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# SUMMARY

Muscle fibre type composition and glycogen depletion pattern and the latter's relation to technological meat quality were studied in longissimus dorsi muscle of halothane-gene-free Swedish Yorkshire pigs fed a high or a low protein diet. The muscle consisted of 8% type I, 9% type IIA and 83% type IIB fibres. Low glycogen levels were found in most type I and type IIA fibres, while a greater variation in glycogen levels was seen within type IIB fibres. The proportion of depleted IIB fibres was positively correlated to ultimate pH and pH at exsanguination and negatively to drip loss and EEL value. In pigs on the low-protein diet the proportion of depleted IIB fibres was positively correlated to plasma lactate levels and negatively to shear force. There were differences in meat quality between pigs fed the two diets. Pigs fed the low-protein diet had lower shear force values and higher intramuscular fat content. Carcasses from pigs on the high-protein diet had a higher lean percentage and less fat. When 30 % or more of the type IIB fibres were depleted of glycogen, the muscles showed a tendency towards DFD (Dry, Firm, Dark), as the meat had higher ultimate pH, higher water-holding capacity and lower reflectance values (darker meat).

Results from this study indicate that most of the type I and IIA fibres and approximately 30% of type IIB fibres had been recruited in these pigs due to the preslaughter treatment. This type of glycogen depletion pattern seemed to have a certain influence on technological meat quality.

# INTRODUCTION

Stress-situations and treatment of pigs prior to slaughter are known to affect muscle glycogen levels and may result in meat with the DFD condition, which is an important factor in meat quality (LISTER and SPENCER, 1983). Little is known about glycogen content within different fibre types at exsanguination, and its relation to meat quality. Muscles from pigs carrying the halothane gene contain numerous glycogen-depleted fibres at slaughter and give meat of poor quality (ESSEN-GUSTAVSSON et al., 1992).

The aim of this work was to study the relationship between muscle glycogen depletion pattern and technological meat quality at slaughter in pigs known to be halothane-gene-free.

# MATERIALS AND METHODS

**Animals:** A group of 37 pigs used in a selection experiment (described by STERN et al., 1990) of halothane-gene-free Swedish Yorkshire (entire males and gilts) were used, where a high protein diet (13.1% CP, 0.96% lysine; n=19) or a low-protein diet (13.1% CP, 0.64% lysine; n=18) was fed. The metabolized energy content was 11.9 MJ/kg in both diets. The pigs were slaughtered the week they reached 103 kg. They were transported 5 km from the research station to the abattoir, where they were kept for 2 h in the lairage before they were electrically stunned with low voltage on the floor.

**Muscle samples:** Immediately after exsanguination, muscle samples from M. longissimus dorsi were taken at the last rib, frozen in liquid nitrogen and stored at -80°C until analysis.

**Histochemical analyses:** Serial sections of the muscle were cut (10 µm) and stained for myo-ATPase after preincubation at pH 4.6 (BROOK & KAISER, 1970) and classified as type I, IIA and IIB fibres, according to the intensity of the staining. The glycogen depletion pattern in muscle was evaluated on sections (20 µm) stained with periodic acid-Schiff (PAS; PEARSE,

1961). Fibres were classified as depleted when unstained, and they were also identified according to fibre type. A computerized image analysis system designed for muscle fibre analysis (BIO-RAD Scan Beam, Hadsund, DK) was used to calculate relative distribution of fibre types in area and number (%) and mean fibre area ( $\mu\text{m}$ ) for each fibre type. At least 200 fibres from each muscle sample were used in the analyses. The volume distribution of IIB fibres was estimated from the product of the proportional area distribution and the volume (weight) of the dissected muscle according to Oksbjerg et al. (1990).

**Biochemical analyses** A piece of the muscle was freeze-dried overnight and dissected free from blood, fat and connective tissue. Glycogen content was then analysed according to ESSEN GUSTAVSSON et al. (1992) and expressed as glucose units on a dry-weight basis. Plasma lactate concentrations were analysed using a lactate analyser (Analox, Analox Instruments Ltd, London, England). Muscle pH at slaughter immediately after exsanguination ( $\text{pH}_s$ ) was analysed by homogenisation of muscle in iodoacetate as described by TARRANT et al. (1972).

