



## PERFORMANCE TRAITS OF PUŁAWSKA PIGS DEPENDING ON POLYMORPHISM IN THE *RYRI* GENE (C.1843C>T)\*

Magdalena Szyndler-Nędza<sup>1</sup>, Katarzyna Ropka-Molik<sup>2\*</sup>, Aurelia Mucha<sup>1</sup>, Tadeusz Blicharski<sup>3</sup>, Marek Babicz<sup>4</sup>

<sup>1</sup>Department of Pig Breeding, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland

<sup>2</sup>Department of Animal Molecular Biology, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland

<sup>3</sup>Department of Immunogenetics, Institute of Genetics and Animal Breeding, Jastrzębiec, Postępu 36A, 05-552 Magdalenka, Poland

<sup>4</sup>Department of Pig Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

\*Corresponding author: katarzyna.ropka@izoo.krakow.pl

### Abstract

The objective of the study was to determine the relationship between polymorphism in the *RYRI* gene (rs344435545) and on-farm test results (fattening, slaughter and reproductive traits) of pigs maintained in conservation herds. The experiment covered 76 boars and 291 gilts kept in 33 herds. Animals aged 150–210 days were weighed and P2 and P4 backfat thickness as well as P4 loin muscle depth (P4M) were measured with an ultrasonic device. Standardised daily gain (g/day) of the animals and carcass meat percentage (%) were determined. The following reproductive traits of sows were also collected: number of teats, age at first farrowing, dates of next farrowing, number of piglets born alive and weaned at 21 days of age. Hair roots were sampled from the gilts, sows and boars to determine the *RYRI* gene polymorphism. It was found that only 3% of the animals in the analysed population had *TT* genotypes, whereas 28.34% were heterozygous (*CT*). This polymorphism (*CT*) was carried by 28.34% of the animals. When analysing the effect of this polymorphism on fattening and slaughter traits in live animals, it was found that sows with *TT* genotype, compared to the others, were characterised by higher backfat thickness only ( $P \leq 0.05$ ). In the boars with *CC* and *CT* genotypes, no significant differences were noted between the values of the analysed traits. For reproductive traits of the sows, it was observed that females with *TT* genotype weaned more piglets until 21 days of age ( $P \leq 0.05$ ).

**Key words:** fattening, slaughter, reproduction, *RYRI*, Puławska

---

\*The work was financed as part of the Biostrateg project (no. 297267) financed by the National Centre for Research and Development.

In pigs, substitution at position 1843 bp of the *RYRI* gene (c.1843C>T; rs344435545) that led to alteration in amino acid sequence from arginine to cysteine (p.Arg614Cys) causes changes in  $\text{Ca}^{2+}$  ion transport from the endoplasmic reticulum into cytosol (Fujii et al., 1991). This polymorphism is recognised as the main genetic determinant of malignant hyperthermia. Animals with *TT* or *CT* genotype are less resistant to stressors which disturb the physiological process in the organism exposed to stress and elicits aggressive behaviour in a herd, thus reducing productivity in pigs and possible reproductive performance of sows, and even causing diseases and deaths (Kurył and Wróblewski, 1992; Reiner et al., 1993; Murray and Johnson, 1998). Under stress conditions, fattening pigs carrying this polymorphism show an increased risk of PSE (pale, soft, exudative) meat which negatively affects the technological quality of the meat (Guàrdia et al., 2004; Janik et al., 2006; Marini et al., 2012). Despite this, because this polymorphism has a concurrent positive effect on increasing the meat content of the carcass cuts, it was decided to retain heterozygous pigs with *C/T* genotype in the breeding stock of some breeds (Orzechowska et al., 2004).

One such breed in Poland was the Puławska. In this breed, analysis of *RYRI* genotypes frequency in 2003 revealed that out of 52 studied animals, 52% had a heterozygous *CT* genotype and 14% the unfavourable homozygous *TT* genotype (Babicz et al., 2003). A recent random screening of the Puławska pig population (330 sows and 81 boars) showed that the proportion of heterozygous animals decreased to 25.5% for the sows and to 34.6% for the boars; for animals with the *TT* genotype, 3% and 1.2%, respectively (Ropka-Molik et al., 2017). However, the large number of heterozygous animals (resulting from the implemented strategy) observed among the boars as well as sows is a cause for concern. This is due to the rule that only one boar is kept in a medium-sized herd of the Puławska breed. Because the offspring produced from one boar can be very numerous, therefore the unfavourable *T* allele may rapidly spread throughout the population and a large group of pigs with *TT* genotype may appear. Given that the unfavourable effect of the *T* allele of this gene on performance and reproductive traits of pigs has been reported by various authors, it has been suggested that the number of heterozygous animals should be reduced in the population (Ropka-Molik et al., 2017). Although it has been shown that local Puławska sows, when compared to more productive Polish Landrace sows, are more resistant to stress-inducing factors (Babicz et al., 2016), the effect of polymorphism in the *RYRI* gene on fattening and slaughter traits of young animals, and primarily on lifetime production of farmed sows, has not been identified in the Puławska breed yet. Therefore, the present study was undertaken to determine the effect of a polymorphism in the *RYRI* gene (rs344435545) on on-farm test results (fattening, slaughter and reproductive traits) of pigs maintained in conservation herds.

