

## *RYRI* gene variability and effect on meat pH in Argentinean hybrids swines

Variabilidad del gen *RYRI* y efecto en el pH de la carne en cerdos híbridos argentinos

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### SUMMARY

The *RYRI* gene is the principal cause of the porcine stress syndrome (PSS) and the presence of pale, soft and exudative meat (PSE) in swine. In Argentina there are no reports about the variability of *RYRI* gene in hybrid swine and its effect on the quality of carcass.

By PCR-HRM we analyzed 403 hybrids swines from Argentina and 65.0% were homozygous *NN*, 30.8% heterozygous *Nn* and 4.2% homozygous *nn*. Additionally, a significant effect ( $p < 0.0001$ ) of the *RYRI* gene was detected on the pH of carcass at 45 minutes after slaughter. Normal genotypes *NN* showed the highest pH values at 45 minutes after slaughter (6.27), heterozygous *Nn* showed intermediate pH values (5.94) and homozygous susceptible *nn* showed the lowest pH values (5.64). Argentina present high incidence of *Nn* and *nn* genotypes for the *RYRI* gene in relation to Brazil, the principal pig producer of Latin America. Additionally the *RYRI* gene affects the pH of the meat, one of the principal parameters in the determination of the PSE meat.

Key words: (Swine), (*RYRI*), (Molecular Markers), (PSE)

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## RESUMEN

El gen *RYRI* es la principal causa del síndrome de estrés porcino (PSS) y la presencia de carne pálida, blanda y exudativa (PSE) en los cerdos. En Argentina no existen informes sobre la variabilidad de dicho gen en cerdos híbridos y su efecto sobre la calidad de la canal.

Por PCR-HRM se analizaron 403 cerdos híbridos de Argentina y el 65,0% eran homocigotos *NN*, 30,8% heterocigotos *Nn* y el 4,2% homocigotos *nn*. Además, se detectó un efecto significativo ( $p < 0,0001$ ) del gen *RYRI* en el pH de la canal a los 45 minutos post faena. Los genotipos *NN* mostraron los mayores valores de pH a los 45 minutos después del sacrificio (6,27), los heterocigotos *Nn* mostraron valores intermedios de pH (5,94) y los homocigotos *nn* presentaron los valores más bajos de pH (5,64).

Argentina presenta alta incidencia de los genotipos *Nn* y *nn* del gen *RYRI* en relación a Brasil, el principal productor de cerdos de América Latina. Además, el gen *RYRI* afecta al pH de la carne, uno de los principales parámetros para la determinación de carnes PSE.

Palabras clave: (Cerdos), (*RYRI*), (Marcadores moleculares), (PSE)

## INTRODUCTION

In recent years, pig production sector, worldwide has endeavored to select lean hogs. However, from the sixties, it was observed that the selection results were linked to high mortality due to stress. The selection of breeding with better lean features and increased muscle development (breeds Pietrain, Poland China and Landrace) corresponded with diseased animals or bearer of the Porcine Stress Syndrome (PSS) or malignant hyperthermia (MH). Sick animals, with the minimum stress can show clinical signs such as tremors and muscle stiffness, purplish spots on skin, increased body temperature and may even lead to death, causing significant losses in primary production<sup>5</sup>. After sacrificing the animal, this disease compromises pork quality due to the rapid decrease in pH, resulting in a pale, soft and exudative meat (PSE). These results in great losses in the industry at obtain a product with altered colors and flavors and lower yield in the cooking. The main cause of this disease is a mutation at nucleotide 1843 of cDNA (GenBank M911451) of the gene encoding the calcium release channel in the

skeletal muscle sarcoplasmic reticulum called ryanodine receptor (*RYRI*), commonly known as the halothane gene (HAL)<sup>7</sup>.