**Meat quality analysis:** Technological meat quality was measured at 24 h post mortem. Meat colour was measured as surface reflectance (EEL, Diffusion Systems Ltd, London, England) and ultimate pH ( $\text{pH}_u$ ; Radiometer PHM63, Radiometer, Copenhagen, DK), drip loss (HONIKEL, 1987) and intramuscular fat content (IMF; Soxtec System H<sup>+</sup>, Höganäs, Sweden). Shear force values were measured on cooked samples aged for 2 days, using the Warner-Bratzler apparatus according to the procedure of LUNDSTRÖM et al. (1987). Lean percentage was calculated as described in KARLSSON et al. (1992).

**Statistical analysis:** The data were analysed by the method of least-squares using the GLM procedure (SAS Institute Inc., 1985). A classification was made depending on the glycogen depleted IIB fibres (more or less than 30 %). The statistical model included the fixed effects of diet and sex, while sire nested within diet was considered as random. The mean-squares for sire were used as error line when diet differences were tested. When analysing differences between glycogen depletion classes, this fixed effect was included in the model above.

## RESULTS and DISCUSSION

Results from the comparison between diets are shown in Table 1. There were no marked differences in the glycogen depletion pattern, fibre type distribution, or fibre mean area in muscle from pigs fed the low or high protein diet. A tendency to a difference between animals on the two diets was seen for the percentage type IIA and type IIB relative fibre type area distribution, where animals fed the low-protein diet showed a higher proportion of IIA and a lower proportion of IIB fibres. There was no difference in total glycogen content or plasma lactate between pigs on the two diets. Differences in technological meat quality were found between animals fed the two diets, where animals fed the low-protein diet had a lower shear force value and higher intramuscular fat content than animals given the high-protein diet. When comparing production traits, animals fed the low-protein diet showed a lower lean percentage (53.7 % vs. 60.6 %,  $P=0.003$ ) and more side fat (22.4 mm vs. 14.2 mm;  $P=0.01$ ) compared with animals given the high-protein diet. Since lean percentage differed between diets, a greater proportion of the total muscle mass consisted of type IIB fibres in pigs on the high-protein diet.

Glycogen was depleted according to the histochemical staining in most type I and type IIA fibres, while approximately 25% of IIB fibres were depleted. A greater variation in glycogen levels was seen in type IIB fibres and the range for this fibre type was from 0 to 60%. The proportion of depleted IIB fibres was positively correlated to ultimate pH (0.37,  $P=0.02$ ) and  $\text{pH}_s$  at exsanguination ( $r=0.41$ ,  $P=0.07$ ) and negatively to drip loss ( $r=-0.30$ ,  $P=0.07$ ) and shear force value ( $r=-0.32$ ,  $P=0.05$ ). In pigs on the low-protein diet the proportion of depleted IIB fibres was positively correlated to plasma lactate levels ( $r=0.36$ ,  $P=0.10$ ) and negatively to shear force ( $r=-0.30$ ,  $P=0.10$ ). To ascertain whether glycogen-depleted fibres would influence the technological meat quality, animals were divided into two groups according to the proportions of glycogen-depleted type IIB fibres (more or less than 30%). Even when there were no differences in total glycogen content, the animals with more than 30% depleted type IIB fibres showed a

