



Relationship between the Porcine Stress Syndrome gene and carcass and performance traits in F₂ pigs resulting from divergent crosses

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Abstract

The *PSS* genotypes of 596 F₂ pigs produced by initial mating of Brazilian native boars commercial sows and were characterized by PCR-RFLP and their carcass and performance traits were evaluated. Among the 596 animals analyzed, 493 (82.72%) were characterized as *NN* and 103 (17.28%) as *Nn*. With respect to carcass traits, *Nn* animals presented higher ($p < 0.05$) right half carcass weight, left half carcass weight, loin depth and loin eye area, and lower shoulder backfat thickness, backfat thickness between last and next to last but one lumbar vertebrae and backfat thickness after last rib at 6.5 cm from the midline compared to *NN* animals. *Nn* animals also showed ($p < 0.05$) higher values for most of the cut yields, indicating higher cutting yields for animals carrying the *n* allele and lower values for bacon depth, confirming lower fat deposition in carcass. In addition, *Nn* animals presented ($p < 0.05$) lower values for the performance trait weight at 105 days of age. These results indicate that animals carrying the *PSS* gene generate leaner carcasses, higher cut yields, and that the effects of the gene can be observed even in divergent crosses.

Key words: carcass, performance, *PSS* gene, PCR-RFLP, pig.

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Introduction

Since the identification of Porcine Stress Syndrome (*PSS*), several studies have been carried out comparing performance and carcass traits between *PSS* genotypes. Most of these studies used the halothane challenge test to separate reactor (*nn*) and non-reactor (*NN* and *Nn*) animals (Leach *et al.*, 1996). The more thorough identification of the three *PSS* genotypes by molecular techniques has received interest in their effects on carcass traits (Fisher *et al.*, 2000c).

The *PSS* gene, also called halothane gene (*Hal* gene), encodes the precursor protein of the calcium release channel of skeletal muscle sarcoplasmic reticulum (Fujii *et al.*, 1991). The *Hal* gene, in recessive homozygosis (*nn*), is associated with the development of *PSS*, and is related to mortality during transportation as well as occurrence of pale, soft and exudative (*PSE*) meat. When in heterozygosis (*Nn*), the *Hal* gene continues to be related to poor meat quality, but recessive homozygous (*nn*) animals

gain more weight when compared to normal homozygous (*NN*) ones (Bastos *et al.*, 2001; Santana *et al.*, 1998).

Zhang *et al.* (1992), studying Pietran x Yorkshire crossed animals, attributed 1 to 10% of the phenotypic variation in meat yield and growth traits to the *PSS* gene locus. Other studies have indicated that *Nn* animals presented advantages in traits such as feed efficiency, carcass yield and lean meat content compared to dominant homozygous animals (*NN*); however, they showed a higher incidence of *PSE* meat (Leach *et al.*, 1996; Fisher *et al.*, 2000c), a source of economic loss in the swine industry.

The objective of the present study was to evaluate carcass and performance traits and to determine their relationship with the *PSS* gene in a F₂ population resulting from divergent crosses.

Material and Methods

The 596 genotyped F₂ animals were produced by outbreed crossing of 18 commercial females, including 11 Landrace x Large White and 7 (Landrace x Large White x Pietrain) with two Brazilian native boars (Piau breed). Both boars and 11 parental females had the *NN* genotype. The F₂

animals were reared and slaughtered at a live weight of 65.03 ± 5.51 kg at the Pig Breeding Farm, Department of Animal Science, Federal University of Viçosa, Viçosa, Minas Gerais State, Brazil.

Animals were deprived of food for 18 h before slaughter, with ad libitum access to fresh water. Afterwards they were electrically stunned (300V/5s) and bled by heart puncture under the animal's left armpit.

