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Influence of the halothane gene (HAL) on pork quality in two commercial crossbreeds

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ABSTRACT. We evaluated the effect of the halothane (HAL) gene on the quality of pork in domestic pigs. Half-carcasses from two different commercial pig (*Sus domestica*) crossbreeds were analyzed, 46 of which were homozygous dominant (HAL^{NN}) and 69 of which were heterozygous (HAL^{Nn}) for the halothane gene. The measures included backfat thickness, lean meat percentage, carcass weight, pH 24 h after slaughtering, color, and drip loss; DNA was extracted from the haunch muscle. Swine with the HAL^{Nn} genotype had less backfat thickness and higher lean meat percentages than swine with the HAL^{NN} genotype. Yet, swine with the HAL^{Nn} genotype had lower quality meat than those with the HAL^{NN} swine. The pH at 24 h was lower in HAL^{Nn} swine. The meat color was paler in HAL^{Nn} animals, the drip loss was greater in those animals bearing the *n* allele, and the amount of intramuscular fat was not related to the halothane genotype. We conclude that bearers of the recessive allele of the halothane gene produce more meat, but with quality parameters that are inferior to those sought by consumers and industry.

Key words: Porcine stress syndrome gene; PSE; PCR-RFLP; Swine

INTRODUCTION

Pork is the most consumed animal meat worldwide; this is due in large part to characteristics that ease its transformation and the subsequent variety of products. Commercialization of pork on the international market has increased considerably; exports doubled in only eight years. Brazil, which contributes 14% of the world production of pork, is the fourth largest exporter of this meat (ABIPECS, 2008). Brazil's pork products are from reasonable to good quality and are produced at low cost, which enables them to be offered at competitive prices; however, these products are mainly exported to countries with few quality requirements and with low remuneration for the final product (Antunes et al., 2001).

The export of pork to markets that are more conscious of issues related to animal sanitation and well-being, and methods of slaughter and meat processing, such as Japan, the United States and Europe, is the main focus of large companies that invest in resources that guarantee improvements in quality and attempt to gain better remuneration for their products. Brazil will soon have access to these more demanding markets, and it is necessary that the country be prepared for the demand for high-quality meat. New goals for meat quality need to be incorporated into selection systems, whether they are quantitative, qualitative or organoleptic (Antunes et al., 2001).

Differences in the quality of pork are due to genetic and environmental factors. There are several variations in the quality of pork that have been identified by slaughterhouses all over the world, such as the porcine stress syndrome, the pale, soft and exudative (PSE) meat and the halothane (HAL) gene (Peloso, 1999).

The HAL gene, besides determining the predisposition to porcine stress syndrome in swine and being related to PSE, which is a serious problem for the meat industry, is also responsible for carcasses with a greater proportion of lean meat. The halothane gene not only affects the meat quality of homozygous recessive (HAL^{nn}) swine but also the quality of heterozygote (HAL^{Nn}) swine carcasses (Sather et al., 1991).

We examined polymorphisms in the HAL gene in two commercial crossbreeds and how they correlate with characteristics of meat quality, including color, drip loss, pH, lean meat percentage, and intramuscular fat.

MATERIAL AND METHODS

Animals

The animals were produced on commercial ranches affiliated with Frigorífico Cotrijuí, which is located near the city of São Luiz Gonzaga, RS, Brazil. They were reared following the sanitary, well-being and animal environment standards required by the competent public organs in Brazil. Two different crossbred groups were studied:

Group A - 58 hybrid animals derived from crossbreeding pure German Pietrain males with commercial females, which results in a Pietrain genotype in the maternal line that is free from HAL.

Group B - 57 hybrid animals derived from crossbreeding 75% German Pietrain and 25% Duroc males with commercial females of the same line that did not have the HAL gene.