High-Resolution Melting (HRM) is a post PCR analysis method used to identify genetic variations in nucleic acid sequences. This method is based on the properties of dissociation (melting) of double-stranded DNA. Different melting profiles are obtained from the transition of double-stranded DNA to simple-stranded DNA due to a gradual increase in temperature after the PCR<sup>10</sup>. The recent development of HRM can be attributed to the generation of new dyes designed for this technique, and technological improvements in the instruments of real-time PCR<sup>13, 8</sup>. HRM is considered the simplest method for genotyping and mutation detection because it is done in the same tube and immediately after the PCR<sup>9, 10, 12</sup>.

In Argentina there is no precedent in the incidence of *RYRI* gene and its effect on the quality of carcass, therefore the objective of this study was to evaluate the variability of this gene and the effect in the pH of the carcass of hybrids pigs from four provinces of Argentina which represents the 60% of pig production in Argentina.

## MATERIALS AND METHODS

For the analysis 403 commercial hybrid pigs were used. The animals come 20 different producers from 4 different states of Argentina (Córdoba, Santa Fe, Chaco and Tucumán). The main of the tested animals are hybrids derived from crossing hybrids females Landrace x Yorkshire with terminal hybrids males composed by different proportions of Duroc, Pietrain, Hampshire and Yorkshire, the tested animals were unrelated in parentage within producers.

The pigs were slaughtered at a live weight between 100 and 110 kg and were deprived of food for around 18 hs before slaughter, but had access to fresh water *ad libitum*. The pigs were electrically stunned and bled by puncture in the jugular vein.

The pH of carcass was measured in the *longissimus dorsi* muscle between the ribs 9 and 11 at 45 min postmortem in the left half of the carcass with a digital pH meter (Testo 205).

Genomic DNA was extracted from hair roots using the protocol detailed in Bastos *et al.* (2000)<sup>2</sup>.

The amplification of PCR products was made using the cycling conditions and primers developed by Brenig and Brem (1992)<sup>4</sup> in a final volume of 25 µl which contained 1X Real Mix (Biodynamics), 0,2 µM of each primer, 5 µl of extraction solution of genomic DNA and mQ H<sub>2</sub>O. The C/T single nucleotide polymorphism (SNP) in the *RYRI* was detected using High Resolution Melting (HRM) using a Thermal Cycler Rotor Gene Real Time Q (Qiagen). HRM consisted of a temperature ramp between 83 and 89 °C, rising by 0.1 °C/2 s. The SNPs detection by HRM is faster than the commonly used PCR-RFLP technique due the elimination of the restriction enzyme and the electrophoresis.

The allelic and genotypic frequencies and analyses of variance were performed using the program Infostat<sup>6</sup>. Least squares means were compared using the Fisher's LSD test with a comparison error rate of 0.05.

## RESULTS

Of the 403 pigs used for this study, 19.6% had the allele "n" associated with susceptibility to porcine stress syndrome (PSS), while 80.4% had the allele "N" normal or resistant to PSS. Genotypic frequencies observed were 65.0% homozygous normal or resistant individuals (*NN*) to the PSS, 30.8% of individuals heterozygous (*Nn*) and 4.2% homozygous susceptible (*nn*) at PSS (Table 2, Fig 1).

Additionally, we observed a highly significant effect ( $p < 0.0001$ ) of the *RYRI* gene on meat pH at 45 min. post slaughter, which is the main parameter used to determine type PSE meat. Individuals homozygous normal (*NN*) showed the higher values of pH (6.27), individuals heterozygous (*Nn*) had intermediate values of pH (5.94) and individuals homozygous recessive (*nn*) showed the lowest values of pH (5.64) (Table 1).