At slaughter the following carcass traits were evaluated: slaughter age (SA), right half carcass weight (RHCW), left half carcass weight (LHCW), carcass yield including feet and head (CY), carcass length according to the American carcass classification method (MLC), shoulder backfat thickness (SBT), midline backfat thickness after last rib (LR), midline backfat thickness between last and next to last but one lumbar vertebrae (LL), midline lower backfat thickness above the last lumbar vertebra (L), backfat thickness after last rib, 6.5 cm from the midline (P2), backfat thickness after last rib, 6.5 cm from the midline, equivalent to P2 (ETO), loin depth (LD), and loin eye area (LEA).

The following cut yields were also evaluated after 24 h of cooling at 4 °C: cold right half carcass weight (CRHCW), total ham weight (THW), skinless and fatless ham weight (HW), total shoulder weight (BSW), skinless and fatless shoulder weight (SFBSW), total picnic shoulder weight (PSW), skinless and fatless picnic shoulder weight (SFPSW), total loin (bone-in) weight (TLW), boneless loin weight (LW), bacon weight (BCW), bacon depth (BCD) and sirloin weight (SW). Evaluated performance traits were: birth weight (BW), live weights at 21 (W21), 77 (W77) and 105 (W105) days of age. Feed intake (FI), average daily gain (ADG), and feed:gain ratio (FG) were measured from 77 to 105 days of age.

Carcass traits and cut yields were determined by the carcass dissection technique described by Nascimento and Mota (2000) and Benevenuto Júnior (2001)

Genotypic analysis were performed at the Laboratory of Animal Biotechnology, Department of Animal Science, Federal University of Viçosa.

DNA was salt extracted from white blood cells collected immediately after slaughter. Briefly, leukocytes were obtained from 10 mL blood collected in 0.5 mL 0.5% EDTA and centrifuged for 15 min at 958.69 g. The plasma was discarded and each pellet was stored in 1 mL NET 100 (100 mM NaCl, 10 mM Tris, pH 8.3, 100 mM EDTA, pH 8.0) at -20 °C for an indeterminate period of time. On the day of extraction, the samples were thawed at room temperature (about 25 °C). The cells were then washed twice in 1X PBS (3.825 g NaCl, 0.469 g NaH₂PO₄·H₂O, 500 mL MilliQ water) and each microtube was filled with 2 mL hemolysis solution (10 mM Tris, pH 7.5, 5 mM MgCl₂, 10 mM NaCl). The cells were then homogenized by vortexing, centrifuged at 958.69 g for 10 min in a refrigerated centrifuge at 4 °C and the supernatant was discarded. This step was repeated

until a white pellet was obtained. The samples were then transferred to new labeled microtubes and the pellets were incubated at 55 °C with 200 µL proteinase K buffer (5X) (0.375 M NaCl, 0.12 M EDTA, pH 8.0), 20 µL proteinase (20 mg/mL), 26 µL 20% SDS (20 g SDS, 100 mL MilliQ water) and 744 µL distilled water, in a final volume of 1 mL, for 4-6 h or overnight. For DNA salt precipitation, each sample was divided into two additional microtubes, 110 µL 5 M NaCl was added, and each sample was homogenized manually for 15 s and then centrifuged at 15,338 g for 5 min at room temperature. The supernatant (DNA solution) was then transferred to a new microtube and 1 mL of absolute ethanol was added to each sample. The samples were resuspended and then centrifuged for 5 min at 15,338 g. The supernatant was discarded and the pellet was dried for 15 to 20 min. at room temperature. After drying, the pellet was resuspended in 100 to 200 µL TR (20 mM Tris, pH 8.3, 0.1 mM EDTA, pH 8.0) and incubated in a water bath at 37 °C for 30 min. Finally, the samples were stored at 4 °C for an indeterminate period of time.

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), which generated a product of 659 bp.

The amplification mixture contained 1 U of Taq DNA polymerase (Phonotria), 0.2 µM of each primer (forward - 5'-TCCAGTTTGCCACAGGTCTACCA-3' - and reverse - 5'-TTCACCGGAGTGGAGTCTCTGAG-T-3'), 2 mM MgCl₂, 20 mM Tris, pH 8.3, 50 mM KCl, 0.2 mM dNTPs, and 25 ng genomic DNA in distinct tubes for each animal, in a final volume of 20 µL, following the standard protocol described by Fujii *et al.* (1991).

