Muscle characteristics in relation to technological meat quality and meat quantity in pigs

ANDERS KARLSSON, ANN-CHARLOTTE ENFÄLT, BIRGITTA ESSEN-GUSTAVSSON, KERSTIN LUNDSTRÖM, INGEMAR HANSSON, LOTTA RYDHMER and SUSANNE STERN
Swedish University of Agricultural Sciences, S-750 07 UPPSALA, Sweden

INTRODUCTION: In commercial pig breeding, carcass meat content and daily gain are traits of great economic importance. Both traits are affected by genetic selection as well as by the diet. Pigs given a high protein diet had a higher weight gain, meat content, and dressing percentage and a lower carcass fat content than pigs given a low protein diet (see e.g. DAVYN and BRESKIN, 1978; POND et al., 1980; ADEOLA and YOUNG, 1989). The lower intramuscular fat content in pigs given a high protein diet may lead to meat with a poorer sensory quality (drier and less tender) than that from fatter pigs reared on a low protein diet (see e.g. LEE et al., 1967; RAMSEY et al., 1990). In previous studies the effects of dietary protein content on production and carcass traits were studied, but not on the relation between muscle characteristics and meat quality.

The purpose of this investigation was to study the relationship between muscle histochemical and biochemical properties and technological meat quality during selection for a higher lean tissue growth rate. Comparisons were made between low and high protein diets and to some degree between generations.

MATERIAL AND METHODS: The animals studied were purebred halothane-gene-free Swedish Yorkshire pigs (entire males and gilts) from a selection experiment (STERN et al., 1991), where a high (HP; 18.5% crude protein (CP), 0.96% lysine) and a low (LP; 13.1% CP 0.64% lysine) protein diet was fed when selecting for increased lean tissue growth rate. In the present study pigs were from generation 1 and 4, and they were sent to slaughter at a live weight of 103 kg. After at least two hours of rest in the lairage the animals were stunned with low voltage electricity on the floor at an abattoir in Uppsala. Sidefat, growth rate and lean percentage was measured according to Stern et al. (1991). From 20 pigs per generation and diet, muscle samples for histochemical and biochemical analyses were taken from M. longissimus dorsi (LD) at the last rib, from the red part (rectus femoris) of M. quadriceps femoris (QF) in generation 1 and 4, and from M. biceps femoris (BF) in generation 4. The samples were taken immediately after exsanguination and were stored at -80°C until analysis. Meat quality was analysed 24h - 48h post mortem on a total of 270 pigs from generation 1 and 4.

Muscle fibre characteristics Muscle fibre types were identified by staining transverse serial sections for myofibrillar ATP-ase activity after preincubation at pH 4.3, 4.6 and 10.3 (BROOKE and KAISER, 1970). Glycogen content and the activities of the enzymes citrate syntase (CS), 3-OH-acyl-CoA dehydrogenase (HAD), lactate dehydrogenase (LDH) and hexokinase (HK) were analysed as described previously (ESSEN et al., 1980; ESSEN-GUSTAVSSON et al., 1984; ESSEN-GUSTAVSSON et al., 1991). These analyses provide a measure of oxidative capacity (CS), lipid oxidation (HAD) and glycolytic capacity (HK and LDH) of the muscles.

Meat quality measurements **Meat colour** was measured as internal reflectance measured with a Hennessy Grading Probe (GP, Hennessy Grading System, Auckland, NZ; 590 nm), and as surface reflectance, measured with EEL using the Y-filter (EEL-Y; Evans Electro Selenium Ltd.,UK; 400-700 nm). **Water holding capacity** was measured as (1) **drip loss**, measured as the loss of water from a 2.5 cm thick slice of muscle, hanging for 4 days in a polyethene bag at +4°C (HONIKEL, 1987) and (2) subjectively as **filter paper wetness** (KAUFFMAN et al., 1986). The score used ranged from 0 to 5, where 0 means a dry filter paper and 5 the other extreme. Extractability of **total muscle proteins** (sarcoplasmic and myofibrillar proteins) and the **sarcoplasmic proteins** were made on minced muscle by a modification of the method described by LUNDSTRÖM et al. (1988). For generation 1 the BCA-method (SMITH et al., 1985), and for generation 4 the biuret method, was used for determining the protein concentrations. From these results the **myofibrillar proteins** were calculated as the difference between total and sarcoplasmic proteins. The **pigment content** was analysed as alkaline hematin according to the method of KARLSSON & LUNDSTRÖM (1991). **Intra muscular fat content** (IMF) and **crude protein** (CP) were analysed in samples from LD in generation 4 using the Soxtex System H$^+$ (IMF) and Kjeltec (CP) equipments (Tecator AB, Höganäs, S). **Ultimate pH** (pH_u) measurements were also performed (Radiometer PHM63, Radiometer, Copenhagen, DK).

Statistical analyses The data were analysed with the Statistical Analysis System (SAS Institute Inc., 1985). The models used included the fixed effects of generation, diet, sex and day of slaughter. When analysing drip loss, the model used was corrected for initial sample weight by regression. The interaction between generation and diet was included in the model when significant.

