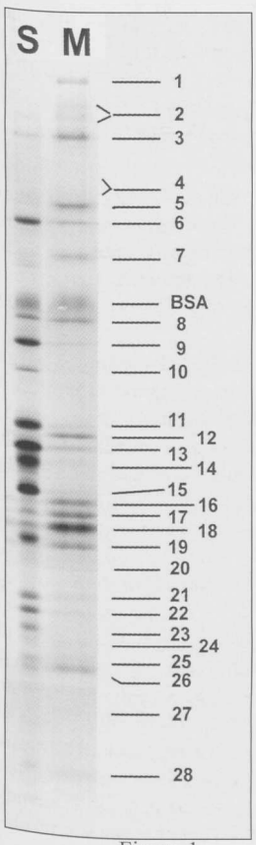

Figure 1
SDS-PAGE separation pattern of extracted sarcoplasmic (S) and myofibrillar (M) proteins

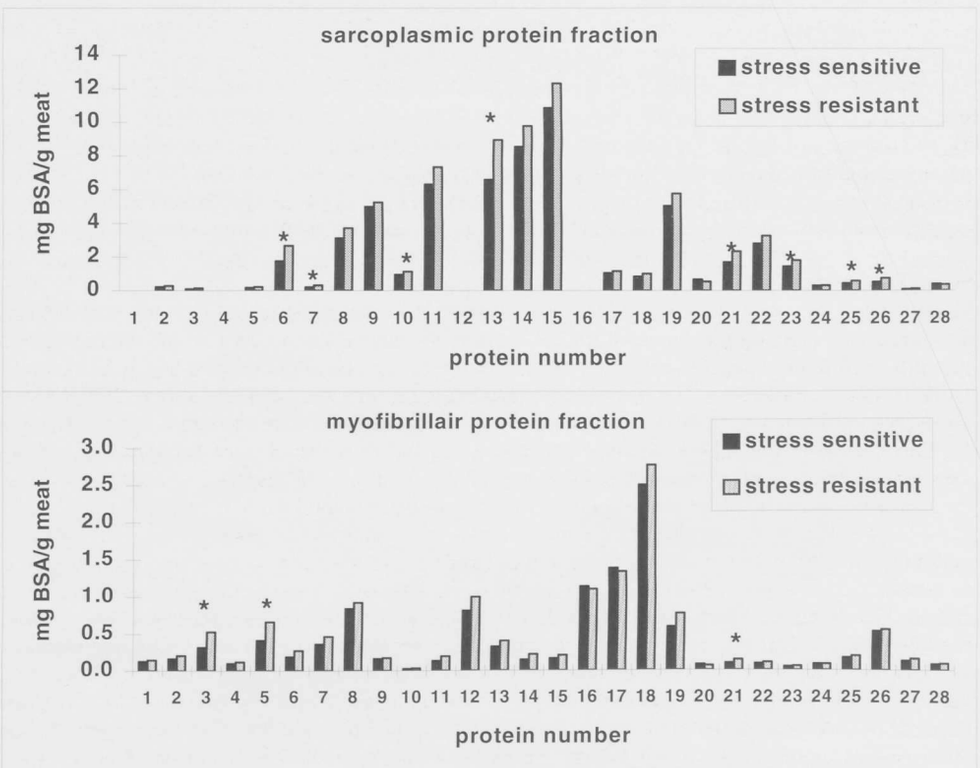

Figure 2
Mean semi-quantitative concentrations of extractable sarcoplasmic and myofibrillar proteins. * = significant difference at $p<0.05$

1: myosin heavy chain; 2: M-proteins; 3: C-protein; 4: unknown; 5: α-actinin; 6: phosphorylase b/phosphorylase b kinase; 7: phosphofructokinase; 8: phosphoglucomutase; 9: pyruvate kinase; 10: phosphoglucose isomerase; 11: enolase; 12: actin; 13: creatin phosphokinase/phosphoglycerate kinase; 14: aldolase; 15: glyceraldehyde phosphate dehydrogenase; 16: troponin T; 17: β-tropomyosin; 18: α-tropomyosin; 19: lactate dehydrogenase; 20: unknown; 21: phosphoglycerate mutase; 22: triose phosphate isomerase; 23: myokinase; 24: myosin light chain; 25: unknown; 26: troponin I; 27: troponin C; 28: myoglobin