water-holding capacity (measured as drip loss), higher ultimate pH and lower EEL values, compared with those having less than 30% depleted IIB fibres. These results show that if a pig has more than 30% of depleted IIB fibres already at slaughter, this will influence the technological meat quality towards DFD meat. In the present study these results were not dependent on the protein diet given to the pigs. One reason for the glycogen depletion here may be that entire males were used, as they fought a lot during lairage. Essén-Gustavsson et al. (1992) who studied the differences in meat quality and muscle metabolism in pigs with different halothane genotypes (NN and nn) found a significant difference in glycogen depletion pattern between the two halothane groups, where nn-pigs showed the highest proportion of depleted IIB fibres (29%). These findings - and ours - show that when 30% or more of the IIB fibres are depleted, this has an effect on meat quality. In the study by Essén-Gustavsson et al. (1992) where pigs carried the halothane gene, they developed PSE, while the halothane-gene-free pigs in our study instead developed meat towards DFD quality. LACOURT and TARRANT (1985) studied the effect of how different forms of stress affected glycogen depletion pattern in different muscle fibre types in Friesian bulls. The different forms of stress induced were (1) mixing with strangers for 5 h or (2) subcutaneous injections of adrenaline. The main findings were that glycogen depletion pattern varied with different kinds of stress. During mixing there was a greater loss of glycogen from type IIA and IIB fibres than from type I fibres. This pattern was similar to that expected after work of high intensity and short duration. Adrenaline caused a very high loss of glycogen in type I fibres compared with the type II fibres. Their conclusion was that fibre type response to stress is determined by the nature of the stressor. A slaughter stress that is physically demanding in terms of muscle activity will tend to be greater in type II fibres than in type I fibres. Additional stress during the pre-slaughter period may result in sympathetic arousal and adrenaline release, and as type I fibres have a better blood supply, this fibre type can have a greater glycogen loss. The glycogen depletion pattern seen in our study seems to be caused by a mixture of both types of stressors described by LACOURT and TARRANT (1985), with a varying proportion in individual animals.

CONCLUSIONS

Our results show that the pre-slaughter handling can cause biochemical and physiological reactions which may lead to the development of DFD meat.

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Table 1. Least-squares means with standard errors for differences in *M. longissimus dorsi* between diets

	Diet		Prob. value
	High	Low	
<u>Relative frequency, %</u>			
Type I	8 ± 1.1	8 ± 1.1	0.92
Type IIA	8 ± 1.2	10 ± 1.2	0.36
Type IIB	84 ± 1.5	82 ± 1.5	0.52
<u>Relative fibre area, %</u>			
Type I	5 ± 0.86	6 ± 0.90	0.28
Type IIA	4 ± 0.63	6 ± 0.65	0.08
Type IIB	90 ± 1.0	87 ± 1.0	0.06
<u>Glycogen-depleted fibres, %</u>			
Type I	58 ± 6.7	82 ± 6.9	0.04
Type IIA	65 ± 9.0	58 ± 9.3	0.62
Type IIB	23 ± 2.2	20 ± 2.3	0.45
<u>Glycogen, mmol*kg<sup>-1</sup> dry wt</u>	220 ± 11	207 ± 12	0.44
<u>Muscle volume, ml</u>			
Type IIA	177 ± 26	224 ± 27	0.25
Type IIB	3970 ± 67	3323 ± 70	0.0003
<u>Technological meat quality</u>			
Drip loss, %	4.0 ± 0.43	4.0 ± 0.45	0.99
EEL value	19.7 ± 1.5	19.4 ± 1.5	0.88
pH <sub>s</sub>	6.18 ± 0.07	6.22 ± 0.06	0.59
pH <sub>a</sub>	5.58 ± 0.07	5.59 ± 0.07	0.86
Shear force, kg/cm <sup>2</sup>	4.82 ± 0.23	4.10 ± 0.23	0.06
Intramuscular fat content, %	1.5 ± 0.18	2.5 ± 0.19	0.005

Table 2. Least-squares means with standard errors for differences in *M. longissimus dorsi* between glycogen depletion classes

	Glycogen class		Prob. value
	Depleted	Non-depleted	
<u>Glycogen-depleted fibres, %</u>			
Type I	61 ± 12.3	73 ± 6.8	0.42
Type IIA	74 ± 9.3	57 ± 5.1	0.12
Type IIB	40 ± 3.1	15 ± 1.7	0.0001
<u>Glycogen, mmol*kg<sup>-1</sup> dry wt</u>	199 ± 16.3	219 ± 9.6	0.30
<u>Muscle volume, ml</u>			
Type IIB	3662 ± 116	3642 ± 64	0.88
<u>Technological meat quality</u>			
Drip loss, %	2.8 ± 0.5	4.4 ± 0.3	0.007
EEL value	16.5 ± 0.9	20.6 ± 0.5	0.0005
pH <sub>s</sub>	6.29 ± 0.07	6.18 ± 0.04	0.38
pH <sub>a</sub>	5.62 ± 0.04	5.52 ± 0.02	0.079
Shear force, kg/cm <sup>2</sup>	4.3 ± 0.3	4.5 ± 0.1	0.44
Intramuscular fat content, %	2.1 ± 0.2	2.0 ± 0.1	0.68