RESULTS: Muscle fibre characteristics No marked changes were seen in the percentage of Type I, IIA or IIB fibres between the diets and generations. A somewhat higher proportion of Type I fibres and a lower proportion of Type IIB fibres was found in QF in LP animals in generation 1, in comparison with the HP animals. In both generation a higher oxidative and lower glycolytic capacity was seen in QF compared with LD (Tables 1 and 2).

LP animals had a significant lower LDH activity in all muscles compared with HP animals in both generations. In generation 4 HP animals had increased LDH activity and decreased HK activity in LD and QF, compared with generation 1. Animals in generation 4 had a slightly higher CS activity than in generation 1. The results are shown in Tables 1 and 2. Glycogen content at slaughter was significantly correlated to ultimate pH for LD and QF in generation 1 (LD, r=-0.46; QF, r=-0.75) and only for BF in generation 4 (BF, r=-0.55).

Carcass characteristics and technological meat quality Sidefat, lean percentage and growth rate differed significantly between HP and LP diets (14 vs 20 mm; 64 vs 59 %; 838 vs 694 g/day respectively; $p<0.001$). Significant differences in the technological meat quality between the diets were found in both generations where HP animals had a significant higher water holding capacity, measured as filter paper wetness, lower reflectance, and a higher protein extracability in LD compared to LP (Table 3). There was no significant difference in the myofibrillar protein extractability between the diets. When a comparison of the protein extractability in generation 4 was made on a fat free basis, i.e. after correction for IMF, the difference between diets decreased but it was still significant for the sarcoplasmic proteins ($p<0.05$).

In LD, the IMF was different between diets (1.1% vs 2.4% in HP and LP, respectively; $p<0.001$). The correlation between IMF and reflectance was zero in HP and positive in LP when measured both as internal and surface reflectance ($r=0.40$ for both; $p<0.05$). There was also a difference in CP in LD between diets (23.1% vs 22.4% in HP and LP, respectively; $p<0.05$).

DISCUSSION: The two muscles LD and QF were choosen as they are known to differ in their fibre composition and metabolic characteristics (LUNDSTRÖM et al., 1989). The results for muscle fibre type composition and enzyme activities in generation 1 in this study show that LD and QF differ in both the contractile and metabolic properties. LD is the most used indicator muscle in meat quality studies, and the main emphasis in the meat quality evaluation here was thus put on this muscle. QF consists of four parts, and is therefore difficult to use in many meat quality analyses. BF was taken in generation 4 in order to include an easily defined ham muscle.

The higher oxidative and lower glycolytic capacity in QF than in LD is to some extent probably related to the higher Type IIA/IIB ratio in QF. Type IIA fibres usually have a higher oxidative capacity than Type IIB fibres (ESSEN-GUSTAVSSON & LINDHOLM, 1984). The same differences in metabolic profile between these two muscles, were also found in generation 4. However, there seemed to be a slightly higher oxidative capacity in generation 4 in both muscles. The greatest change in oxidative capacity was found in animals fed the LP diet. In HP animals the glycolytic capacity was increased in the 4th generation, whereas the capacity for phosphorylation of glucose was however, lowered as indicated by the HK activity. These results suggest that the concentration of protein in the diet could influence the metabolic profile of the muscle. In this study it was also seen that the amount of glycogen at slaughter is important for ultimate pH. In both generations the glycogen content differed between muscles, with a much higher glycogen content in LD in comparison with QF. This is likely related to the differences seen in fibre typing and metabolic profile between the muscles. It has been shown that Type I and IIA fibres were depleted of glycogen in connection with slaughter, while Type IIB fibres still had glycogen left (ESSEN-GUSTAVSSON et al., 1991). All muscles studied showed a negative correlation between glycogen content and pH_u, which shows the importance of the muscle glycogen concentration at slughter for the ultimate meat quality.

It can be concluded that technological meat quality was slightly better in animals fed the

HP compared with the LP diet indicated by a higher water holding capacity, lower reflectance and higher protein extractability. This result was consistent in both generations. A high meat content in the carcass has often been related to poorer meat quality, but this was not found here. An explanation for this may be that in studies where a high meat content was negatively correlated to meat quality, animals with the halothane gene were usually included. The preselection against the halothane gene which was done before the Swedish selection experiment started may be the explanation why this negative relationship was not found here.

