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MUSCLE CHARACTERISTICS AND METABOLIC RESPONSE AT SLAUGHTER IN DOMESTIC PIGS REARED EITHER OUTDOORS OR INDOORS.

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INTRODUCTION

Through years of selection pig producers have succeeded in developing a fattening pig with rapid muscle growth and a large muscle mass. During recent years, however, there have been several discussions about animal welfare and criticism has been raised against intensive breeding of pigs. Some pig producers therefore take greater interest nowadays in free range pigs, both for ecological and economic reasons. Among consumers there is also an increasing demand for pork meat which has been produced from pigs that have been brought up under more natural conditions than in the conventional pig industry.

Conventionally raised pigs in contrast to free range pigs, are kept indoors in small pens, which make them almost totally physically inactive. Physical activity in these pigs occurs mainly in connection with transportation and handling at slaughter, events which must thus be considered as physically stressful. A good marker for physical stress is the lactate concentration in the blood which increases with the intensity of exercise (Kallweit, 1982; Jensen-Waern and Nyberg, 1993). Stressful events prior to slaughter, such as handling, transportation and lairage time are important factors that are known to influence the quality of meat (see Fernandez and Thornberg, 1991).

In pigs, as in other mammals, the oxidative capacity of the skeletal muscles increases if the animal is physically active (Essén-Gustavsson, 1986; Essén-Gustavsson *et al.*, 1988). Trained pigs have a higher oxidative capacity in their muscles and tolerate physical stress better, as they show a lower blood lactate response to submaximal exercise compared with untrained pigs (Lindberg *et al.*, 1973; Essén-Gustavsson and Lindholm, 1983). The purpose of this study was to determine whether free range pigs, which are adapted to some exercise, tolerate pre-slaughter treatment better than fattening pigs which have been reared conventionally. From both groups, blood samples were collected in connection with exsanguination and muscle samples were obtained pre- and post-slaughter for analysis of muscle characteristics and the metabolic response.

MATERIALS AND METHODS

Animals

Twenty crossbreed (Yorkshire. Swedish Landrace. Hampshire) pigs, with a mean weight of 22kg, were divided into two groups with ten animals in each. One group was kept outdoors (O group) in an enclosed pasture, covering a hilly area of 6000m^2. Two huts with straw as bedding were used as shelters for the animals. The other group was housed indoors (I group) in conventional pens. Both groups were handled and fed by the same people. The animals were given a barley-based diet twice daily, according to the feeding scale used at the Swedish University of Agricultural Sciences (Andersson, 1985).

Experimental procedure

After 19 weeks, when the mean weight of the pigs was 107kg, they were transported by lorry for 30 minutes to the slaughterhouse. The animals were then rested in the lairage for three hours before the CO_2-stunning and exsanguination. One week prior to slaughter, muscle samples were obtained from the *longissimus dorsi* muscle by the needle biopsy technique (Bergström, 1962). During the biopsy procedure, the pigs were kept in a small pen for a few minutes. The incision was made at a point between the last rib and the iliac crest. Samples were also taken from this muscle and the papillary muscle of the left cardiac ventricle 45 to 60 minutes after exsanguination. The skeletal muscle sample was divided into two pieces. One piece was taken for histochemical analysis and rolled in talcum powder before being frozen in liquid nitrogen and the other piece and the papillary muscle were used for biochemical analysis and frozen immediately in liquid nitrogen. All samples were stored at -80°C until analyzed.

Blood samples for determination of the concentrations of lactate and potassium were collected in heparanised tubes at exsanguination.

Histochemical analyses

Serial cross-sections (10μm) were obtained by using a cryostat microtome at -20°C. Sections were stained for myofibrillar ATPase after both alkaline (pH10.3 for 10 minutes at 37°C) and acid (pH4.3 for three minutes at room temperature [RT] and pH4.6 for five minutes at RT) preincubation (Brooke and Kaiser, 1970). At least 200 fibres were identified from photomicrographs as types I, IIA and IIB.

Biochemical analyses

Muscle samples for enzyme analyses were freeze-dried, dissected free of connective tissue, fat and blood under a dissection microscope, weighed and homogenised in a phosphate buffer (pH7.3) with an ultrasonic disintegrator. The activities of the following enzymes were assayed by fluorometric techniques at 25°C: citrate synthase (CS), 3-OH-acyl-CoA-dehydrogenase (HAD), lactate dehydrogenase (LDH) and hexokinase (HK) (Essén *et al.*, 1980; Essén-Gustavsson *et al.*, 1984). The samples for glycogen analyses were weighed and then boiled in 1M HCL in sealed tubes to obtain glucose residues. The samples for lactate analyses were weighed and then extracted in perchloric acid and neutralised with K_2HCO_3. The contents of glucose and lactate were measured fluorometrically (Lowry and Passoneau, 1973). Muscle pH was analyzed on a pH-meter after homogenisation of muscle in iodoacetate neutralized to pH7.0.

