SPECIAL INFLUENCE OF THE MUTATION IN PORCINE RYANODINE RECEPTOR TO THE PH-1 IN M. LONGISSIMUS DORSI

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SUMMARY

In 1991 a single point mutation in the ryanodine receptor was discovered to be associated with malignant hyperthermia. By the indirect halothan challenge test only the phenotypes hal^+ and hal^- could be segregated. The analysis of this point mutation by an DNA-based test enabled scientists to distinguish clearly the homozygous positive from the negative and heterozygous genotypes. This possibility led to discussions about new ways in Austrian pig breeding. The purpose of this study was to find out an influence of this mutation on meat quality parameters of Austrian crossbreeds.

The relationship between meat quality, characterised by pH-1, conductivity and water holding capacity (WHC) in M. longissimus dorsi and M. semimembranosus, and genotyping the mutation in the ryanodine receptor showed a special link between the genotype and the pH-1 in M. longissimus dorsi.

The examination of 33 NN, 29 nn and 87 Nn pork loins led to highly significant differences between nn/Nn and nn/NN within all physicochemical parameters in both muscles but only the pH-1 in M. longissimus dorsi differed highly significant between NN/Nn-genotypes.

Meat quality characteristics of Austrian hogs of the late seventies - before the halothantest was established to Austrian pork breeding - and today's quality parameters made clear that today's mean values of pH-1 in M. longissimus dorsi have risen by 0,3 pH-units. In contrast to this no differences could be seen in pH-values of M. semimembranosus.

Introduction

At the end of the last century bacon was a precious part of pork. But today the industrial society prefers fat reduced meat and that tendency is supported by nutrition experts who tell people to eat less than 30% of the daily calories as fat (American Heart Association, 1988; Kris-Etherton et al., 1988). So an extremely lean pig was produced but these pigs showed a high stress lability. And in stress situations for example slaughter transports those pigs are likely to get metabolic disturbance that is followed by a development of P (pale) S (soft) E (exsudativ) meat after slaughter. Less tenderness, juiciness, and flavourless of PSE meat for the consumer and limited value for meat processing lead scientists to reduce PSE-incidence. Those stress situation can be induced by the narcotic halothan. So the halothan challenge test was introduced to pig breeding for dam selection in Austria in the late 70s. The heterozygous genotypes could only be found by further checks of their offspring. In 1991 Fuji et al. associated this reaction with a single point mutation in the calcium release channel in muscles sarcoplasmic reticulum . By this analysis of the point mutation in the ryanodin receptor by an DNA-based test (PCR and RFLP techniques) the heterozygous genotypes could be identified more easily (Fuji et al., 1991; Otsu et al., 1992). As a result pork meat quality could be easily compared to the genotype (Pommier and Houde, 1992).

The idea was to work out the status in the MHS genotype of Austrian slaughter pigs and at the same time to analyse the quality of those carcasses. The comparison between the most significant parameter the pH_1 which determines PSE in pork muscle and the different genotypes in typical Austrian breeding was of special interest. The metabolic disturbance was measured by analysing the Creatinkinase and the Aldulase activity of slaughter blood. Furthermore other physicochemical parameters like water holding capacity and conductivity were examined. Very important was the comparison between slaughter weight and muscle percentage of the different genotypes.

Materials and Methods

150 pigs were analysed from March to July 1992 in Austrian slaughterhouses. 50 5 minutes post mortem physicochemical parameters were examined and of each slaughter pig a 50 g muscle sample for DNA analyses were removed. This samples were frozen immediately to -20°C. The native DNA was extracted following the instructions of Sambrook et al. (1991). For PCR and the restriction of the amplificates the method of Fuji et al. (1991) was used. For the separation in a PAG (Polyacrylamidgel) and the visualisation by silver staining the paper of Bassam et al. (1991) was slightly varied. The slaughter weight and lean percentage of every carcass was determined by LSQ (Lendenstärke-Speckquotient).

The pH_1 was determined with a Knick Portamess 654 using an electrode Lot 406-M6-DXK-S7/25 pH2..11 Temp. 0..80°C2 of Ingold. The measuring point of M. longissimus dorsi was between the processus spinosus of vertebrae thoracicae 12 and 13 in 5 cm depth. A second measurement point was situated in M. semimembranosus also in a depth of 5 cm.

Conductivity was examined at the same location as the pH_1 because of comparability.

Water holding capacity was detected in M. semimembranosus after 60 min. p.m. by using the method of Grau and Hamm (1952).

Aldulase activity was determined by a test combination of Boehringer Mannheim GmbH No. 123 838. For measuring the Creatinkinase activity the test kit CK NAC activated No. 1273 248 of Boehringer Mannheim GmbH was used. For both analyses EDTA slaughter blood samples were collected.

Results and discussion

In Austria the halothan challenge test was introduced to pig breeding for dam selection in the late 70s. Comparing three different studies from 1978, 1985 and 1992 dealing with the pH_1 values of M. semimembranosus and M. longissimus dorsi for PSE diagnoses it was discovered that these values differ very significantly in M. longissimus dorsi but not in these of M. semimembranosus (Table 1.). The MHS-gentest provides the possibility to examine the different genotypes and to compare them with the pH_1 values of their carcasses. As demonstrated in table 2. the different genotypes differ in the mean values of their pH_1 data. Looking at table 6. all values differ significantly between NN and nn genotypes and they also differ between Nn and nn genotypes. But the NN and Nn genotypes differ only in the pH_1 value of M. longissimus dorsi. So the obvious idea is that the halothan genotypes influence mainly the quality, concerning PSE, of the M. longissimus dorsi but not as much the quality of M. semimembranosus. This is opposed to the study of Pommier and Houd (1992) who found significant differences in colour, water holding capacity and pHu (Ultimate pH) between NN and Nn but none between Nn and nn. This may be due to the different slaughter techniques.

The metabolic disturbance measured by analysing the Creatinkinase activity of slaughter blood is different in all genotypes (Table 6.). This is not surprising because the M. longissimus dorsi represents 12% of the carcass muscle mass (Kauffman and St Clair, 1965). With the damage of muscle cell membranes which in sensitive animals starts already before slaughter a high activity of this enzyme is found in blood. In slaughter pigs we found Creatinkinase activities from 590 U/l up to 86 500 U/l. The mean values of the different genotypes is documented in table 3.

The distribution within the weight groups and the lean percentage classes was influenced by the different genotypes although the fattening period of all examined pigs had been the same. Table 4. shows the results of the lean percentage which was highest for nn hogs, lower for Nn and lowest for NN pigs (Table 4). The weight groups were contrary (Table 5.)

Conclusion

This study makes clear that meat quality parameters of M. longissimus dorsi shows, besides its principal sensitivity because of the special fibre composition (red - white ratio), also a close relation to the halothangen.

As for the economic aspect is concerned in Austria the nn and Nn pigs have leaner carcasses but less body weight over the same fattening period than NN hogs.

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Table 1. Development of pH_1 of M. semimembranosus (SM) and M. longissimus dorsi (LD) of slaughter pigs in 1978, 1985 and 1992 in Austria.
Table 2. Mean values (Ø) of pH_1 of M. semimembranosus (SM) and of M.longissimus dorsi (LD) of different genotypes.
Table 3. Mean values of the Creatinkinase activity in different genotypes
Table 4. Lean Percentage (Pfeiffer und Falkenberg) of carcasses with different genotypes
Table 5. Slaughter weight of different genotypes
Table 6. The comparison of MHS-genotypes