INVESTIGATIONS ON THE RELATIONSHIP BETWEEN MALIGNANT HYPERTHERMIA IN PIGS AND THE PSE-CHARACTERISTICS IN PORK

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Introduction: Stress susceptible pigs experiencing stress shortly before or at slaughter show very fast biochemical changes in their mu post mortem. Within one hour the pH drops from about 7,0 to values between 5,5 and 5,3. At prevailing high temperatures (> 35°C) the pH decrease causes denaturation of sarcoplasmic and myofibrillar proteins in combination with the desintegration of inner membranes lea to **PSE** (pale, soft, exudative)-meat (Briskey, 1964). **Malignant hyperthermia** (MH) is triggered either by physical stress or pharmacolo agents like halothane or other volatile anaesthetics. It is characterized by rise in body temperature within a very short time, metabolic acid and skeletal muscle contracture (Ellis and Heffron, 1985). The exact sequence of reactions leading to the MH-syndrome is still unknown it is combined with a calcium homeostasis in the cell, which is regulated by the different cellular membranes (Carafoli, 1987). Acidosis as as an increased body temperature are the result of defective sarcoplasmic membranes, leading to an efflux of Ca^{2+} into the sarcoplasma, w is responsible for an accelerated carbohydrate metabolism running into accumulation of lactic acid in the muscle cells. It is proposed that which is observed in pigs *in vivo* (Mickelson, 1989) and the PSE-syndrome developing in pig muscles post mortem (Eikelenboo Minkema, 1974) are caused by the same heritable genetic defects. The objective of these studies was to prove this hypothesis.

Materials and Methods: For the investigations on the relationship between MH in pigs and the PSE-characteristics in pork 19 halot tested pigs were used in total. The animal material for the studies was selected from four cross breeds: Pietrain x Schwerfurter [PS (Pietrain x Hampshire [PH (4)] and Large White x Large White [LL (1)]. The application of the halothane challenge (Fischer et al., resulted in 7 MH^+ and 12 halothane negative (MH^-) animals. The criterion for the differentiation between pigs developing normal or meat was the pH_1-value measured 45 min post mortem in M. longissimus dorsi thoracis et lumborum (MLD) in the region of the 12 vertebrae. Animals showing a pH_1 below 5,8 were categorised as PSE-pigs, whereas all the other pigs with a pH_1 above this limit belo to the group of normal pigs. The genotype of the pigs was molecular genetically determined by means of PCR (polymerase chain reactio combination with a specific restriction endonuclease digestion in the coding sequence of the ryanodine receptor gene (Houde and Pomi 1993). With regard to the enzymological investigations MLD was removed from the carcass 45 min post mortem and pyruvate kinase isolated as described by Schwägele et al. (1996). The number of PK isoforms of the different muscle samples was determined by analy isoelectric focusing techniques (IEF) according to Frey et al. (1986).

Results and Discussion: Four groups of pigs were selected, which were halothane challenged as piglets and tested for the appearence of PSE-syndrome after slaughter by determination of pH_1. They were M^-P^- (halothane negative, MH^-, no PSE); M^-P^+ (halothane negative, PSE); M^+P^- (halothane positive; MH^+, no PSE) and M^+P^+ (halothane positive; MH^+, PSE). In addition to the phenotype, the crossbreed the results of the above mentioned genotypisation for the different groups of animals are shown in table 1. Homozygous not mutated pigs were not found among the selected animal material. As shown by Haschke (1992) the glycolytic enzyme pyruvate kinase (PK) can se as an indicator for the differentation between normal and PSE-muscles. In the case of PK from PSE-muscles there exist three isofo instead of two, which are detected for the enzyme from normal pig muscles. After isolation of the enzyme PK from MLD of every animal separated PK was characterized concerning the number of isoforms by IEF (Frey et al., 1986). The relationship between the phenotype the number of occuring PK isoforms on one side as well as the connection between the phenotype and the genotype on the other side presented in figure 1.With respect to the ryanodine receptor gene there exist three possible genotypes: NN (homozygous not mutated), (heterozygous mutated) and nn (homozygous mutated). In the case of the phenotypes M^+P^+ and M^+P^- the ryanodine receptor gene coding a calcium release channel was without any exception homozygous mutated (nn). PK isolated from the muscle tissue of both animal gro showed with only one exception in group M^+P^+ three PK isoforms.