Slaughter

Slaughter was carried out at Frigorífico Cotrijuí, which is located in São Luiz Gonzaga, RS, Brazil, and has a Federal Inspection Service. The animals were transported to the slaughterhouse in special trucks, using correct pre-slaughter handling. Animals were maintained in a corral for 12 h with only water available. Before slaughter, the animals passed through a corridor with showers, which minimizes heat and reduces stress. The corridor was electrified at two points, the first of which was 438 V and 0.69 A and the second was 106 V and 0.57 A over a 5-s interval. Afterwards, bloodletting, scalding at 62°C for 3 s, toilette (hair extraction), and evisceration were carried out. The animals were hung by the left leg and stored in a refrigerated chamber kept at temperatures between 4° and 10°C.

Collection and preparation of the samples

Twenty-four hours after slaughter, the pH, backfat thickness and lean meat percentage were measured in the carcasses, and samples of meat (haunch muscle tissue from the semimembranosus muscle) were taken for the analyses of color, intramuscular fat, drip loss, and DNA. All measurements and samples were obtained from the right half carcass of the animals.

pH assessment

The pH of the haunch muscle from the right half carcass was measured 24 h after slaughtering using a portable Tradelab Testo 205 pH meter, which is specific for analyzing the pH of meat.

Lean meat percentage

The ruler and pistol methods were used to determine the lean meat percentage of the animals. In the ruler method, backfat thickness was measured in the right half-carcass, 24 h after slaughtering (Antunes, 2002). The pistol method was run with a New-Zealand pistol Henessey Grading Probe.

Color measurement

The meat sample color was visually classified according to the Nippon Ham industry standard, which is used worldwide. This standard has a scale from 1 to 6, with number 3 being the ideal pork color. Colors 1 and 2 are weaker and can indicate PSE pork. Number 4 is still within the acceptable limits, although it is a little dark. The numbers 5 and 6 are common in cases of dark, firm and dry pork and in heavy and old animals.

Drip loss

Twenty-four hours after slaughtering, a 4 x 4 x 4-cm cube of meat from the semimembranosus muscle of each animal was collected and weighed, obtaining initial weights between 80 and 100 g, following the Pork Composition and Quality Assessment Procedures of the American Meat Science Association (NPPC, 2000). The meat cubes were packaged in plastic

bags and hung for 48 h at 2° to 4°C, with the lost liquid (blood) captured in the package.

After 48 h, the meat was dried using paper towels and weighed again to obtain final weights after the drip loss. The percentage drip loss was calculated by multiplying the difference between the final and initial weights by 100. The samples were packed in plastic bags with identification tags and frozen for further analyses of intramuscular fat and DNA extraction for halothane genotyping.

Intramuscular fat (ether extract)

The percentage of intramuscular fat was estimated from the ether extract using the Goldfish method, according to the Manual for Analytical Proceedings of the Brazilian Animal Feed Compendium (Ministério da Agricultura e Abastecimento, 1998). After drip loss analysis, the frozen samples were thawed, a small quantity (10 mg) was taken for DNA extraction and the remaining sample was cut in cubes to be dried in an incubator at 55°C for 72 h to prepare it for grinding in a mill. Afterwards, the protocol below was followed:

1. Five grams of the dried and grinded sample, packed in a cartridge extractor, was weighed and placed in a drying oven at 105°C for 2 h;

2. The cartridge was put in the extractor, petroleum ether was added in appropriate quantities and the extractor was attached to the condenser and to a volumetric balloon supported on an electrically warmed metal sheet;

3. The fat was extracted for 6 h at a condensation speed of 2 to 4 drips/s;

4. The balloon containing the extracted fat was placed in a drying oven at 105°C for 30 min, chilled in a desiccator and weighed;

5. The fat percentage was calculated according to the following equation:

$$\text{Ether extract \%} = \frac{(A - B) \times 100}{C}$$

where *A* is the balloon weight with fat; *B* is the empty balloon weight, and *C* is the weight of the sample put in the cartridge extractor.

DNA extraction

The muscle sample collected for the DNA extraction was stored in 2.5-mL plastic tubes containing TriZOL® and forwarded to the Laboratório Biogenetics (Biogenetics Laboratory), Uberlândia, Minas Gerais, for DNA extraction, according to the protocol described below.

