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Adenine nucleotide breakdown products in muscle at slaughter and their relation to meat quality in pigs with different halothane genotypes

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SUMMARY: Adenine nucleotides and their breakdown products were analysed in muscle samples from pigs with different halothane genotypes (NN and nn). The muscle samples were taken immediately after exsanguination. The total adenine nucleotide pool was 27% lower in nn-pigs than NN-pigs and an inverse relationship was seen between adenine nucleotide content and IMP levels. High IMP concentrations were observed when initial muscle pH was below 6.4. Values for EEL and drip loss showed a close relationship with adenine nucleotide and IMP concentrations. These results underscore that a sufficient capacity of the muscle to regenerate ATP in connection with stress-situations preslaughter is of importance for obtaining meat of good quality.

INTRODUCTION: It is well known that meat from halothane-positive pigs often is pale, soft and exudative (PSE) and thus of poor quality (WEBB et al., 1982). The development of PSE-meat is said to be related to a rapid ATP breakdown and a high rate of glycolysis postmortem, inducing a lowering of pH and therefore denaturation of muscle proteins (BENDALL and WISMER-PEDERSEN, 1962). PSE-meat is characterized by reduced water-holding capacity (BRISKEY, 1964). High values for drip loss and reflectance are often observed in PSE-meat (BARTON-GADE, 1979). Recent studies have shown that, after exsanguination, stress-susceptible pigs with genotypes Hal^n Hal^n (nn-pigs) have lower ATP, CP and glycogen concentrations and higher lactate concentrations and also lower initial muscle pH, compared with non stress-susceptible pigs with genotypes Hal^N Hal^N (NN-pigs) (LUNDSTRÖM et al., 1989, ESSÉN-GUSTAVSSON et al., 1991). The highest values for drip loss and reflectance were also found in the muscle samples from the nn-pigs. Muscle metabolic response in these pigs was further investigated with a special aim to study adenine nucleotide breakdown products and their relation to initial muscle pH and meat quality.

MATERIAL AND METHODS: The nineteen crossbred pigs (Swedish Yorkshire x Swedish Landrace) used in this study are the same as have been described in a previous report (ESSÉN-GUSTAVSSON et al., 1991). Ten of the pigs had the genotype NN and nine had the genotype nn. All pigs were fed, raised and handled under similar conditions at a research station. The pigs were slaughtered when they reached a body weight of 100 kg, following 5 km transport from the research station to the slaughter house. They were kept in lairage for 2 h without mixing with pigs from other herds and electrically stunned with a low voltage shock on the floor, thereby avoiding the use of a restrainer. Muscle samples were obtained from M. longissimus dorsi immediately at exsanguination. The samples were stored at -70°C until biochemical analyses were performed. In connection with carcass evaluation about 24 h after slaughter, meat quality measurements were performed on M. longissimus dorsi.

Biochemical analyses: Part of the muscle was freeze-dried and dissected free from blood, fat and connective tissue. One to two mg of muscle was then extracted in perchloric acid and neutralised with KOH. After centrifugation, extracts were stored at -70°C until analysed for ATP, ADP, AMP, IMP, hypoxanthine, xanthine and uric acid with HPLC-technique using a reversed phase C18 column (Biophase ODS C18, 250x4.6 mm 5 µm). Separation of nucleotides and their breakdown products was performed under isocratic conditions with 50 mM Na-citrate buffer (pH 6.0) containing 10 mM tetrabutylammonium dihydrogenphosphate and 2.5% acetonitril, at a flow rate of 0.8 ml/min, UV-detection at 254 nm and oven temperature of 35°C. Concentrations of the nucleotides and their breakdown products were calculated from chromatograms by comparison to external standards. Muscle pH on the sample taken immediately at exsanguination (pHe) was analysed by homogenisation of muscle in iodoacetate (TARRENT et al., 1972).

