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Characterization of swine stress gene by DNA testing using plucked hair as a source of DNA

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Abstract

The swine stress gene (*hal*) in recessive homozygotes (*nn*) leads to porcine stress syndrome (PSS), and is associated with pale, soft, exudative pork (PSE). In heterozygosis (*Nn*) it is linked to poor carcass quality. A total of 179 pigs (86 Large White, 69 Landrace, 12 Duroc and 12 Pietrain) were characterized as normal homozygotes (*NN*), heterozygotes or recessive homozygotes following amplification of a target region of the *hal* gene using the polymerase chain reaction (PCR), followed by a restriction endonuclease assay. Plucked hair was used as a source of genomic DNA. The resulting PCR was digested with the restriction enzyme *Cfo*I, followed by agarose gel electrophoresis. Of 179 animals tested, 70% were *NN*, 28% were *Nn*, and 2% were *nn*. The frequency of heterozygotes was higher ($P < 0.05$) in Landrace (0.43 for *Nn*) than in Large White pigs (0.09 for *Nn*). Nine of the 12 Pietrain animals were *Nn* and three were *nn*, suggesting a high frequency for the *n* allele in this breed. These results may be related to the incidence of PSS and PSE in these two breeds, both of which are widely used in breeding programs. The utilization of plucked hair as the source of genomic DNA was a non-invasive and quick method to screen farm animals.

INTRODUCTION

The swine stress gene (*hal*) is located on chromosome 6 (p1.1-q2.1) and codes for ryanodine receptors, which are Ca^{2+} release channels of skeletal muscle sarcoplasmic reticulum (Otsu *et al*., 1991). Porcine stress syndrome (PSS) is an inherited myopathology in which skeletal muscle contraction, hypermetabolism and an elevation in body temperature are triggered by inhaled anesthetics and environmental stress (Louis *et al*., 1990; Santoro and Faucitano, 1996). Comparison of sequence of full-length *hal* cDNA (Genbank M91451) from PSS susceptible and PSS non-susceptible pigs has revealed 18 single nucleotide polymorphisms between these two types of pigs. One of the polymorphisms involves the substitution of cytosine (PSS non-susceptible) by a thymine (PSS susceptible) at nucleotide 1843. This alteration results in the replacement of an arginine at position 615 by a cysteine (Fujii *et al*., 1991). As a consequence, in recessive homozygotes (*nn*) the gene *hal* leads to PSS and the major *post-mortem* manifestation of pale, soft and exudative pork (PSE). In heterozygosis (*Nn*) the *hal* gene produces lower carcass quality (Prommier and Houde, 1993; Cheak *et al*., 1994; Webb, 1996), but possibly higher carcass weight (Zhang *et al*., 1992; Leach *et al*., 1996; Fávero, 1997). The polymorphism at nucleotide 1843 of the *hal* gene has recently been characterized by a DNA test using blood or a muscle biopsy as the source of genomic DNA (Houde *et al*., 1993; Cheak *et al*., 1994).

In this study, we assessed the validity of the DNA test in commercial pigs using PCR amplification of the target region of *hal* gene followed by a restriction endonuclease assay (REA). We also examined the feasibility of using plucked hair as source of genomic DNA, and determined the frequency of each *hal* genotype in Landrace, Large White, Duroc and Pietrain pig breeds.

MATERIAL AND METHODS

Animals

One hundred seventy-nine pigs (86 Large White, 69 Landrace, 12 Duroc and 12 Pietrain) were studied. All animals came from a single commercial farm in the State of Rio Grande do Sul, Brazil. Plucked hair was collected from randomly chosen pigs.

Extraction of genomic DNA

Genomic DNA was extracted from the hair roots using two protocols. In protocol I (Bauerová *et al*., 1995), 10 hair roots were suspended in 100 µl of lysis buffer (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, 2% SDS, 70 mM DTT and 0.4 mg/ml of proteinase K), and then incubated at 45°C overnight. Five microliters was used in the PCR reaction. In protocol II, modified from Drissing *et al*. (1996), 1-3 hair roots were incubated at room temperature for 5 min in 0.5 µl of 0.1 M NaOH, after which 4.5 µl of 0.02 M Tris-HCl, pH 7.4, was added. The PCR reaction mixture (20 µl) was then added to the tube containing the above extract. As alternative sources of DNA, blood and semen samples were also used and the DNA was extracted according to the method described by Sambrook *et al*. (1989).