The TriZOL® solution was transferred to a 1.5-mL tube, taking care not to transfer pieces of the muscle. A total of 0.2 mL chloroform was added to each 1 mL TriZOL®, which was vigorously agitated in a vortex until a milky pinkish solution was obtained. The solution was centrifuged at 13,000 *g* at 4°C for 15 min and separated into three phases: the aqueous containing the RNA, the white and milky interphase, which mainly contained the DNA, and the lower pinkish phase predominantly containing proteins.

The aqueous phase was removed and 0.3 mL absolute ethanol was added per 1 mL TriZOL® that was initially used, and the solution was mixed by inversion. Samples were incubated at room temperature for 3 min and centrifuged at 1900 *g* for 5 min at 4°C. The supernatants were removed and the resulting pellets were washed with 0.1 M sodium citrate in 10%

ethanol per 1 mL TriZOL®. Solutions were incubated at room temperature for 30 min, agitated by vortexing every 10 min and centrifuged at 2000 *g* for 5 min at 4°C. The pellets were suspended in 2 mL 75% ethanol for each 1 mL TriZOL® that was initially used, incubated at room temperature for 15 min, agitated by vortexing every 5 min, and centrifuged at 2000 *g* for 5 min at 4°C. The supernatants were carefully removed, and the tubes were inverted in a laminar flow hood over filter paper for 2 to 10 min to dry the pellets, which were dissolved in 450 µL 8 mM NaOH for each 60 mg tissue used. Samples were centrifuged at 13,000 *g* for 10 min to obtain the supernatant containing the DNA. After the extraction, the quality of the DNA was assessed by electrophoretic separation on 1% agarose gels, and the total DNA quantity was estimated with a spectrophotometer at 260 nm.

Genotyping

Characterization of the HAL gene was carried out at the Laboratório Biogenetics (Biogenetics Laboratory), which used primers with the following sequences: 5'CCTGGGACATCATCCTTCTG3' and 5'GGTGGTGGAGGGTTCTAAGC3' taken from Franco et al. (2008). For the polymerase chain reaction (PCR), 50-150 ng DNA was added to a reaction mixture consisting of 2 nM $MgCl_2$, 1U *Taq* DNA polymerase, 8 nmol dNTPs, and 10 pmol of each primer, in a final volume of 20 µL. The temperature cycling program consisted of the following steps: 95°C for 5 min; 34 cycles of 94°C for 30 s, 56°C for 35 s, 72°C for 30 s, 94°C for 30 s, and a final extension step at 72°C for 4 min. The amplified fragment of the halothane gene was enzymatically digested with the *Hha*I restriction enzyme, which recognizes and cuts the sequence 5'GCGC3' within the normal allele, or with the *Bsi*HKAI enzyme, which recognizes and cuts the 5'GA/TGCA/TC3' sequence from the mutant allele. Digested fragments were electrophoretically separated on 2.5% agarose gels and visualized by staining with ethidium bromide and exposure to ultraviolet light.

Statistical analysis

An analysis of variance was carried out using a completly randomized design with a 2 x 2-factorial scheme (2 HAL genotypes x 2 types of crossbreed) and the Tukey test was used for comparison among averages, both at 5% significance. The variates were corrected through the covariance, considering the hot carcass weight as a covariate. All the analyses were performed with the Statistica 8.0 program (Statsoft, 2007).

RESULTS AND DISCUSSION

Genotypic and allelic frequencies of the halothane gene

Of the 115 carcasses, 40% contained the normal halothane genotype (HAL^{NN}), including 15% from the A crossbreed and 25% from the B crossbreed. There were 60% heterozygous animals (HAL^{Nn}); 36% were from the A crossbreed and 24% were from the B crossbreed. None of the animals had a double recessive halothane genotype (HAL^{nn}) in either crossbreed; 76.5% of the animals possessed normal dominant alleles and 23.5% of the animals had a recessive allele (Tables 1 and 2).

Table 1. Genotypic frequency of the HAL gene of 115 swine of two commercial crossbreeds, A and B, São Luiz Gonzaga, RS, Brazil, 2008.