Plasma lactate and potassium

The concentration of lactate in plasma was determined with a lactate analyzer (Analox GM7, Analox Instruments Ltd, London, UK). The plasma potassium concentration was assayed using a seralyser reflectance photometer (Ames).

Statistical analyses

The Mann-Whitney U test was used to compare the results between the O and I groups. Differences were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

The fibre type distribution of the *longissimus dorsi* muscle was similar in both groups (Table 1). In the biopsy specimens of the *longissimus dorsi* muscle, the activities of the enzymes CS and HAD, markers for oxidative capacity,

were significantly higher in the O group than in the I group (Table 1). In the muscle samples obtained post-slaughter, the activity of HAD was significantly elevated in the O group compared with the I group (Table 1). There was no significant difference in CS but only one pig in the I group in contrast to five pigs in the O group showed CS activity of above 10mmol/kg/min. The activities of the enzymes LDH and HK markers for glycolytic capacity were of the same magnitude in both groups (Table 1). There was a slight difference in HAD and LDH activities between pre- and post-slaughter samples. One explanation for this may be that the samples were not taken from exactly the same site. The pattern of the enzyme activities in the myocardium was similar to that in the skeletal muscle, with higher CS activity in the O group. The activities of the enzymes CS, HAD and HK were much higher in the myocardium than in the skeletal muscles, whereas the LDH activity was lower (Table 1). The fibre type composition and enzyme activities in the *longissimus dorsi* muscle in this study are in accordance with earlier reports (Essén-Gustavsson and Fjelkner-Modig, 1985; Enfält *et al.*,1993). Compared with many animal species, the porcine muscle and especially the *longissimus dorsi* contains a high proportion of type IIB fibres, which have a low oxidative capacity (Essén-Gustavsson and Lindholm, 1984). These fibres therefore have to rely on anaerobic metabolism of carbohydrates for their energy production. A high plasma lactate concentration can be expected during physical exercise when these fibres are recruited. However, pigs trained on a treadmill have a lower lactate response during exercise compared with untrained pigs and this is associated with a higher oxidative capacity (Essén-Gustavsson and Lindholm, 1983, Essén-Gustavsson *et al.*, 1988). Pigs trained on a treadmill also had lower lactate levels at exsanguination compared with untrained pigs (Lindberg *et al.*, 1973). When pigs raised in small pens ($0.5m^2$) were compared with those raised in larger pens ($0.9m^2$) no difference was observed in the lactate response at exsanguination (Lannek *et al.*, 1974). In a recent study, domestic pigs were allowed to move up to 735m indoors in the piggery for five days a week during their fattening period (Enfält *et al.*, 1993). This type of activity was too modest, however, to cause any adaptational changes in the muscle metabolic profile in comparison to litter mates kept in pens. In the present study, the pigs kept outdoors carried out moderate physical activity during the whole fattening period, which seems to have had a training effect. The oxidative capacity as indicated by the enzyme activities in both the myocardium and the longissimus dorsi muscle was also greater in most of the pigs kept outdoors compared with those kept indoors. The lactate concentration in the plasma at exsanguination was also significantly lower in the O group than in the I group (Table 2). This indicates that the pigs kept outdoors tolerated the stress imposed on them by the transport and handling better than the pigs kept indoors. This is also supported by the lower potassium concentration seen at exsanguination in pigs kept outdoors (Table 2). A correlation was found between the lactate and potassium concentrations ($r=0.79$, $P<0.001$). The results agree with those in man, calves and horses where potassium has been found to increase with the intensity of exercise and to be lower in trained than untrained subjects (Kjeldsen *et al.*, 1990; Fosha-Dolezal and Fedde, 1988; Sjogaard *et al.*, 1985; Harris and Snow, 1986). High potassium concentrations can give rise to ventricular tachycardia and cardiac arrest (Poole-Wilson, 1984). Pigs sometimes die during transportation to the abattoir and in the lairage and hyperkalaemia may thus be one reason for these sudden deaths.

