

The occurrence of the unfavorable allele of the *RYR1* gene in the population of Puławska breed

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Crossing animals in low-number populations or one-sided selection aimed at improving a specific group of traits may lead to a reduction in the genetic diversity of the population and the appearance of rare, often unfavorable, allelic variants (mutations) in populations. This is a serious problem in the case of conservative breeds, in which the number of individuals is usually small, and the ability to select animals for crossing is limited.

Currently, the development of molecular genetics methods enables identification of the genetic basis of selected disease syndromes present in populations of breeding animals, as well as their rapid identification. In pigs, the mutation in the *RYR1* gene, causing the amino acid sequence change at position 614 of the resulting protein from arginine to cysteine (p.Arg614-Cys; c.1843C>T; rs118192172), was considered to be the main genetic factor for the occurrence of malignant hyperthermia. The *RYR1* gene (ryanodine receptor 1) encodes the subunit of the sarcoplasmic calcium channel located in the skeletal muscle, and plays a key role in the maintenance of calcium homeostasis (Tilgen et al., 2001).

Studies conducted in humans have confirmed the association of mutations in the *RYR1* gene locus with the occurrence of muscular system diseases, such as malignant hyperthermia and central core disease (Lunardi & Monnier, 2004; Robinson et al., 2006).

In case of pigs, it was demonstrated that the presence of the T allele in the mutation locus

in the *RYR1* gene (c.1843C>T) is associated with a change in the transport of Ca²⁺ ions in the sarcoplasmic reticulum, which in turn leads to malignant fever. The presence of mutations in the *RYR1* gene (HAL) determines the reduction of animal resistance to stress factors, which affects the disruption of vital processes of the body subjected to stress, the emergence of aggressive behaviors in the herd, and consequently leads to a reduction in pig production, and even disease and death. Furthermore, the meat obtained from such pigs has a disadvantage of PSE (pale, soft and exudative meat) and is characterized by a reduced technological value (Janik et al., 2006; Kapelański et al., 1999). In 2009–2010, genotype evaluation was carried out in the locus of the *RYR1* gene related to the susceptibility of pigs to stress, which was performed for pigs of breeds popularly bred in the country (pbz, wbp, Pietrain, Duroc breeds). This allowed the elimination of sensitive pigs from breeding and a reduction in the number of heterozygous carriers of the studied mutation. This, in turn, reduced the number of animal deaths caused by stressors (transport, chasing, high temperature, etc.) and significantly increased the efficiency of production. In the native Puławska breed, in 2003, the frequency test for this genotype was performed on 52 gilts from five paternal lines (Babicz et al., 2003). In the studied group of animals, it was shown that 52% of the subjects had a heterozygous *CT* genotype, and individuals with an unfavorable homozygous *TT* genotype

constituted 14%. Over many years of breeding pigs of this breed there was a strategy adopted, allowing the maintenance of *RYRIC/CT* heterozygotes in order to improve the meatiness of the carcass. However, due to the problems related to the quality of meat and breeding of gilts and sows with *CT* genotype (Antosik et al., 2006, Bogdzińska, 2006), actions should be taken in order to eliminate both genotypes (*CT* and *TT*) from the breeding population.

The aim of the conducted research was to estimate the frequency of occurrence of an unfavorable *RYRI* gene allele in the currently genetic resource protection program, covering the the Puławska breed pig population. The information obtained can be taken into account in the selection of animals for crossing and will allow the elimination of the tested mutations from the breeding of carriers.

Material and methods

The study material consisted of hair roots collected in the years 2016–2017 from Puławska breed pigs in the amount of 411 animals (81 boars and 330 gilts from herds covered by the genetic resources conservation program). Genomic DNA was isolated from roots using the Sherlock (A & A Biotechnology) kit. The quality and concentration of the obtained DNA were evaluated using the Nanodrop apparatus. The polymorphic variants of the c.1843C>T mutation were determined by the PCR-RFLP method using the *HinPI* restriction enzyme (Fujii et al., 1991).

Results and discussion

The domestic pig breeds covered by the protection programs are characterized by high variability and genetic diversity, which determines the occurrence of valuable features, such as very good quality of meat. However, recently the number of these animals in breeding varies and is small, which may contribute to an increase in homozygosity of the population, and an increase in the occurrence of unfavorable allele variants. This is the case with the *RYRI* gene and the T allele associated with pig susceptibility to stress conditions. Stress

sensitive animals (*TT* homozygous pigs) are characterized by an increased number of deaths during the fattening period and reduced reproductive efficiency. The spread of the unfavorable allele in the population was due to the fact that heterozygous individuals (in terms of the discussed mutation – *CT*) are characterized by a higher content of meat in the carcass, and lower fat content in relation to mutation-free pigs – *CC* (Koćwin-Podiada et al., 1994, 2000).