Crossbreed	Genotype						Total	%
	HAL^{NN}	%	HAL^{Nn}	%	HAL^{nn}	%		
A	17	15	41	36	0	0	58	51
B	29	25	28	24	0	0	57	49
Total	46	40	69	60	0	0	115	100

Table 2. Allelic frequency of the HAL gene of 115 swine of two commercial crossbreeds, A and B, São Luiz Gonzaga, RS, Brazil, 2008.

Allele	Number of alleles	%
N	161	70
n	69	30
Total	230	100

Bastos et al. (2001) genotyped 160 hybrid commercial swine and found a genotypic frequency of 52.6% HAL^{NN}, 41.8% HAL^{Nn} and 5.6% HAL^{nn} animals. Culau et al. (2002) examined 151 carcasses; 61.6% had the normal halothane genotype (HAL^{NN}), 33.8% were heterozygotes (HAL^{Nn}) and 4.6% had the recessive halothane genotype (HAL^{nn}).

According to Murray (1994), in a randomized swine population, the expected frequency of the HAL gene should be 23% for HAL^{Nn} and 1.8% for HAL^{nn} animals. Such a large difference between the expected and observed frequencies in the HAL gene may be due to management practices, in which the HAL gene has undergone selection as a means to increase the quantity of the meat in the carcass by crossing heterozygous males (HAL^{Nn}) with homozygous dominant females (HAL^{NN}). This procedure would result in 50% HAL^{Nn} and 50% HAL^{NN} progeny, with an increase from 1 to 2% of total meat content and, supposedly, without any change in its quality (Fávero and Belláver, 2007).

Analysis of the characteristics of meat quality

A crossbreed animals with the *n* allele had significantly less backfat thickness (measured with a ruler) than the HAL^{NN} animals (Table 3).

Table 3. Averages of the backfat thickness (mm) measured by ruler in 115 carcasses of commercial hybrid swine (A and B crossbreeds) according to the genotype of the HAL gene, São Luiz Gonzaga, RS, Brazil, 2008.

Crossbreed	Genotype		
	HAL^{NN}	HAL^{Nn}	
A	20.67^{Aa}	17.70^{Ab}	18.57^{A}
B	18.66^{Aa}	18.68^{Aa}	18.66^{A}
	19.39^{a}	18.09^{a}	

Averages followed by identical small letters in the line do not differ statistically among them by the Tukey test at 0.05. Averages followed by identical capital letters in the column do not differ statistically among them by the Tukey test at 0.05.

Culau et al. (2002) did not find a significant difference between the backfat thicknesses of these same genotypes, as did Sather and Jones (1996). However, they found that

normal swine (HAL^{NN}) had greater backfat thickness than the heterozygote (HAL^{Nn}) animals for the $P < 0.06$, as also found by Antunes (1997).

We found that HAL^{Nn} animals had a higher percentage of lean meat (measured by ruler) compared to homozygous dominant animals (Table 4), as reported by Culau et al. (2002). These authors also found that heterozygous swine have a greater percentage of lean meat than normal swine.

Table 4. Averages of the backfat thickness percentage measured by formula in 115 carcasses of commercial hybrid swine (A and B crossbreeds) according to the genotype of the HAL gene, São Luiz Gonzaga, RS, Brazil, 2008.

Crossbreed	Genotype		
	HAL^{NN}	HAL^{Nn}	
A	57.46^{Aa}	58.87^{Ab}	58.46^{A}
B	58.42^{Aa}	58.40^{Aa}	58.42^{A}
	58.06^{a}	58.68^{a}	

Averages followed by identical small letters in the line do not differ statistically among them by the Tukey test at 0.05. Averages followed by identical capital letters in the column do not differ statistically among them by the Tukey test at 0.05.

De Smet et al. (1998) reported that the lean meat content was greater in HAL^{nn} recessive swine carcasses, moderate in heterozygous swine (HAL^{Nn}) and lower in the normal swine, with heterozygous swine closer in lean meat content to the recessive swine (HAL^{nn}) than the normal swine. However, Sather and Jones (1996) reported similar values for normal and heterozygous swine (HAL^{NN} and HAL^{Nn}). They observed that heterozygote swine contained only 1% more lean meat compared to homozygous animals, whereas Culau et al. (2002) found a difference of 2.38% more lean meat in heterozygous swine than in normal swine.