Samples were distributed into microtubes, each labeled with the number of the animal to be analyzed. These microtubes, containing the reagent mixture described above, were centrifuged at 7,826 g for 10 s to guarantee that the samples were at the bottom of each microtube. The microtubes were then added to a 96-sample tray of a MJ-Research PTC-100 thermocycler. 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/45 s, 68 °C/1 min and 72 °C/1 min, and a final polymerization step at 72 °C for 5 min.

For mutation analysis of the previously amplified samples, the *BsiHKA I* restriction enzyme (New England Biolabs) was used. This enzyme cleaves the DNA 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 animals (*nn*). After digestion, the samples were analyzed on 8% silver nitrate-stained polyacrylamide gels and the animals 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 performed using the SAS General Linear Models (SAS, 1997) program, according to the following model:

$$Y_{ijklm} = m + G_i + S_j + L_k + b(C_{ijklm} - \bar{C}) + e_{ijklm}$$

where Y_{ijkl} = observed trait in l animal, of i genotype, j sex and k batch; 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); b = linear regression coefficient of trait as a function of the covariate; C_{ijkl} = observed covariate value in l animal, of i genotype, j sex, k batch, and e_{ijkl} = random error.

For carcass traits and cut yields, carcass weight and cold right half carcass weight (CRHCW) were used as covariates, respectively. For traits FI, W105, ADG, and FG, W77 was used as covariate.

Results

The RFLP patterns observed were as expected. Animals homozygous for the mutation (*nn*) were characterized by bands of 358, 166 and 135 bp. Normal animals (*NN*) showed the 524-bp band and the complementary 135-bp band. Heterozygous animals showed bands of 524 and 358 bp and smaller bands of 166 and 135 bp.

The frequencies of the *NN* and *Nn* genotypes were 493 (82.72%) and 103 (17.28%) animals, respectively. Since only one *nn* animal was identified, it was not considered for analysis. These unusual frequencies in F₂ crosses were found due to divergent mating patterns, in which parental boars were not carriers and the F₁ generation was randomly mated despite their *PSS* genotype.

Average results for carcass traits and number of observations within each genotype are shown in Table 1. Traits showing significant differences between *NN* and *Nn* genotypes were RHCW, LHCW, SBT, LL, P2, LD and LEA. Higher ($p < 0.05$) RHCW, LHCW, LD and LEA and lower ($p < 0.05$) SBT, LL and P2 were observed for *Nn* animals, indicating a higher lean content and lower fat deposition.

Average results and the number of observations for cut yields within each genotype are shown in Table 2. Significant differences between *NN* and *Nn* animals were observed for CRHCW, THW, HW, SFBSW, PSW, SFPSW, LW and SW. *Nn* animals showed higher ($p < 0.05$) values for all these cut yields compared to *NN* animals, confirming the carcass trait results, indicating a higher lean mean content in *Nn* animals. For BCD *Nn* animals presented a significantly ($p < 0.05$) lower value, confirming their lean carcass trait.

Average results and the number of observations for each performance trait within each genotype are shown in Table 3. Among the performance traits evaluated, only

Table 1 - Carcass traits (means and standard deviations) obtained for each *PSS* genotype (*NN* and *Nn*).

Trait	Genotype			
	<i>NN</i>		<i>Nn</i>	
	N ¹	Mean ± sd	N ¹	Mean ± sd
RHCW (kg) *	420	26.85 ± 2.63	78	27.30 ± 4.04
LHCW (kg)*	422	26.74 ± 2.59	78	27.21 ± 2.89
SA (days)	416	147.51 ± 10.02	77	149.83 ± 10.37
CY (%)	408	82.09 ± 1.99	75	81.94 ± 2.28
MLC (cm)	416	71.71 ± 3.08	77	71.87 ± 3.80
SBT (mm)*	420	40.86 ± 5.41	78	39.08 ± 5.69
LR (mm)	422	20.00 ± 4.82	78	19.72 ± 4.71
LL (mm)*	422	28.92 ± 5.89	78	27.30 ± 6.14
L (mm)	373	44.95 ± 2.05	70	44.98 ± 1.92
P2 (mm)*	420	17.24 ± 3.74	78	15.51 ± 3.66
LD (mm)*	368	43.43 ± 4.23	66	45.48 ± 4.16
LEA (cm ²)*	374	26.09 ± 3.74	66	28.14 ± 3.39