It was confirmed that the T allele increases the rate of pH decrease in muscle tissue after death, which significantly affects the frequency of meat with PSE-type defect. Studies of many authors confirmed much higher percentages of subjects with meat characterized by the PSE defect (36–100%) in the group of homozygous *TT* pigs, compared to homozygous *CC* animals (0–14%) (Koćwin-Podiada et al. 1994, 2000, Przybylski et al., 1994). The necessity to eliminate individuals with an unfavorable T-allele from the breeding is also supported by the fact that c.1843C>T polymorphism of the *RYRI* gene significantly affects the reproductive characteristics of pigs. The studies of Kuryl & Wróblewski (1992) confirmed the negative effect of the discussed mutation on the number of piglets born in the litter. On the other hand studies by Buczyński et al. carried out in the Żłotnicka white pig breed showed a significant relation between the T-allele and the piglets' death before the 21st day of life.

The genotype of the c.1843C>T mutation located in the *RYRI* gene was determined for all animals in the conducted studies. It was found that in the population of the native Puławska pig breed, *CC* genotype has the highest frequency 70%. Heterozygous individuals accounted for 27% and identified 3% of pigs with the *TT* genotype (recessive homozygotes). In case of analysis which included the sex, almost identical percentage share of individual genotypes was found in the group of gilts, as well as in the entire population (Tab. 1).

Table 1. Percentage share of *RYRI* genotypes in Puławska breed

Breed	Genotype			Number of examined animals
	CC	CT	TT	
Examined population	70% (288)	27% (112)	3% (11)	411
<i>Boars</i>	64.2% (52)	34.6% (28)	1.2% (1)	81
<i>Sows</i>	71.5% (236)	25.5% (84)	3% (10)	330

On the other hand, up to 9% higher frequency of heterozygous pigs and 7% lower frequency of homozygous CC pigs was confirmed in the group of boards, as compared to gilts. The prevalence of pigs with one copy of the unfavorable allele observed in the boar group is probably due to the fact that heterozygous pigs are characterized by a greater amount of meat in the carcass, and are therefore selected for crossing as a parental generation with valuable phenotypic traits. Due to the fact that offspring obtained from one boar can be very numerous, and usually there is a one boar kept in the herd – the demonstrated distribution of genotypes indicates the possibility of rapid spreading of the unfavorable T allele in the population.

The conducted study confirmed the presence of an unfavorable T-allele of the *RYRI* gene in the Puławska breed. The high percentage of heterozygous individuals and the presence of recessive homozygotes susceptible to the occurrence of malignant fever is disturbing. The obtained results should be taken into account when selecting the animals for crossing and enable the elimination of stress-sensitive pigs from breeding (TT homozygotes), as well as limit the heterozygous carriers of the mutation.

Such activities will reduce the number of animal deaths caused by stressors (transport, chasing, high temperature, etc.) and increase production efficiency.

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THE OCCURRENCE OF THE UNFAVORABLE ALLELE OF THE *RYR1* GENE IN THE POPULATION OF PUŁAWSKA BREED

Summary

Conservative breeds are usually characterized by small number of individuals and thus the selection of animals to cross is very limited. *RYR1* gene coding for ryanodine receptor 1 which is as a calcium release channel in the sarcoplasmic reticulum. RYR1 protein plays a key role in calcium homeostasis in skeletal muscle and mutation in *RYR1* gene is associated with malignant hyperthermia susceptibility. The purpose of the study was to estimate the frequency of the occurrence of the unfavorable allele of the *RYR1* gene in the currently protected genetic resources of the puławska pig population. The analysis was performed on 411 pigs (330 sows and 81 boars). The *RYR1* genotypes were identified by PCR-RFLP method. The obtained results showed the highest frequency of CC genotype (70%). Heterozygous individuals accounted for 27% and 3% of pigs with TT genotype (recessive homozygotes) were identified. In the case of gender, the 9% higher number of heterozygous and 7% lower homozygote CC pigs were detected when compared to gilts group. The prevalence of boars with one copy of the unfavorable allele is probably because heterozygous pigs have more meat in carcasses and are therefore they have been chosen for crossbreeding as a parental race with valuable phenotypic characteristics. Given the fact that the offspring obtained from one boar may be very numerous showed distribution of genotypes indicates the possibility of rapidly spreading the unfavorable T allele in the population. The information obtained may be considered during selecting animals for crossing.

Key words: *RYR1*, malignant hyperthermia, Puławska breed, conservation breed

Puławska breed
(phot. M. Szyndler-Nędza)

