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Research Article

Relationship between the Porcine Stress Syndrome gene and pork quality traits of F_2 pigs resulting from divergent crosses

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Abstract

The *PSS* genotypes of 596 F_2 pigs produced by initial mating of Brazilian commercial sows and native boars were characterized by PCR-RFLP and the pork quality traits were evaluated. Among the 596 pigs studied, 493 (82.7%) were *NN* and 103 (17.3%) were *Nn*. There were no differences between *NN* and *Nn* pigs in the following pork qualities: pH_u (5.71 ± 0.16 *vs* 5.70 ± 0.11), intramuscular fat (1.55 ± 0.64% *vs* 1.65 ± 0.67%), shear force (5552 ± 878 g/1.2 cm *vs* 5507 ± 826 g/1.2 cm), lightness (44.96 ± 2.05 *vs* 45.01 ± 1.92), redness (0.64 ± 0.60 *vs* 0.79 ± 0.55), yellowness (6.62 ± 0.56 *vs* 6.65 ± 0.48), hue (84.28 ± 5.53 *vs* 83.41 ± 4.85), or chroma (6.68 ± 0.52 *vs* 6.73 ± 0.52). However, pork from *Nn* pigs had a significantly ($p < 0.05$) lower pH_{45} (6.41 ± 0.27 *vs* 6.51 ± 0.26) and greater drip (3.92 ± 1.90% *vs* 3.06 ± 1.60%), cooking (33.29 ± 2.26% *vs* 32.50 ± 2.54%) and total (35.67 ± 2.48% *vs* 34.01 ± 2.58%) loss compared to that of *NN* pigs. These results indicate that, even in divergent crosses, *PSS* gene carriers produce pork of poorer quality.

Key words: meat quality, PCR-RFLP, pork, PSE, PSS, pig.

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Introduction

Meat is one of the main sources of protein in the human diet, and pork is one of the most produced and consumed worldwide (Franco *et al.,* 1998). One of the concerns during pork production is its quality. Historically, considerations about pork quality have been restricted to aspects related to health, processing, nutrition and, to a lesser extent, sensory traits. However, in recent years, pork consumers have become increasingly concerned about the safety of this meat, its ease of preparation, and their satisfaction during consumption. Consumer satisfaction during pork consumption is related to sensory perception of attributes such as meat color, juiciness and tenderness, which are adversely affected by the development of the PSE (pale, soft and exudative) condition after death. One of the main economic losses in the pig industry is related to PSE pork, which originates from animal stress and depends upon pig genetics and animal handling conditions before and during slaughtering. Stress conditions can activate malignant hyperthermia in pigs homozygous for the *PSS* (Porcine Stress Syndrome) gene (*nn*) and may even cause death (Fisher *et al.,* 2000a). Since *PSS* gene carriers have a higher probability of presenting poor quality pork (Santana *et al.,* 1998), there is a constant concern about pig welfare during handling and transport before slaughter (Geers *et al.,* 1994).

Recent advances in our understanding of the regulation of skeletal muscle contraction has led to the identification of a mutation in the ryanodine receptor in the sarcoplasmic reticulum calcium release channel that has been correlated with PSS and malignant hyperthermia. Stress-susceptible pigs respond to halothane anesthesia with limb muscle rigidity, increased anerobic metabolism and increased body temperature (Rempel *et al.,* 1993). The increased anerobic metabolism in muscle produces a sudden decrease in pH after death (pH < 5.5 24 h after slaughter) that, together with an increase in muscle temperature, leads to protein denaturation and adversely affects pork quality traits such as color and water-holding capacity and results in excessive water loss during pork preparation (Fávero, 2002).

Theoretically, at the same finishing level (same fat depth), heavier pigs should show a greater tendency to develop PSE because heavier carcasses take longer to cool because of their larger volume/surface area ratio. This is a

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problem directly related to the muscles of the ham that cool more slowly than muscles such as the *longissimus dorsi*. Consequently, heavier pigs show a greater tendency to develop PSE pork. Moreover, the muscle glycogen content is higher in heavier pigs, leading to rapid postmortem increases in glycolysis and, consequently, greater decreases in muscle pH after slaughter (Ellis and Bertol, 2001).

A high water-holding capacity of pork used to manufacture hams and sausages has a direct impact on the quality of these processed products because it reduces drip loss in fresh and frozen products, and also reduces cooking loss and maintains the product's juiciness. Substances such as phosphates and rusk increase the water-holding capacity and are used to produce hams and sausages from PSE pork. However, Fisher *et al.* (2000c) have shown that the addition of phosphate does not increase pork water absorption, but causes pork products to bind water more completely, thereby minimizing losses during processing.

Genetic factors are among the traits that affect pork quality, and the identification of major genes and molecular markers is a promising approach for improving economic traits, such as pork quality, that are not measurable in breeding animals (Fávero, 2002).