In the case of the lean meat calculated by the pistol technique (Table 5), significant differences were found between A and B crossbreeds, which may be explained by the data lost during collection due to the difficulty in acquiring these values. Another possibility is that the formula used for the calculation is specific to the animals of an extinct company and may not be representative of the animals used in this trial. This is a current problem in the swine production industry. The independent producers that slaughter their animals in commercial slaughterhouses that remunerate the producers (integrated or not) by means of a typification formula are subject to significant differences in the remuneration due to data on animals that is embedded in the formula of the company.

Table 5. Backfat thickness calculated by pistol (HGP) of 115 carcasses of commercial hybrid swine (A and B crossbreeds) according to the genotype of the HAL gene, São Luiz Gonzaga, RS, Brazil, 2008.

Crossbreed	Genotype		
	HAL^{NN}	HAL^{Nn}	
A	47.96^{Aa}	53.62^{Ab}	51.96^{A}
B	55.70^{Ba}	54.69^{Aa}	55.20^{B}
	52.84^{a}	54.05^{a}	

Averages followed by identical small letters in the line do not differ statistically among them by the Tukey test at 0.05. Averages followed by identical capital letters in the column do not differ statistically among them by the Tukey test at 0.05.

The pH at 24 h was greater in the crossbreed B animals (Table 6). In addition, there was also a significant difference between the HAL^{NN} and HAL^{Nn} swine, with higher pH values

in the heterozygous animals. This is expected because the animals with lower pH also have lower quality meat. With this in mind, it can also be said that the HAL^{Nn} animals have more meat and less fat, but lower meat quality. The median values found are above those described by Lawrie (1958) as acceptable (between 5.3 and 5.7), so that the meat has good palatability, softness and a desirable color.

Table 6. Averages of the quantitative measurements of pH taken 24 h after slaughtering (pH 24 h) in 115 carcasses of commercial hybrid swine (A and B crossbreeds) according to the genotype of the HAL gene, São Luiz Gonzaga, RS, Brazil, 2008.

Crossbreed	Genotype		
	HAL^{NN}	HAL^{Nn}	
A	5.85^{Aa}	5.72^{Aa}	5.76^{A}
B	5.94^{Aa}	5.80^{Aa}	5.87^{B}
	5.90^{a}	5.76^{b}	

Averages followed by identical small letters in the line do not differ statistically among them by the Tukey test at 0.05. Averages followed by identical capital letters in the column do not differ statistically among them by the Tukey test at 0.05.

The HAL gene exercises influence over the initial pH and, even in heterozygous animals it provokes a decrease in the initial pH values and results in a greater frequency of PSE meats (Russo et al., 1994; Sather and Jones, 1996; De Smet et al., 1998; Culau et al., 2002). The final pH of the carcasses does not differ between the three halothane genotypes, demonstrating that the HAL gene does not influence the final pH (De Smet et al., 1998; Tam et al., 1998; Culau et al., 2002).

With regard to the meat color characteristics, a significant difference was observed between the HAL^{NN} and HAL^{Nn} animals in the A crossbreed and between the HAL^{NN} and HAL^{Nn} animals overall, in which the HAL^{Nn} animals were paler (Table 7). This result was expected because there is an interaction between pH, temperature and color, in which temperatures above 55°C cause a significant increase in the denaturation of metmyoglobin. At temperatures between 25° and 55°C, there is an interaction between pH and temperature, with temperature alone not capable of denaturating metmyoglobin. At temperatures well below 50°C and pH levels below 5.6, a significant denaturation of metmyoglobin can occur in the meat, provoking a pale appearance (Zhu and Brewer, 2002).

Table 7. Averages of the quantitative measurements of color according to the Japanese standard (being 1 the palest and 6 the darkest) in 115 carcasses of commercial hybrid swine (A and B crossbreeds) according to the genotype of the HAL gene, São Luiz Gonzaga, RS, Brazil, 2008.