A low water holding capacity is an indication of PSE (Pale, Soft, Exudative) meat. There was no significant difference in drip loss between the two diets, but the difference in filter paper wetness was significant. In the study of KAUFFMAN et al. (1986), which included PSE carcasses, a high correlation between these two methods (r=0.90) was found. In our study, the correlation was significant but lower (r=0.42; p<0.001). In meat from PSE carcasses the intracellular space is reduced as the myofibrills are more compressed than in normal meat (OFFER et al., 1988). This will result in a transport of the muscle fluid to the extracellular space from which it can be released as drip loss. The difference in significance levels between the methods used here for measuring water holding capacity may be explained by the fact that there was a low PSE frequency in our material. A significant difference between diets was found in the extractability of sarcoplasmic proteins but not of the myofibrillar proteins. The sarcoplasmic proteins, which account for 30% of the total muscle proteins, can bind about 3% of the muscle water content (KUNZ and KAUFFMAN, 1974, cit. LOPEZ-BOTE et al., 1989). When these proteins denature, the liberated water can still be held in the muscle by capillary forces, and may therefore not be detected as drip loss. The significant differences in filter paper wetness may be explained by the fact that the capillary force which occurs when the filter paper is applied on the meat surface, can help to release the water which was liberated when the sarcoplasmic proteins were denatured (E. TORNBERG, 1991, personal communication). A possible explanation for the difference in extractability of the sarcoplasmic proteins between diets may be the large difference in subcutanous fat, thus decreasing the cooling rate for carcasses from pigs on the LP diet.

When measuring the meat colour as reflectance the choice of wave length is of importance, as the reflectance is affected by factors such as pigment content, protein structure and IMF content. Surface reflectance measured with the EEL-Y will be influenced by all these three factors. Internal reflectance measured with the GP is only slightly influenced by pigment content when the PSE-frequency is low (LUNDSTRÖM et al., 1988).

CONCLUSIONS: The changes seen in enzyme activities between generations indicate that selection for high meat content and/or the protein level of the diet may influence muscle metabolic profile.

The difference between diets for technological meat quality was consistent between generations, with a slightly better meat quality in carcasses from the HP line, probably due to a slower rate of cooling in the fat LP pigs. A high meat content is usually related to a decrease in meat quality, but this was not found here. The preselection made on the animals used in this study to eliminate the halothane gene before the selection experiment was started could be an explanation for that. A slight increase in the water-holding capacity between the generations was seen. If this was due to selection or to an improvement in handling of the pigs before slaughter cannot be determined.

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Table 1. Least-squares means and levels of significance for muscle characteristics between the two protein diets in generation 1 and 4

	Generation 1					Generation 4					
	LD			QF		LD			BF			QF	
Variable	HP	LP		HP	LP	HP	LP		HP	LP		HP	LP
Fibre type (%)													
Type I	11	8	n.s.[1]	7	15	**	7	7	n.s.	25	21	#	18	16 n.s.
Type IIA	8	9	n.s.	22	26	n.s.	9	8	n.s.	10	11	n.s.	23	30 *
Type IIB	81	83	n.s.	72	60	*	82	83	n.s.	65	67	n.s.	57	53 n.s.
IIA/IIB[2]	0.1	0.1	n.s.	0.2	1.0	*	0.1	0.1	n.s.	0.2	0.2	n.s.	0.7	0.5 n.s.
Enzyme activity ($mmol*kg^{-1}*min^{-1}$)														
CS	6	6	n.s.	15	17	n.s.	9	10	n.s.	16	16	n.s.	17	24 **
HAD	10	14	***	14	15	n.s.	14	14	n.s.	15	16	n.s.	16	20 *
HK * 100	46	35	*	56	49	n.s.	32	40	n.s.	39	45	n.s.	40	52 *
LDH	2975	2515	***	1970	1661	***	3379	2716	***	3238	2610	***	2298	1848 ***
Glycogen ($mmol*kg^{-1}$)	160	221	**	93	75	n.s.	245	204	*	180	179	n.s.	115	71 n.s.

[1] Levels of significance: n.s. = p>0.10; # = p<0.10; * = p<0.05; ** = p<0.01; *** = p<0.001.
[2] Ratio between type IIA and IIB fibres

Table 3. Least-squares means and levels of significance[1] for meat quality parameters between lines in generation 1 and 4

	Generation 1			Generation 4		
Variable	LP	HP		LP	HP	
M.longissimus dorsi						
Drip loss (%)	4.7	4.5	n.s.	4.0	3.6	n.s.
Filter paper wetness	2.8	0.7	**	1.2	0.8	*
EEL-Y	21.6	19.3	*	18.2	17.9	n.s.
GP	97	72	***	95	86	***
Protein extractability (mg/g wet wt.)						
Total	146.7	166.6	#	128.9	146.0	#
Sarcoplasmic	57.4	62.0	n.s.	66.0	73.2	**
Pigment (ppm)	32.4	36.6	n.s.	38.6	40.0	n.s.
pH_u	5.63	5.75	#	5.55	5.53	n.s.
M.biceps femoris						
Drip (%)	-	-		2.2	2.7	n.s.
Filter paper wetness	-	-		0.7	0.8	n.s.
GP	-	-		84	88	#
EEL-Y	-	-		15.4	16.1	n.s.
Protein extractability (mg/g wet wt.)						
Total	-	-		141.7	146.0	n.s.
Sarcoplasmic	-	-		62.4	69.6	***
pH_u	5.88	5.87	n.s.	5.88	5.74	#
M. quadriceps femoris						
pH_u	6.15	5.95	#	6.15	5.89	*

[1] See table 1.