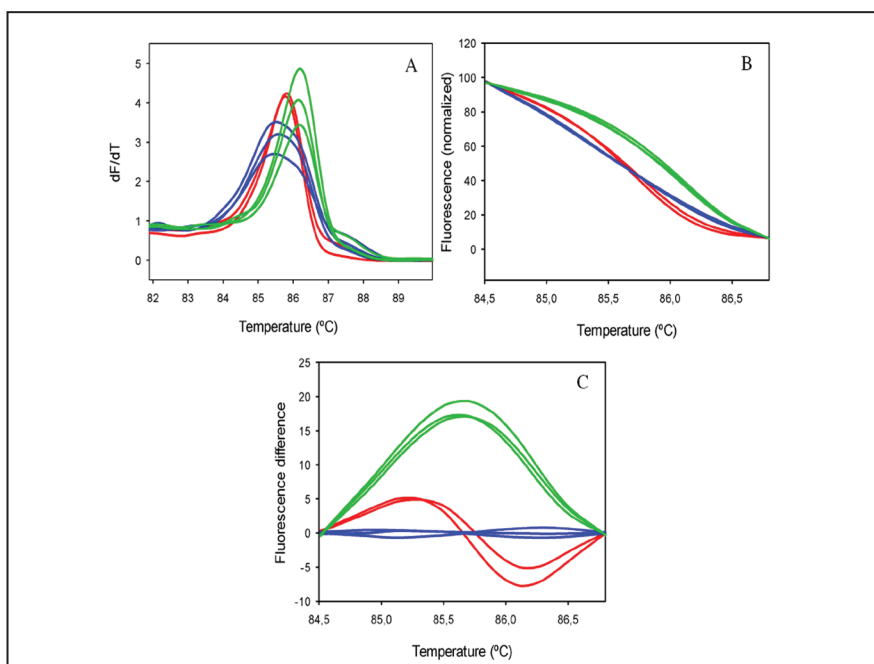
## DISCUSSION

When we compare the frequencies observed in this work and the observed in another works from Brazil<sup>1,3,11</sup> (the principal pig-producer of Latin America), we observe higher incidence of *Nn* and *nn* genotypes (Table 2).

The probable causes of this situation may be due to the lack of knowledge about the allele constitution by the breeders, the use of different pure lines in the formation of hybrids and the lack of planning of crosses made.

The molecular marker assisted selection is an interesting tool that can help to reduce the frequencies of *nn* genotypes as well as the presence of PSE meat in Argentinean hybrid pig populations. This will allow to Argentina to be a producer of pork with good quality.

Additionally the DNA extraction from plucked hairs and detection of the *RYRI* C/T polymorphism by HRM are interesting approaches to extend the detection of this SNP to producers and breeding companies in order to reduce the high frequency of susceptible allele (*n*) in Argentinean swines.



**Figure 1.** High-resolution melting profile showing the resolved *RYRI* genotypes. Homozygous *NN* (green line), heterozygous *Nn* (blue line) and homozygous *nn* (red line). a: Melting curves, b: Normalized melting profile and c: Fluorescence difference plot

**Table 1.** pH of meat in different *RYRI* genotypes

<i>RYRI</i> Genotypes	pH	n
<i>NN</i>	6.27±0.58 A	262
<i>Nn</i>	5.94±0.46 B	124
<i>nn</i>	5.64±0.32 C	17

Means with different letters are significantly different at  $p < 0.05$

**Table 2.** Allelic and genotypic frequencies (%) of *RYRI* gene in Argentina and Brazil

Genotypic frequencies	Argentina		Bastos <i>et al.</i> (2001) <sup>3</sup>		Band <i>et al.</i> (2005) <sup>1</sup>		Silveira <i>et al.</i> (2011) <sup>11</sup>		Brazil (average)	
	n	%	n	%	n	%	n	%	n	%
<i>NN</i>	262	65.0	84	52.6	493	82.6	46	40.0	623	71.4
<i>Nn</i>	124	30.8	67	41.8	103	17.2	69	60.0	239	27.5
<i>nn</i>	17	4.2	9	5.6	1	0.2	0	0.0	10	1.1
Allelic frequencies										
n		19.6		26.9		8.8		30.0		14.9
N		80.4		73.1		91.2		70.0		85.1

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