The glycogen content of the skeletal muscles and of the myocardium showed no differences between the groups (Table 2), but a large inter-individual variation was noted. In the myocardium only one pig in the I group but six pigs in the O group had glycogen content of over 20mmol/kg. The large variation in glycogen content between individuals in both the O and I groups may be related both to genetic factors and to different exposure to physical stress during pre-slaughter treatment. Recently it has been suggested that a major gene RN^-/rn^+ influences the glycogen content of muscle (see Fernandez and Thornberg, 1991). Comparisons involving Hampshire pigs indicate that these are carriers of the RN^- allele and have a higher glycogen content in *longissimus* when compared to other breeds. Since the pigs in the present study were Hampshire crosses, this may be a factor of importance for the large variation found in glycogen content in both the O and I groups. A correlation was found between the pre- and post-slaughter glycogen contents of the *longissimus dorsi* muscle ($r=0.80$, $P<0.001$). This supports that genetic factors could play a role. The glycogen content was lower in post- than in pre-slaughter samples and the difference was correlated to the post-slaughter muscle lactate content ($r=0.65$, $P<0.01$). The muscle lactate content was slightly higher in the O group than in the I group (Table 2). That lactate production influence muscle pH is shown by the negative correlation between muscle pH and lactate content ($r=0.85$, $P<0.001$). Glycogen is an important substrate for energy production in muscle during pre-slaughter treatment and the post-mortem process. The quality defect dark, firm and dry meat (DFD) is a consequence of low muscle glycogen content at slaughter causing high muscle pH post-mortem. It has been shown that free range pigs have a calmer behaviour at lairage and show lower pH_2-values and a lower incidence of DFD than pigs raised

indoors (Barton-Gade and Blaabjerg, 1989). In the present study muscle pH post-slaughter varied between pigs but did not differ between groups. Of interest was that three pigs had high pH values (>6.3) and low glycogen content in both the myocardium and *longissimus dorsi* muscle and they belonged to the group that had been kept indoors.

CONCLUSION

The results of the present study show that the exercise attained by free range pigs will give rise to an adaptational response in muscle that will lead to an improved aerobic metabolism and cause lower blood lactate and potassium concentrations at slaughter. This indicates that free range pigs can tolerate pre-slaughter treatment better than conventionally raised pigs.

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Table 1. Enzyme activity (µmol/g/min) and fibre types (%) in muscle samples from pigs kept indoors (I group) and outdoors (O group). Values are given as means ± SD.

	I group	O group	Level of significance
m.longissimus pre-slaughter	n = 10	n = 10	
CS	7 ± 1	9 ± 1	P<0.05
HAD	21 ± 6	31 ± 9	P<0.01
HK	0.5 ± 0.3	0.5 ± 0.3	NS
LDH	3057 ± 391	2860 ± 130	NS
m.longissimus post-slaughter	n = 8	n = 10	
CS	8 ± 2	9 ± 2	NS
HAD	11 ± 2	14 ± 3	P<0.05
HK	0.6 ± 0.4	0.8 ± 0.2	NS
LDH	2014 ± 355	1928 ± 207	NS
Myocardium post-slaughter	n = 9	n = 10	
CS	96 ± 8	105 ± 9	P<0.05
HAD	91 ± 6	96 ± 9	NS
HK	12 ± 2	13 ± 3	NS
LDH	527 ± 71	591 ± 68	NS
Fibre types m.longissimus pre-slaughter	n = 7	n = 10	
I	14 ± 4	14 ± 4	NS
IIA	9 ± 2	8 ± 4	NS
IIB	77 ± 5	78 ± 4	NS

Table 2. Plasma lactate and potassium concentrations (mmol/l) at exsanguination, and glycogen and lactate concentrations (mmol/kg) and pH in muscle samples from pigs kept indoors (I group) or outdoors (O group). Values given as means ± SD.

	I group	O group	Level of significance
Blood samples	n = 10	n = 10	
Plasma lactate	11.9 ± 3.2	7.8 ± 1.1	P<0.01
Plasma potassium	11.2 ± 0.7	10.3 ± 0.5	P<0.01
m.longissimus pre-slaughter	n = 10	n = 10	
Glycogen	342 ± 129	422 ± 135	NS
m.longissimus post-slaughter	n = 8	n = 10	
Glycogen	235 ± 142	279 ± 113	NS
Lactate	279 ± 100	399 ± 111	P<0.01
pH	6.17 ± 0.27	5.96 ± 0.18	NS
Myocardium post-slaughter	n = 9	n = 10	
Glycogen	19 ± 11	31 ± 21	NS
pH	6.19 ± 0.10	6.14 ± 0.13	NS