MUSCLE METABOLISM AND GLYCOGEN DEPLETION WITHIN FIBRETYPES AT SLAUGHTER IN PIGS WITH DIFFERENT HALOTHANE GENOTYPES. RELATION TO MEAT QUALITY PROPERTIES.

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SUMMARY

Muscle samples were taken immediately after exsanguination from *M.longissimus dorsi* of pigs with different halothane genotypes (NN and nn). Fibre type composition was similar between groups but the nn-genotype had larger mean fibre areas and lower capillary density than the NN-genotype. The nn-genotype had higher lactate and ammonia and lower glycogen and phosphagen concentrations and twice as many glycogen depleted type IIA and IIB fibres as compared with the NN-genotype. Values were highest for EEL and drip loss in the nn-genotype. Glycogen depleted type IIB fibres were negatively correlated to ATP and CP and positively to lactate and ammonia concentrations and EEL and drip loss. Drip loss was negatively correlated to ATP and CP and positively to ammonia concentrations. These data indicate that stress situations pre-slaughter of pigs with the nn-genotype give rise to phosphagen breakdown, lactate and ammonia formation and a high degree of glycogen depleted type II fibres.

INTRODUCTION

One condition of abnormal meat of poor quality is when it appears as pale, soft and exudative (PSE). This condition is known to be related to increased ATP turnover and a high rate of glycolysis with lactate formation which will give a rapid fall in pH post-mortem (Honikel and Kim 1986). PSE-meat often develop in pigs with the genotype Hal^nHal^n (nn-genotype) which are known to be more stress-susceptible than pigs with genotype Hal^NHal^N (NN-genotype) (Webb et al. 1982). In a recent study it was shown that nn-genotype had lower phosphagen (ATP + CP) and glycogen concentrations and higher lactate concentrations when slaughtered as compared with the NN-genotype (Lundström et al. 1985). When there is I high ATP turnover rate the purine nucleotide cycle may be stimulated and give rise to IMP and ammonia formation (Lowenstein 1972). In metabolic stress situations as when rats and horses perform intense exercise there is a high demand for ATP and IMP and ammonia concentrations increase in their muscles (Meyer et al. 1980; Snow et al. 1985; Essén-Gustavsson and Valberg 1986). From studies on contracting rat muscles it has also been indicated that muscles containing type I (slow twitch) fibres respond differently in their management of phosphagen stores and purine nucleotide cycle metabolites as compared with muscles containing type II (fast twitch) fibres (Terjung et al. 1985). It was therefore of interest to further study the muscle metabolism and especially ammonia formation as well as glycogen depletion within fibretypes in muscles of pigs which were raised and slaughtered under similar conditions but had different halothane genotypes. Furthermore, the aim was to study how the muscle metabolic response at slaughter was related to meat quality properties.

MATERIALS AND METHODS

A group of 19 crossbred pigs (Swedish Yorkshire x Swedish Landrace) were used in this study. The halothane genotypes were determined by means of halothane anesthesia and blood typing (Gahne and Juneja 1985). Ten of the pigs had the genotype NN and nine had genotype nn. Samples from *M. longissimus dorsi* were taken immediately after exsanguination. The piece of muscle was divided into two parts. One piece for biochemical analyses was immediately frozen in liquid nitrogen, while the piece for histochemical analyses was rolled in talcum powder before freezing to prevent artifacts. Both samples were stored at -80°C until analysed.

Biochemical analyses:

A piece of the muscle was freeze dried over night and dissected free from blood, fat and connective tissue. A portion (1-2 mg) of the dissected muscle was weighed for glycogen analyses. The samples were then put into a tube containing 1 ml of 1M HCL and heated to 100°C for 2 h. Glycogen was analysed as glucose residues with fluorimetric techniques (Lowry and Passonneau 1973). Another part of the muscle (1-2 mg), was analysed for lactate, ATP and CP (Lowry and Passoneau 1973).

Table 1. Mean ± SD of fibre type composition, mean fibre area and capillary density in M. longissimus dorsi of pigs with different halothane genotypes

	NN-genotype	nn-genotype
Fibre types (%)		
I	14±6	11±2
IIA	8±3	7±2
IIB	78±7	82±3
Mean fibre area ($m^2 \cdot 10^2$)	51±14[b]	75±17
Capillary density (cap/mm^2)	240±58[c]	184±38

Significant differences b: $p<0.01$; c: $p<0.05$.

Table 2. Mean ± SD of lactate, ammonia, ATP, CP and glycogen concentrations (mmol/kg) and EEL and drip loss (%) in M. longissimus dorsi of pigs with different halothane genotypes.

	NN-genotype	nn-genotype
Lactate	90±47[a]	165±29
Ammonia	0.38±0.20[a]	1.16±0.46
ATP	23±3[a]	15±4
CP	48±23[a]	12±10
Glycogen	232±78[b]	136±57
EEL	21.7±1.8[b]	27.0±3.7
Drip loss	2.4±1.2[a]	5.3±1.1

Significant difference between groups a: $p<0.001$, b: $p<0.01$.

Table 3. Mean ± SD of glycogen depleted fibres (%) from M. longissimus dorsi of pigs with different halothane genotypes.

	NN-genotype	nn-genotype
depleted fibres (%)	8±7[a]	21±8
depleted + nearly depleted fibres (%)	19±10[b]	35±10
depleted fibres (%)		
type I	38±28	47±42
IIA	40±37[c]	78±25
IIB	14±9[a]	29±8

Significant difference between groups
a: $p<0.001$, b: $p<0.01$, c: $p<0.05$.

Ammonia analyses were performed on wet muscle (Kun and Kearney 1974).