Meat quality analyses: Surface reflectance value was measured with an EEL reflectance spectrophotometer (Evans electroselenium Ltd, Halstead, UK). The Y-filter was used giving a measure of visual brigthness (Lundström, 1975). Drip loss was determined as the percentage weight loss of a sample (about 600 g) cut at the last rib and backwards. The samples were kept in trays at 4°C for 24 h (LUNDSTRÖM and MALMFORS, 1985).

Statistics: Conventional statistical methods have been used to calculate the mean values and standard deviations. Differences between NN- and nn-genotypes were tested for significance using the unpaired Students t-test. Correlations were made on the whole material including both genotypes.

RESULTS: Data from the biochemical analyses and meat quality parameters are shown in table 1. It was possible to detect peaks corresponding to ATP, ADP, AMP and IMP in all the muscle samples, whereas peaks for hypoxanthine,

xanthine and uric acid were not detectable in most of the samples. Only in a few samples, small peaks for hypoxanthine were seen at concentrations less than 0.01 mmol/kg d.w. The mean IMP concentration in nn-pigs was 4 times higher and the ATP concentration half of what was observed in NN-pigs. No marked differences were seen in ADP and AMP concentrations between the groups. The total adenine nucleotide pool (ATP+ADP+AMP) was 27% lower in nn- than NN-pigs. A strong inverse relationship was seen between the total adenine nucleotide pool and IMP concentrations ($r=-0.96$). High IMP concentrations were only seen when pH values were below 6.4 (fig. 1). IMP concentrations were correlated to the meat quality parameters, EEL ($r=0.65$) and drip loss ($r=0.74$).

DISCUSSION: The results of this study clearly show that in spite of similar slaughtering conditions, concentrations of adenine nucleotides and IMP differ already at exsanguination in pigs with different halothane genotypes. Interestingly, the sum of the concentrations of IMP and adenine nucleotides did not differ between the nn- and NN-pigs. This suggests that a reduction in the total adenine nucleotide pool in the nn-pigs occur in connection with slaughter. Previous studies in which muscle samples have been taken within one hour after slaughter have also shown higher IMP and lower ATP concentrations in stress-susceptible pigs in comparison with normal pigs (HONIKEL AND FISCHER, 1977, TSAI et al., 1972). Hypoxanthine levels were also shown to rise with time postmortem (HONIKEL and FISCHER, 1977). High concentrations of hypoxanthine and xanthine were not observed in the present study, which indicates that in this early post-mortal phase, IMP had not further been degraded.

The muscle samples were taken only a few minutes after the pigs had been electrically stunned, which implies that the differences in metabolic response observed in these muscles were related to preslaughter treatment. Since nn-pigs are more stress-susceptible they are likely to react more strongly to physical activity and mental stress prior to slaughter. This is in accordance with the high catecholamine levels that have been observed in halothane sensitive stress-susceptible pigs (HÄGGENDAL et al., 1988). It was previously shown that the nn-pigs of this study have more glycogen depleted fibres, higher lactate and ammonia concentrations and larger fibre areas and lower capillarization in muscle compared with NN-pigs (ESSÉN-GUSTAVSSON et al., 1991). The lower capillarization will result in a limitation in oxygen supply to and efflux of lactate and ammonia from the fibres when the pigs are alive. This suggests, that muscle fibre properties may be an important factor that influence ATP turnover. When the shortage of oxygen limits oxidative metabolism of ATP it can be regenerated by breakdown of CP and glycogenolysis with lactate formation. The anaerobic ATP regeneration was indicated in the nn-pigs by a high percentage of the glycogen depleted fibres and the markedly lowered CP concentrations (ESSÉN-GUSTAVSSON et al., 1991). ATP regeneration from ADP through the myokinase reaction with formation of AMP, may therefore become the most important metabolic pathway for the demand of energy in the muscle fibres. If AMP, however, is deaminated there will be a loss in the total pool of adenine nucleotides. In this study it was notable, that high IMP concentrations were only observed when muscle pH was below 6.4, close to where the activity of AMP-deaminase has its pH-optimum (WHEELER and LOWENSTEIN, 1979). In the AMP deaminase reaction not only IMP but also ammonia is produced (LOWENSTEIN, 1972). A close relation was seen between IMP and the previously measured ammonia concentrations ($r=0.89$). It is therefore likely that the high ammonia concentrations seen in nn-pigs are produced by AMP-deamination.