Franco *et al.* (1998) demonstrated that the presence of the *n* allele led to higher pork drip loss in the *semimembranous* muscle, thereby reducing its quality. Lundstrom *et al.* (1995) showed that the *PSS* gene affected the quality of the *longissimus dorsi* and resulted in a less tender, less desirable pork. According to Fisher *et al.* (2000a), a lower initial pH (within 45 min) in *Nn* pigs, and especially in *nn* pigs, compromises pork quality because of rapid glycolysis after slaughter.

The use of genomic markers to help in the selection of pork quality is one of the most promising developments in the pig industry. PSE has been known for some time to be associated with variations in the recessive *PSS* gene (Plastow, 2000), and the availability of a test based on the identification of the causative mutation in the *PSS* gene described by Fujii *et al.* (1991) was a key step in marker-assisted screening of pork quality. The test allows breeders to accurately separate all three *PSS* genotypes, instead of just reactors (*nn*) from non-reactors (*NN* and *Nn*), and has allowed more detailed studies of the effect of this mutation on pork quality.

The aim of this study was to determine the *PSS* genotypes in F_2 pigs derived from divergent crosses and to determine their relationship to pork quality traits.

Materials and Methods

The 596 genotyped F_2 pigs were produced by outbreed crossing using 18 commercial females (11 Landrace x Large White and seven Landrace x Large White x Pietrain) with two Brazilian native boars (Piau breed). Both boars and 11 parental females had the *NN* genotype. The F_2 pigs were reared and slaughtered on the Pig Breeding Farm maintained by the Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil. The pigs were slaughtered at a live weight of 65.0 ± 5.5 kg and were deprived of food for 18 h before slaughter, but had access to fresh water *ad libitum*. The pigs were electrically stunned (300V/5 s) and bled by cardiac puncture under the left armpit.

For each pig, the pH (pH_{45}) of the *longissimus dorsi* was measured 45 min postmortem in the left half of the carcass before cooling and in the right half of the carcass after cooling at 4 °C for 24 h in horizontal freezers. Samples of the *longissimus dorsi* were then obtained to measure other pork quality traits.

Pork quality traits were evaluated in the Meat Laboratory of the Department of Food Technology, Universidade Federal de Viçosa, using the procedures described by Benevenuto Júnior (2001) for pH 24 h postmortem (pH_u), intramuscular fat (IMF), drip, cooking and total loss, shear force, and objective meat color (lightness, redness, yellowness, hue and chroma).

Genotypic analysis was done in the Laboratory of Animal Biotechnology of the Department of Animal Science, Universidade Federal de Viçosa. DNA was salt-extracted from white blood cells collected immediately after slaughter using a standard laboratory protocol. The sequence of the *ryr-1* gene that contains the C → T mutation responsible for triggering PSS (Fujii *et al.,* 1991) was amplified by PCR-RFLP using the primers cited by O'Brien *et al.* (1993) and generated a 659 bp product.

The amplification mixture contained 1 U of *Taq* DNA polymerase (Phoneutria), 0.2 M of each primer (forward - 5'-TCCAGTTTGCCACAGGTCCTACCA-3' - and reverse - 5'-TTCACCGGAGTGGAGTCTCTGAG-T-3'), 2 mM $MgCl_2$, 20 mM Tris, pH 8.3, 50 mM KCl, 0.2 mM dNTPs, and 25 ng of genomic DNA in a final volume of 20 µL, according to the standard protocol described by Fujii *et al.* (1991).

The samples were distributed into previously labeled microtubes containing the reagent mixture described above and were centrifuged at 7,826 g for 10 s to ensure that the samples were at the bottom of each tube. The microtubes were then placed in the 96-sample tray of the thermocycler (MJ-Research PTC-100). The amplification program, modified from Fujii *et al.* (1991) and Houde *et al.* (1993), consisted of initial denaturation at 94 °C for 3 min and 35 cycles at 94 °C for 45 s, 68 °C for 1 min and 72 °C for 1 min, with a final polymerization step at 72 °C for 5 min.

Mutation analysis of the samples amplified as described above was done using the restriction enzyme *BsiHKA* I (New England Biolabs). This enzyme cleaves the 659-bp sequence containing the PSS mutation and generates fragments of 524 and 135 bp in normal homozygotes (*NN*), fragments of 524, 358, 166 and 135 bp in heterozygotes (*Nn*), and fragments of 358, 166 and 135 bp in mutant homozygous pigs (*nn*). After digestion, the reaction prod-

ucts were analyzed on 8% silver nitrate-stained polyacrylamide gels and the pigs were classified as normal homozygotes (*NN*), heterozygotes (*Nn*) and recessive homozygotes (*nn*) according to the size of the DNA fragments.

Statistical analysis of the association of the genotypes with the traits evaluated was done using the SAS General Linear Models (SAS, 1997) program, according to the following model:

$$Y_{ijkl} = m + G_i + S_j + L_k + e_{ijkl}$$

where Y_{ijkl} = observed trait in an animal of genotype i, sex j and batch k, m = general mean, G_i = genotype effect (*NN* or *Nn*), S_j = sex effect (1 = castrated male and 2 = female), L_k = batch effect (k = 1, 2, 3, 4 and 5) and e_{ijkl} = random error.