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PCR and REA

The PCR reactions were done in a final volume of 25 µl containing 200 µM of each dNTP, 2.5 µl of PCR buffer with a final concentration of 1.5 mM $MgCl_2$, 0.2 µM of each primer (forward 5'-GTTCCCTGTGTGGCAATGGTG-3' and reverse 5'-ATCTCTAGAGCCAGGGAGCAAGTTCT CAGTAAT-3') (Fujii *et al.*, 1991), 1 U of Taq DNA polymerase (CENBIOT/RS, UFRGS) and 5 µl of extraction solution containing genomic DNA (Innis *et al.*, 1990). The PCR reaction was carried out in a Perkin Elmer 2400 thermocycler with the following settings: 1 min at 95°C, 1 min at 56°C, and 1 min at 72°C for 30 cycles with a final cycle of 7 min at 72°C. Five microliters of the PCR product was digested with 5 U of *Cfo*I (Boehringer Mannhein) for 3 h at 37°C. Digestion by *Cfo*I produced two DNA fragments of 49 pb and 32 pb for the *N* allele and an intact 81 pb for the "n" allele. The digested product was submitted to electrophoresis on a 4% agarose gel containing 0.5 µg of ethidium bromide/ml (Sambrook *et al.*, 1989), visualized with an UV transilluminator and photographed using a Kodak DC40 digital camera.

Gene frequency

The genotype frequencies of the *hal* gene (*NN*, *Nn*, and *nn*) among the breeds were analyzed using the chi-square test (χ^2).

RESULTS AND DISCUSSION

PCR amplified a specific 81-base pair (bp) DNA fragment from the *hal* gene using different sources of pig DNA. Collection and transportation of plucked hair were quite easy so this was the source used throughout this study. Protocol II for DNA extraction from plucked hair gave the most consistent results, and since it was simpler and easier to perform than protocol I, this method was adopted as standard. A single hair root was enough to allow amplification of the expected DNA fragment (Figure 1). The impurities present in the DNA samples apparently did not affect amplification of the target DNA. The size of the fragment amplified by PCR was the same, 81 bp, in all samples analyzed.

Digestion of the PCR product with *Cfo*I produced two fragments of 49 bp and 32 bp for normal homozygotes (*NN*), three fragments of 81 bp, 49 bp and 32 bp for heterozygotes (*Nn*) and an undigested fragment of 81 bp for recessive homozygotes (*nn*) (Figure 2).

The gene frequency of the *n* allele of the *hal* gene was higher ($P < 0.05$) in Landrace than in Large White animals (Table I). The frequency of heterozygotes was higher ($P < 0.05$) in Landrace pigs than Large White pigs.

Comparisons between Duroc and Pietrain pigs were not done, because of the small sample size. However, there was a tendency towards a high frequency of the *n* allele in Pietrain pigs and a low frequency of this allele in Duroc pigs. No Pietrain animal was characterized as *NN*.

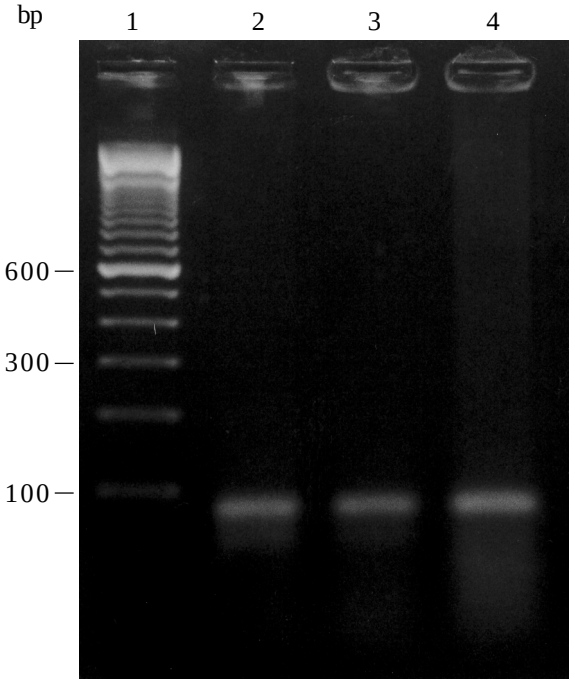

Figure 1 - PCR products obtained using DNA extracted with protocol II. **Lane 1**: 100-bp ladder molecular weight marker (Gibco BRL); **lane 2**: DNA extracted from a single hair root; **lane 3**, DNA extracted from two hair roots, and **lane 4**: DNA extracted from three hair roots.