CONCLUSIONS: Concentrations of adenine nucleotides and IMP differ already after exsanguination in pigs with different halothane genotypes. High values for EEL and drip loss are used as indicators for poor meat quality. The correlations found in this study between these parameters and adenine nucleotides and IMP therefore further underscore, that a sufficient capacity of the muscle to regenerate ATP in connection with stress-situations preslaughter, is of importance for obtaining meat of good quality.

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Table 1. Mean ± SD for ATP, ADP, AMP and IMP concentrations (mmol/kg), pHe, drip loss(%) and EEL in M. longissimus dorsi of pigs with different halothane genotypes

	Halothane genotype		
	NN	nn	Level of significance
ATP	17.5±3.0	9.3±4.8	***
ADP	5.9±1.3	5.4±1.3	n.s.
AMP	1.8±0.9	3.7±2.6	*
IMP	2.1±1.4	9.3±3.5	***
pHe	6.6±0.2	6.3±0.1	***
Drip loss	2.4±1.2	5.3±1.1	***
EEL	21.7±1.8	27.0±3.7	**

Level of significance: *** $p<0.001$; ** $p<0.01$; * $p<0.05$; n.s. $p>0.05$.

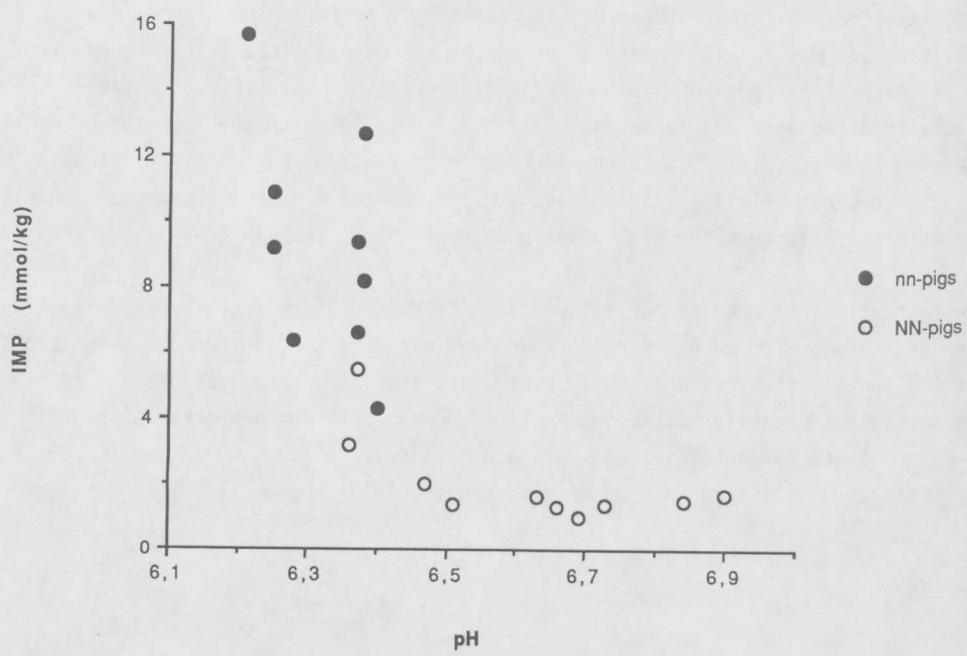

Fig. 1. Relationship between IMP concentration and muscle pH immediately after exsanguination in M. longissimus dorsi of pigs with different halothane genotypes.

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