## Material and methods

Analysis included 367 Puławska pigs (76 boars and 291 gilts) maintained in 33 conservation herds located in the Lubelskie, Mazowieckie and Kujawsko-Pomorskie

provinces. Males and females were weighed (body weight, kg) on the farm at the age of around 180 days (range of 150 to 210 days) and their P2 and P4 backfat thickness as well as P4 loin muscle depth (P4M) were measured with an ultrasonic device (Piglog 105; Frontmatec A/S, Kolding, Denmark). Positions P2 and P4 are located behind the last rib, at the juncture of the thoracic and lumbar vertebrae, 3 cm (P2) and 8 cm (P4) off the midline. These data were used to determine weight gain standardised to 180 days of age (g/day) and carcass meat percentage standardised to 180 days of age (%), in accordance with the method for live evaluation of Puławska pigs (Eckert and Szyndler-Nędza, 2017; Eckert et al., 2017). The animals were maintained on family farms in a semi-intensive system, where from one sow an average of 1.6 litters were obtained per year. The following reproductive performance data of the 213 sows were also collected: number of teats, age at first farrowing, dates of next farrowing, number of piglets born alive and weaned at 21 days of age. It was used to calculate the farrowing interval (interval between litters) for each sow and piglet mortality during lactation.

Biological material in the form of hair roots was collected from the sows and boars to determine their polymorphism in the *RYR1* gene. Genomic DNA was isolated from the roots using a Sherlock kit (A & A Biotechnology, Gdynia, Poland). The quality and concentration of obtained DNA was assessed with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Polymorphic variants of the *RYR1* gene (c.1843C>T, rs344435545) were determined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) using *HinPI* restriction enzyme (Fujii et al., 1991).

The collected data were statistically analysed using procedures of Statistica ver. 10 (StatSoft Inc., 2011). Between-group differences for the results of fattening and slaughter performance were estimated according to the following statistical model:

$$y_{ijk} = a_i + b_j + c_k + f_{(ab)} + e_{ijk}$$

where:

- $y_{ijk}$  = estimated traits of *ijk-th* individual,
- $a_i$  = fixed effect of *i-th* genotype *RYR1* ( $i = 1, 2, 3$ ),
- $b_j$  = fixed effect of *j-th* sex ( $j = 1, 2$ ),
- $c_k$  = random effect of *k-th* herd ( $k = 1-33$ ),
- $f_{(ab)}$  = interaction between factors ( $a$  – genotype *RYR1*,  $b$  – sex).

The following model was used for reproductive traits:

$$y_{ijk} = a_i + b_j + c_k + e_{ijk}$$

where:

- $y_{ijk}$  = estimated traits of *ijk-th* individual,
- $a_i$  = fixed effect of *i-th* genotype *RYR1* ( $i = 1, 2, 3$ ),
- $b_j$  = random effect of *j-th* litters ( $j = 1-15$ ),
- $c_k$  = random effect of *k-th* herd ( $k = 1-33$ ).

Differences between means of individual traits were tested using NIR Fisher's multiple range test at  $P \leq 0.01$  and  $P \leq 0.05$  levels of significance.

## Results

Table 1 presents the frequency of genotypes and alleles in the pig population under study. In both males and females, the homozygous *CC* genotype and the heterozygous *CT* genotype were most common. The unfavourable *TT* genotype was found in the least number of animals in both sexes – 3.44% (10 head) in sows and 1.32% (1 head) in boars. The analysis showed that the observed genotypic and allelic frequencies were in agreement with the Hardy-Weinberg equilibrium.

Table 1. Genotypes and alleles frequency of the *RYRI* gene in the population of Puławska gilts and boars

<i>RYRI</i>	Genotype	Number of animals	Frequency		H-W P-value
			genotype	allele	
Gilts	<i>CC</i>	205	70.45	C 84.51	ns
	<i>CT</i>	76	26.12		
	<i>TT</i>	10	3.44	T 16.49	
Boars	<i>CC</i>	47	61.84	C 80.26	ns
	<i>CT</i>	28	36.84		
	<i>TT</i>	1	1.32	T 19.74	
Total	<i>CC</i>	252	68.66	C 82.83	ns
	<i>CT</i>	104	28.34		
	<i>TT</i>	11	3.00	T 17.17	

ns – not statistically significant.