Results

The RFLP patterns were as expected. Pigs homozygous for the mutation (*nn*) were characterized by fragments of 358, 166 and 135 bp, normal pigs (*NN*) showed the 524-bp and the complementary 135-bp fragment, and heterozygous pigs showed fragments of 524, 358, 166 and 135 bp. The *NN* and *Nn* genotypes were found in 493 (82.7%) and 103 (17.3%) pigs, respectively. Since only one *nn* pig was identified, it was not considered in the analysis. These unusual frequencies in F_2 crosses were found to be the result of divergent matting patterns in which parental boars were not carriers and the F_1 generation was randomly mated regardless of the *PSS* genotype.

The mean results and number of observations for each pork quality trait in each genotype are shown in Table 1. The traits showing significant differences between the *NN* and *Nn* genotypes were pH_{45} and drip, cooking and total losses. *Nn* pigs had a higher glycolytic rate postmortem, as indicated by their lower ($p < 0.05$) pH_{45} values and higher ($p < 0.05$) drip, cooking and total losses.

Discussion

The significantly lower pH_{45} and higher drip, cooking and total losses seen in *Nn* pigs agreed with other reports in the literature (McPhee and Trout, 1995; Lundstrom *et al.,* 1995; Leach *et al.,* 1996; Monin *et al.,* 1999; Fernandez *et al.,* 2002; Green, 1997; Franco *et al.,* 1998; Miller *et al.,* 1999; Jeremiah *et al.,* 1999; Fisher *et al.,* 2000b) and showed that the presence of the *n* allele negatively affected pork quality traits by producing greater muscle acidity that led to greater losses during storage and cooking, and produced less juicy pork.

Drip loss, an indicator of the muscle's water holding capacity, is negatively correlated with pH_{45} (Benevenuto, 2001) and is highly dependent on the initial denaturation of pork myofibrillar proteins.

Table 1 - Pork quality traits for each *PSS* genotype (*NN* and *Nn*).

Trait	Genotype			
	NN		*Nn*	
	N	Value	N	Value
pH_{45}*	424	6.51 ± 0.26	80	6.41 ± 0.27
pH_u	434	5.71 ± 0.16	80	5.70 ± 0.11
IMF (%)	398	1.55 ± 0.64	71	1.65 ± 0.67
DL* (%)	437	3.06 ± 1.60	79	3.92 ± 1.90
CL* (%)	432	32.50 ± 2.54	78	33.29 ± 2.26
TL* (%)	354	34.01 ± 2.58	64	35.67 ± 2.48
SF (g/1.2 cm)	349	5552 ± 878	60	5507 ± 826
L	393	44.96 ± 2.05	72	45.01 ± 1.92
A	384	0.64 ± 0.60	71	0.79 ± 0.55
b	390	6.62 ± 0.56	71	6.65 ± 0.48
h	332	84.28 ± 5.53	61	83.41 ± 4.85
c	343	6.68 ± 0.52	63	6.73 ± 0.52

The values are the mean ± S.D. of the number (N) of observations for each trait and genotype.
*$p < 0.05$ between genotypes (F-test).
pH_{45} - pH 45 min after slaughter; pH_u - pH 24 h after slaughter; IMF - intramuscular fat; DL - drip loss; CL - cooking loss; TL - total loss; SF - shear force; L - lightness; a - redness; b - yellowness; h - hue; c - chroma.

The lack of a significant difference in objective color indices and tenderness between the *Nn* and *NN* genotypes has also been observed by others (Bastos *et al.,* 2001; Fernandez *et al.,* 2002; Miller *et al.,* 1999; Jeremiah *et al.,* 1999). However, discrepancies in pork quality traits have also been reported. Differences in lightness (McPhee and Trout, 1995; Leach *et al.,* 1996; Green, 1997; Bastos *et al.,* 2001), pH_{24} (Leach *et al.,* 1996; Green, 1997), shear force (Fisher *et al.,* 2000a) and intramuscular fat (Zhang *et al.,* 1992; Leach *et al.,* 1996) between the *NN* and *Nn* genotypes have been considered to be indicative of the negative effect of the *n* allele on protein denaturation and pork quality (Fisher *et al.,* 2000a). According to the latter authors, pork from *Nn* pigs is less tender and paler than pork from *NN* pigs because of higher shear force and lightness values ($p < 0.05$), both of which are associated with a higher incidence of PSE pork as a result of sarcoplasmic protein denaturation and a consequent reduction in pork quality.

In the present study, *PSS* gene carriers (*Nn* pigs) had a poorer pork quality, as indicated by their lower pH_{45} and higher drip, cooking and total losses. The effect of the *PSS* gene was demonstrable in pigs resulting from divergent crosses, which are generally less susceptible to stress. These results indicate that this gene is one of the main genes to be studied in relation to pork quality.

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