GENETIC AND HALOTHANE TESTING AS INDICATORS OF MEAT QUALITY IN CROSSBRED PIGS

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Introduction

Porcine stress syndrome (PSS; malignant hyperthermia) refers to an inherited neuromuscular disease that appears when PSS-susceptible animals are exposed to either inhalational anestetics or stress associated with transport, restraint, fighting, mating, vigorous exercise and hot, humid weather (2). When triggered, malignant hyperthermia (MH) is characterized by a sustained muscle rigidity and fever. The primary defect is belived to lie in abberant gating of Ca^{2+} release channels (ryanodine receptors) of sceletal muscle sarcoplasmic reticulum. The RYR1 gene encoding the sceletal muscle ryanodine receptor have been isolated and linked to mutation causing MH-susceptible phenotype in swine (1). The losses of revenue associated with MH sudden death of pigs during the rearing period are elevated by post-mortem development of inferior pork, referred to as Pale, Soft and Exudative (PSE). The PSS mutation is considered unfavorable for both inferior pork (4) and slower growth rates (3). We report here the influence of the MH genotypes on the meat quality of crossbred pigs selected by PCR testing and by the halothane challenge test.

Materials and Methods

Male crossbred pigs of (Large White x German Landrace)F_1 x Belgian Landrace at weight ranging from 25 to 30 kg were submitted to inhalation of a mixture of 4% halothane and oxygene for 5 minutes or until the first symptoms of MH appeared. Halothane negative (Hal^-) pigs were than subjected to DNA-based test for identification of heterozygous RYR1 mutation. Briefly, genomic DNA was isolated from 0.5 ml blood sample which was added to 50 ml of TE buffer pH8, and spun for 10 sec at 1300 g. Then the pellet was washed 3 times with TE and resuspended in 100 μl of Perkin Elmer Cetus gelatin-free buffer suplemented with 0.5% Tween 20 and 10 μg/ml Proteinase K. The mixture was incubated at 60°C for 30 minutes and reaction terminated by immersion in boiling water for 10 min. This mixture was used to isolate 659 bp fragment by PCR amplification. The reaction was done in Perkin Elmer Cetus PCR buffer containing 1mM $MgCl_2$. Genomic DNA (200-400 ng) and 100 ng of each of primers were added to the mixture and program carried at 94°C of 1 min, 53°C for 2 min, and 72°C for 3 min. The forward primer was 5'-TCCAGTTTGCCACAGGTCCATACCA-3' and the reverse primer was 5'-ATTCACCGGAGTGGAGTCTCTGAG-3'. The amplified sequence was cut with HgIAI to detect the presence of C/T mutation and restriction fragments were resolved on 3% agarose gel. Tested animals were allocated in Hal^+ homozygous (nn), MH^- homozygous (NN) and MH^- heterozygous (Nn) genotypes. Animals were fed ad lib and examined over a slaughter weight range of 80 to 130 kg. Meat quality was estimated by standard methods: colour by Göfo-measurement, water-holding capacity by drop loss method, and pH by glass electrode, respectively.

Results

Genomic testing for detecting pigs that carry a genetic predisposition to the PSS has shown to be a powerful tool which, unlike halothane screening, allows to indentify a combination of normal and stress genes in pigs genetic makeup. Table 1 shows the effectivenes of such testing in detection of heterozygous genotype among halothane negative crossbred pigs. As shown, halothane challenge tested pigs may be clasified as halothane positive (10,33%) or halothane negative (89.67%). However, genomic testing revealed that among Hal^- animals 15.61 percentage belonged to heterozygous nonreactors (Nn), and even two animals have shown to be recesive homozygotes (nn). It is likely that the presence of three genotypes related to PSS may influence animal market value.

Table 1. Effectiveness of DNA testing in detection of heterozygous genotype among halothane$^-$ crossbred pigs[a]

Number of animals tested	Phenotype from halothane test No and (%)		Genotype from DNA based animals of Hal^-		
	Hal^+	Hal^-	NN	Nn	nn
300	31 (10.33)	269 (89.67)	225 (84.12)	42 (15.61)	2 (0.74)

[a](Large white x German Landrace)F_1 x Belgian Landrace

Table 2. Influence of different genotypes on meat quality

TRAIT	Meat quality of different genotypes estimated at slaughtered weight of					
	105 kg			130 kg		
	NN	Nn	nn	NN	Nn	nn
Colour	47.2[a]	46.9	46.8	48.1	46.1	45.9
Water-holding capacity (drop loss)	6.25	6.95	7.3	6.52	7.81	7.75
pH45	6.57	6.25	6.24	6.58	7.9	8.1
pH_u	5.78	5.45	5.48	5.72	6.2	6.9

[a]Absolute numbers without standard deviations

No differences in meat quality were found between animals of 80 kg and 105 kg (80 kg omitted)

According to this research, the effect of the presence of the gene inside a pig appears to change according to the weight at which animal is slaughtered. Table 2 shows that meat traits from animals of various genotypes at different slaughter weights vary a lot among the groups. The heterozygous pigs having Nn genotype showed the most surprising observations concerning their meat quality characteristics; at a weight of 80 kg, the meat quality of Nn genotypes was comparable to that of NN animals not carrying the mutation in RYR1 gene. Around 105 kg liveweight colour and water-holding capacity of meat was pretty much the same in heterozygous (Nn) and homozygous recessive (nn) pigs. However, at 130 kg the meat of Nn resembled closely to that of PSS- sensitive stock for the normal quality parameters such as colour and drop loss.

Discussion

There has been a common opinion that the gene for PSS should be eliminated only from one parental line, say from the mother, but retained in the other parent because of its association with better carcass yield and leanness. Studies concerning the pigs slaughtered at around 100 kg have suggested that this approach would carry no more than a small risk of perpetuating quality defects in the progeny grown for meat. The data presented have shown that the elimination of both carriers of recessive homozygotes (nn) and heterozygotes (Nn) of mutated RYR1 gene among the progeny grown for meat should be of great importance for the meat quality of pigs at higher market weights. The molecular method described here offers a new tool for quick and inexpensive detection of all carriers of mutated RYR1 gene in pigs.

References

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