Table 1: Crossbreeds, phenotypes and genotypes of the pigs. PS = Pietrain x Schwerfurter; PH = Pietrain x Hampshire; LL = Large white x Large white; M^+P^+ (halothane positive; MH^+; PSE); M^+P^- (halothane positive; MH^+; no PSE); M^-P^+ (halothane negative; MH^-; PSE); M^-P^- (halothane negative; MH^-; no PSE); (Nn) heterozygous mutated;(nn) homozygous mutated.

Pig number	Breed	Phenotype	Genotype
1	PS	M^+P^+	nn
2	LL		nn
3	PS		nn
4	PS		nn
5	PS		nn
6	PS		nn
7	PS	M^+P^-	nn
8	PS	M^-P^+	nn
9	PS		nn
10	PS		Nn
11	PS		Nn
12	PS		nn
13	PH		Nn
14	PH	M^-P^-	Nn
15	PS		Nn
16	PH		nn
17	PH		Nn
18	PS		Nn
19	PS		Nn

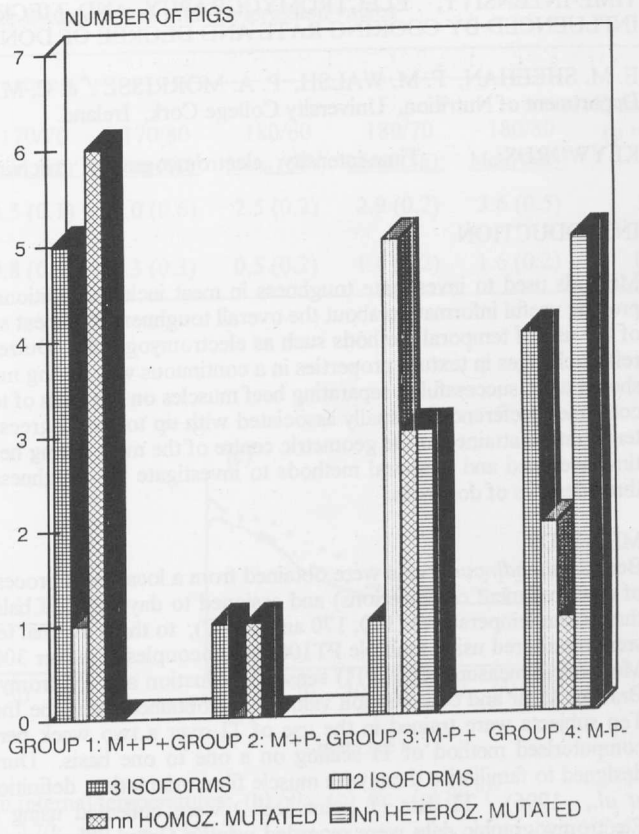

Figure 1: Relationship between number of PK isoforms, genotype and phenotype. Abbreviations see legend of table 1.

Five of six pigs belonging to the phenotype M^-P^- could be characterized as heterozygous mutated (Nn), whereas three of six pigs of the phenotype M^-P^+ were (Nn) and also the remaining three (nn). The enzyme PK isolated from animals of the phenotype M^-P^+ consisted with one exception of two PK isoforms. In the case of six animals in total of the phenotype group M^-P^- the enzyme of four pigs showed three PK isoforms. As expected a close relationship was found between the genotype (nn) and the existence of three PK isoforms in the phenotype groups M^+P^+ and M^+P^-. On the other hand there exists no clear relationship between the genotype and the number of appearing PK isoforms for the phenotype groups M^-P^+ and M^-P^-. Concerning the type of the ryanodine receptor gene halothane negative pigs (MH^-) can be both (Nn) and (nn), whereas halothane positive pigs are exclusively (nn).

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