*Significant differences ($p < 0.05$) between genotypes by the F-test.

RHCW - right half carcass weight; LHCW - left half carcass weight; SA - slaughter age; CY - carcass yield including feet and head; MLC - carcass length determined according to the American carcass classification method; SBT - shoulder backfat thickness; LR - backfat thickness after last rib, at midline; LL - backfat thickness between last and next to last but one lumbar vertebra, at midline; L - lower backfat thickness after last lumbar vertebra, at midline; P2 - backfat thickness after last rib at 6.5 cm from the midline; LD - loin depth; LEA - loin eye area.

Table 2 - Cut yields (means and standard deviations) obtained for each *PSS* genotype (*NN* and *Nn*).

Trait	Genotype			
	<i>NN</i>		<i>Nn</i>	
	N ¹	Mean ± sd	N ¹	Mean ± sd
CRHCW (kg)*	422	26.45 ± 2.85	78	26.99 ± 3.32
THW (kg)*	420	7.29 ± 0.81	77	7.40 ± 0.94
HW (kg)*	420	4.96 ± 0.58	76	5.23 ± 0.70
BSW (kg)	422	2.34 ± 0.35	78	2.38 ± 0.40
SFBSW (kg)*	420	1.68 ± 0.27	77	1.75 ± 0.28
PSW (kg)*	419	4.88 ± 0.59	78	5.00 ± 0.71
SFPSW (kg)*	422	2.68 ± 0.37	77	2.87 ± 0.44
TLW (kg)	419	3.49 ± 0.48	76	3.44 ± 0.49
LW (kg)*	418	1.01 ± 0.18	77	1.11 ± 0.20
BCW (kg)	418	2.71 ± 0.45	76	2.68 ± 0.46
BCD (mm)*	414	25.38 ± 6.60	77	23.68 ± 6.90
SW (kg)*	420	0.22 ± 0.04	77	0.24 ± 0.04

*Significant differences ($p < 0.05$) between genotypes by the F-test.

CRHCW - cold right half carcass weight; THW - total ham weight; HW - skinless and fatless ham weight; BSW - boston shoulder weight; SFBSW - skinless and fatless boston shoulder weight; PSW - picnic shoulder weight; SFPSW - skinless and fatless picnic shoulder weight; TLW - total loin (bone-in) weight; LW - loin weight; BCW - bacon weight; BCD - bacon depth; SW - sirloin weight.

Table 3 - Pig performance traits (means and standard deviations) obtained for each *PSS* genotypes (*NN* and *Nn*).

Trait	Genotype			
	<i>NN</i>		<i>Nn</i>	
	N ¹	Mean ± sd	N ¹	Mean ± sd
BW (kg)	481	1.22 ± 0.28	103	1.20 ± 0.24
W21 (kg)	441	4.96 ± 1.09	102	5.08 ± 1.02
W77 (kg)	467	21.64 ± 4.16	103	20.99 ± 3.74
FI (kg)	456	40.49 ± 8.00	103	39.20 ± 7.39
ADG (kg)	449	0.54 ± 0.13	102	0.53 ± 0.12
FG (kg)	446	2.81 ± 0.67	101	2.75 ± 0.56
W105 (kg)*	450	36.82 ± 6.40	100	36.01 ± 5.39

¹Number of observations for each trait and genotype.

*Significant differences ($p < 0.05$) between genotypes by the F-test.