Histochemical analyses:

Transverse serial sections were cut in a cryostat and stained for myofibrillar ATP-ase after preincubation at pH 4.3, 4.6 and 10.3 (Brooke and Kaiser 1970). The percentage of each fibretype was calculated from a minimum of 200 fibres. The amylase-PAS staining was used to visualise capillaries (Andersen 1975). Capillary density was evaluated and fibre areas were measured on photomicrographs of the amylase-PAS stains using a MOP-digiplan analyser. Glycogen depletion pattern in the muscle was studied using periodic acid-Schiff's (PAS) stained sections (Pearse 1961). Fibres were classified as depleted when unstained or nearly depleted when lightly stained, and they were also identified according to fibretype. The percentage of each fibre type classified as depleted was calculated.

EEL:

Surface reflectance value was measured with an EEL reflectance spectrophotometer (Evans electroselenium Ltd, Halstead, U.K.). The Y-filter was used given a measure of visual brightness (Lundström 1975). Drip loss: Drip loss was determined as the percentage weight loss of a sample (about 650 g) from *M. longissimus dorsi* cut at the last rib and backwards. The samples were kept in trays at +4°C for 24 hours (Lundström and Malmfors 1985).

RESULTS

Data from histochemical analyses are shown in table 1. Fibre type composition did not differ between genotypes but mean fibre areas were larger and capillary density lower in the nn-genotype compared with the NN-genotype.

Data from biochemical analyses and meat quality parameters are shown in table 2. Glycogen, ATP and CP concentrations were lower and lactate and ammonia concentrations higher in the nn-genotype compared with the NN-genotype. The highest values for EEL and drip loss were obtained in meat from the nn-genotype. Glycogen depletion pattern are shown in table 3. The pigs with the nn-genotype had a higher amount of depleted fibres than the pigs with the NN-genotype. There was no difference in the percentage of depleted type I fibres whereas almost twice as many type IIA and IIB fibres were depleted.

DISCUSSION

The data of this study indicate that in stress-susceptible pigs in connection with slaughter, not only glycogenolysis but also the purine nucleotide cycle is activated to a great extent. The purine nucleotide cycle in which AMP is deaminated to IMP with subsequent ammonia formation has been shown to be of importance when energy demand is excessive as when rats or horses exercise or when muscles are electrically stimulated (Essén-Gustavsson and Valberg 1986; Snow et al . 1985; Terjung et al. 1985). It was therefore of interest to note that the stress in connection with slaughter seemed to cause not only lower glycogen and phosphagen stores and greater lactate concentrations but also that higher ammonia concentrations were found in muscles of pigs with the nn-genotype. In the rat studies, ammonia was also shown to be produced especially in those muscles which contain a high percentage of non-oxidative type II (fast twitch) fibres (Terjung et al. 1985). In pigs, *M. longissimus dorsi* consists of as much as 80-90% non-oxidative type IIB fibres (Essén-Gustavsson and Lindholm 1984).

The data of the present study also showed that glycogenolysis was marked in type IIB fibres, and that these were depleted to a higher degree in stress-susceptible pigs. Positive correlations were seen between the percentage of depleted type IIB fibres and both muscle lactate and ammonia concentrations, and

negative correlations to both ATP and CP concentrations. In agreement with type IIB fibres having a low capacity for oxidative metabolism these fibres therefore seem to release energy mainly through glycogenolysis, breakdown of creatine phosphate and ATP. Furthermore, the data indicate that ATP was regenerated from ADP through the myokinase reaction, and that AMP was deaminated to IMP by adenylate deaminase, giving rise to the high ammonia concentrations. The myokinase reaction and deamination of AMP may have played an important role for ATP regeneration especially in those fibres which were depleted of glycogen. Of note was also, that ammonia concentrations were markedly increased when lactate concentrations were high which is associated with low muscle pH. The activity of adenylate deaminase has been shown in vitro to increase with lowering of pH (Wheeler and Lowenstein 1979). An extreme Cellular acidosis has also been shown to enhance adenylate deaminase activity during intense contractions of rat muscle (Dudley and Terjung 1985). Muscle IMP can further be degraded to uric acid and give rise to free radical formation which is said to induce cellular damage, especially of membranes (Banister et al. 1985). This may therefore be one important factor that could influence meat quality characteristics and especially drip loss. It was therefore interesting to note the positive correlation between ammonia levels and drip loss. A great drip loss and a high value for EEL are both indicating meat of poor quality. That EEL and drip loss which both are measured the day after slaughter are influenced by the metabolic response obtained in connection with slaughter was clearly shown in this study. Both drip loss and EEL were negatively correlated to ATP and CP concentrations and positively correlated to lactate and ammonia concentrations. A positive correlation was also seen to the amount of type II fibres that were glycogen depleted. Thus, at slaughter when there is a marked glycogenolysis in many type IIB fibres with subsequent ammonia formation, this will have a negative influence on meat quality characteristics. Another interesting finding, is that stress-susceptible pigs have large mean fibre areas and thus a low muscle capillarisation compared with the NN-genotype. This may indicate, that in stress-susceptible pigs the diffusion of muscle lactate into blood is limited because muscle fibres are large. Lactate can thus rapidly accumulate and give rise to low pH which may activate both myokinase giving rise to AMP and adenylate deaminase giving rise to IMP, with subsequent ammonia formation. These metabolic changes may then be related to damages of muscle cell membranes which are said to be responsible for a rapid drip loss (Honikel and Kim 1986). It was also shown that mean fibre areas correlated to both lactate, ATP and CP levels as well as drip loss.

ACKNOWLEDGEMENT

This study was made possible by grants from the Swedish Council for Forestry and Agricultural Research.

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