Crossbreed	Genotype		
	HAL^{NN}	HAL^{Nn}	
A	3.04^{Aa}	2.46^{Ab}	2.63^{A}
B	2.71^{Aa}	2.39^{Aa}	2.56^{A}
	2.84^{a}	2.43^{b}	

Averages followed by identical small letters in the line do not differ statistically among them by the Tukey test at 0.05. Averages followed by identical capital letters in the column do not differ statistically among them by the Tukey test at 0.05.

Culau et al. (2002) observed that the average color of the pork was 2.41 (± 0.56), which is slightly paler than the normal score, which is equal to 3.0. They found that the pork chops of the heterozygote (HAL^{Nn}) and recessive (HAL^{nn}) swine were significantly paler than those of the homozygous dominant animals, similar to findings by Simpson et al. (1987); Lunsdtröm et al. (1989); Sather et al. (1991); Russo et al. (1994), and De Smet et al. (1995).

We found significant differences in drip loss values between the HAL^{NN} and HAL^{Nn} genotypes overall; they were greater in the animals with the *n* allele (Table 8). Culau et al. (2002) found that the average liquid drip loss was greater in recessive swine; however, drip loss did not differ in the other groups, different from what was reported by Russo et al. (1994) and De Smet et al. (1996), who found significant differences between all genotypes.

Table 8. Averages of the quantitative measurements of drip loss in 115 carcasses of commercial hybrid swine (A and B crossbreeds) according to the genotype of the HAL gene, São Luiz Gonzaga, RS, Brazil, 2008.

Crossbreed	Genotype		
	HAL^{NN}	HAL^{Nn}	
A	1.63^{Aa}	2.19^{Aa}	2.02^{A}
B	1.70^{Aa}	2.47^{Ab}	2.07^{A}
	1.67^{a}	2.30^{b}	

Averages followed by identical small letters in the line do not differ statistically among them by the Tukey test at 0.05. Averages followed by identical capital letters in the column do not differ statistically among them by the Tukey test at 0.05.

The undesirable pH and temperature conditions found in the meat of stressed animals normally lead to an inferior water holding capacity (Rosenvold et al., 2002). This indicates that, even in populations free from the HAL gene, pH and temperature are of great importance in the formation of exudate. Along these lines, Schäfer et al. (2002) reported that only 2% of the range of loss by exudation is explained by the pH at 24 h. Henckel et al. (2002) also reported that the pH at 24 h is a weak indicator of the water holding capacity in the populations free of the HAL gene. Considering that 75% of pork is destined for processing, the industrialization of meat that has low water holding capacity becomes economically unfeasible (Angerami, 2004).

Baulain et al. (2000), Hermesh et al. (2000) and Schworer et al. (2000) mention that high levels of intramuscular fat are key to the production of high-quality meat; however, we found no significant differences between the crossbreeds and the genotypes (Table 9), as also reported by Antunes (2002).

Table 9. Averages of the quantitative measurements of intramuscular fat in 115 carcasses of commercial hybrid swine (A and B crossbreeds) according to the genotype of the HAL gene, São Luiz Gonzaga, RS, Brazil, 2008.

Crossbreed	Genotype		
	HAL^{NN}	HAL^{Nn}	
A	1.83^{Aa}	2.00^{Aa}	1.95^{A}
B	1.96^{Aa}	2.08^{Aa}	2.02^{A}
	1.91^{a}	2.04^{a}	

Averages followed by identical small letters in the line do not differ statistically among them by the Tukey test at 0.05. Averages followed by identical capital letters in the column do not differ statistically among them by the Tukey test at 0.05.

CONCLUSIONS

We found that swine with the heterozygous HAL^{Nn} genotype had lower backfat thickness and greater lean meat percentage than those with an HAL^{NN} genotype; however, animals with the recessive allele produce an inferior quality meat. The pH at 24 h was lower in swine bearing the recessive HAL allele; only in this characteristic. Meat color was also paler in swine bearing the recessive allele of the halothane gene, and the drip loss was greater in animals with the *n* allele. Finally, the amount of intramuscular fat in the animals was not related to the halothane gene.

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