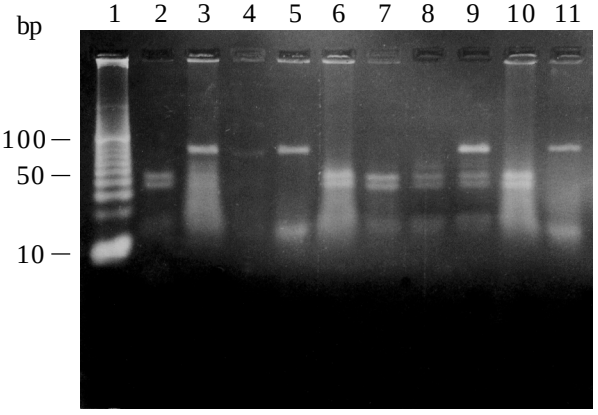

Figure 2 - Electrophoretic profile of PCR products digested with *Cfo*I. **Lane 1**: 10-bp ladder molecular weight marker (GIBCO BRL); **lanes 2**, **6**, **7**, **8**, and **10**: normal homozygotes (*NN*) (two Large White pigs, two Landrace pigs, and one Duroc pig); **lanes 3**, **4**, and **9**: heterozygotes (*Nn*) (two Landrace pigs and one Pietrain pig); **lanes 5** and **11**: recessive homozygotes (*nn*) (two Pietrain pigs).

Plucked hair was a suitable source of DNA for PCR, and it was easily collected and transported from the farm to the laboratory. This source of DNA should facilitate the characterization of the *hal* genotype in farm animals. Collection and use of blood or semen as a source of DNA is

Table I - Frequencies of the *hal* gene in different breeds of pigs.

Breeds	No.	Gene frequency (%)		
		NN (No.)	*Nn* (No.)	*nn* (No.)
Large White	69	0.91[a] (78)	0.09[a] (8)	-
Landrace	86	0.56[b] (38)	0.43[b] (31)	-
Duroc	12	0.83 (10)	0.16 (2)	-
Pietrain	12	-	0.75 (9)	0.25 (3)
Total	179	0.70 (126)	0.28 (50)	0.02 (3)

NN - Normal homozygotes; *Nn* - heterozygotes; *nn* - recessive homozygotes; [a,b]Different letter means $P < 0.05$.

laborious and, in some cases, dangerous because of the stress caused to the animal during sample collection. Death may occur if the genotype for the *hal* gene is *nn*.

The high frequency of heterozygotes (*Nn*) in Landrace pigs and the high frequency of heterozygotes (*Nn*) and recessive homozygotes (*nn*) in Pietrain pigs may be related to the incidence of PSS and PSE, since both of these breeds are used widely in breeding programs and commercial farms. A study to correlate the presence of the *n* allele with carcass traits in these animals was done by Bastos *et al*. (2000), and no correlation was found between the presence of the *n* allele and higher carcass weight, contrary to findings of Zhang *et al*. (1992), Leach *et al*. (1996) and Fávero (1997).

As the presence of the *hal* gene, both in heterozygosity and recessive homozygosity, does not seem to be associated with better carcass weight, and the utilization of these animals may lead to lower carcass quality, breeding programs should aim at eliminating this genotype from the herd. The use of hair roots as a source of DNA should facilitate the analysis of the genotype of *hal* gene of different breeds and crossbreed pigs.

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RESUMO

O gene do estresse suíno (*hal*) em homozigose recessiva (*nn*) ocasiona a síndrome do estresse porcino (PSS), e está relacionado com a ocorrência da carne pálida, mole e exudativa (PSE). Em heterozigose (*Nn*) está associado com diminuição da qualidade de carcaça. Um total de 179 suínos (86 Large White, 69 Landrace, 12 Duroc e 12 Pietrain) foram caracterizados como homozigotos normais (*NN*), heterozigotos e homozigotos recessivos por análise do DNA usando a reação de polimerização em cadeia (PCR), seguida de um ensaio com endonuclease de restrição. Foi utilizado folículo piloso como fonte de DNA genômico. O produto do PCR foi digerido com a enzima de restrição *Cfo*I, seguindo-se análise dos fragmentos por eletroforese em gel de agarose. Dentre os 179 animais analisados, 126 (70,0%) foram caracterizados como *NN*, 50 (28,0%) como *Nn*, e 3 (2,0%) caracterizados como *nn*. A freqüência de heterozigotos foi maior ($P < 0,05$) em animais da raça Landrace do que em animais da raça Large White. Nove animais da raça Pietrain foram classificados como heterozigotos e tres como homozigotos recessivos. Estes resultados podem estar relacionados com a incidência de PSS e PSE nestas raças, as quais são largamente utilizadas em programas de cruzamento. A utilização de folículo piloso como fonte de DNA genômico foi um método rápido e não-invasivo que viabiliza a execução da caracterização do gene *hal*.

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