BW - birth weight; W21 - weight at 21 days of age; W77 - weight at 77 days of age; FI - feed intake from 77 to 105 days; ADG - average daily gain from 77 to 105 days; FG - feed gain ratio from 77 to 105 days; W105 - weight at 105 days of age.

weight at 105 days of age was significantly different ($p < 0.05$) between *NN* and *Nn* genotypes, with higher values being observed for *NN* animals.

Discussion

In contrast to some authors (Eggert *et al.*, 1996; McPhee and Trout, 1995), *Nn* animals showed better carcass traits compared to *NN* animals, with higher ($p < 0.05$) RHCW, LHCW, LD and LEA and lower ($p < 0.05$) SBT, LL and P2 values, thus confirming the effect of the *PSS* gene in terms of higher lean meat content and lower fat deposition (Fisher *et al.*, 2000a, 2000b; Leach *et al.*, 1996; Lundstrom *et al.*, 1995.). However, Bastos *et al.* (2001) did not find significant differences in hot carcass weight, backfat thickness, loin depth or lean meat content among the three genotypes (*NN*, *Nn* and *nn*) when studying animals of the Large White, Landrace, Duroc and Pietrain breeds. The results of the present study differ from those described by those authors in terms of hot carcass weight and loin depth, which were higher in *Nn* than in *NN* animals ($p < 0.05$).

Hamilton *et al.* (2000), Fisher *et al.* (2000a), Lundstrom *et al.* (1995) and McPhee and Trout (1995) found shorter carcasses in *Nn* animals compared to *NN* animals, in contrast to the present study in which no significant difference in MLC traits was observed between genotypes ($p > 0.05$). However, Miller *et al.* (1999) also observed no difference in carcass length between *NN* and *Nn* genotypes. These authors suggested that this gene might have been removed from pig populations, since their results indicated only a small effect of the *PSS* gene on lean meat content.

Lundstrom *et al.* (1995), studying F₂ crosses between European wild pigs and Large White animals, found no sig-

nificant difference in growth rate between *PSS* genotypes, as also observed in the present study.

Higher ($p < 0.05$) skinless and fatless cut yields (HW, SFBSW and SFPSW) in *Nn* than in *NN* animals were also reported in the literature (Fisher *et al.*, 2000a; Leach *et al.*, 1996; Zhang *et al.*, 1992). However, in contrast to Fisher *et al.* (2000b) *Nn* animals also showed ($p < 0.05$) lower BCD than *NN* animals. However, Fisher *et al.* (2000b) has slaughtered heavier (86 kg) pigs. In this case the lack of difference in BCD between *NN* and *Nn* animals in their experiment may have been due to the fact that their *Nn* animals had already attained a growth phase of fast fat deposition which could diminish the difference in fat depth between these two genotypes. Lower BCD, associated with no difference in BCW between the two genotypes, suggests higher lean meat content in *Nn* animals with a live weight up to 65 kg.

The lack of statistical differences ($p > 0.05$) in average daily gain between *NN* and *Nn* animals disagrees with the results reported by Zhang *et al.* (1992) and McPhee *et al.* (1994), and is supported by Leach *et al.* (1996), Miller *et al.* (1999) and Jin *et al.* (2002). On the other hand, the lack of a difference ($p > 0.05$) in the feed:gain ratio between *NN* and *Nn* animals disagrees with Leach *et al.* (1996), but agrees with the results reported by McPhee *et al.* (1994) and Miller *et al.* (1999). Taken together, the lack of statistical differences in average daily gain and the feed:gain ratio indicates that the *n* allele does not influence these traits in pigs fed up to 65 kg of live weight. The lack of a difference ($p > 0.05$) in the W21 trait between *NN* and *Nn* animals confirms the results of Jin *et al.* (2002).

In the present study, the *PSS* gene had a positive effect on lean meat deposition and cut yields, but not on performance traits of pigs grown up to 65 kg of live weight. In addition, the effect of the *PSS* gene was even observed in animals resulting from divergent crossings, thus confirming studies regarding the major effects of this gene.

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