Table 2 lists the mean results for fattening and slaughter traits of the sows and boars depending on the *RYRI* gene polymorphism. In the case of sows, animals of *TT* genotype, compared to the other genetic forms, were characterised by highest P2 backfat thickness ( $P \leq 0.05$ ) and mean backfat thickness ( $P \leq 0.05$ ). Only heterozygous sows (*CT*) were characterised by ( $P \leq 0.05$ ) lower loin eye height compared to the sows with *CC* genotype. The *RYRI* gene polymorphism had no significant effect on the value of daily gains, P4 backfat thickness and carcass meat percentage standardised to 180 days of age in females. For boars, the statistical analysis was performed for only two polymorphic groups (*CC* and *CT*) and the results for the *TT* genetic group were ignored because of low frequency of *TT* genotype. The data presented in Table 2 indicate a lack of statistically significant differences between the boars of *CC* and *CT* genotypes in the values of live performance traits of the pigs.

Table 3 shows data for the reproductive traits of the sows according to polymorphism in the *RYRI* gene locus. The *RYRI* gene polymorphism had no statistically significant effect on most of the analysed traits. Only the number of piglets weaned

throughout the lifetime of sows (number of piglets at 21 days of age) in sows with *TT* genotype was 1.29 higher ( $P \leq 0.05$ ) than in sows with *CC* genotype.

Table 2. Mean values ( $\pm$ SD) of fattening and slaughter traits depending on polymorphism in the *RYR1* gene

Traits/Gender	<i>RYR1</i> genotype		
	<i>CC</i>	<i>CT</i>	<i>TT</i>
Gilts			
Daily gain (g)	579.83 $\pm$ 72.90	572.21 $\pm$ 83.04	556.40 $\pm$ 69.95
P2 backfat thickness (mm)	13.52 $\pm$ 2.65 b	14.01 $\pm$ 2.82 b	15.79 $\pm$ 3.35 a
P4 backfat thickness (mm)	12.89 $\pm$ 2.77	12.80 $\pm$ 2.70	14.24 $\pm$ 4.16
Mean backfat thickness (mm)	13.20 $\pm$ 2.51 b	13.41 $\pm$ 2.57 b	15.02 $\pm$ 3.28 a
Loin eye height (mm)	52.79 $\pm$ 5.46 b	51.12 $\pm$ 6.33 a	52.49 $\pm$ 8.08
Carcass meat percentage	55.16 $\pm$ 3.48	54.45 $\pm$ 3.48	54.34 $\pm$ 4.24
Boars			
Daily gain (g)	623.17 $\pm$ 74.74	639.54 $\pm$ 69.40	504.00 $\pm$ 0.00
P2 backfat thickness (mm)	12.03 $\pm$ 3.11	11.56 $\pm$ 2.53	11.30 $\pm$ 0.00
P4 backfat thickness (mm)	11.48 $\pm$ 3.24	11.41 $\pm$ 2.35	10.20 $\pm$ 0.00
Mean backfat thickness (mm)	11.75 $\pm$ 3.07	11.48 $\pm$ 2.31	10.75 $\pm$ 0.00
Loin eye height (mm)	51.16 $\pm$ 4.91	53.21 $\pm$ 4.92	48.40 $\pm$ 0.00
Carcass meat percentage	56.12 $\pm$ 3.19	56.73 $\pm$ 2.93	58.00 $\pm$ 0.00

a, b – means  $\pm$  SD in rows marked with different small letters differ significantly at  $P \leq 0.05$ .

Table 3. Mean values ( $\pm$ SD) of reproductive traits depending on polymorphism in the *RYR1* gene

Traits	Sows (n)	<i>RYR1</i> genotype		
		<i>CC</i> (145)	<i>CT</i> (58)	<i>TT</i> (10)
Age at first farrowing (days)		364 $\pm$ 51.89	351 $\pm$ 35.82	347 $\pm$ 21.65
Mean farrowing interval (days)		200 $\pm$ 45.70	193 $\pm$ 47.11	181 $\pm$ 11.85
Number of teats		14.30 $\pm$ 0.89	14.22 $\pm$ 0.77	14.10 $\pm$ 0.32
Mean number of piglets born alive (head)		9.73 $\pm$ 2.35	10.41 $\pm$ 2.01	10.93 $\pm$ 1.44
Mean number of piglets weaned at day 21 (head)		8.68 $\pm$ 2.22 b	9.24 $\pm$ 1.90	9.97 $\pm$ 1.00 a
Piglet losses until 21 days of age (%)		10.27 $\pm$ 10.83	10.50 $\pm$ 10.06	8.04 $\pm$ 5.95

a, b – means  $\pm$  SD in rows marked with different small letters differ significantly at  $P \leq 0.05$ .

## Discussion

The Puławska breed is a local Polish breed included in a conservation programme of genetic resources, which is aimed to maintain stable within-breed variation, genetic diversity of the breed and a number of valuable phenotypic traits. This breed

is characterised by higher fatness and lower meatiness compared to the Polish Large White and Polish Landrace breeds, but surpasses them in meat quality (Kasprzyk et al., 2013). Studies concerning the effect of polymorphism in the *RYRI* gene on performance traits of the pigs were mostly performed with highly productive breeds which are selected for carcass meatiness. In many of them, however, this polymorphism was reported to have no effect on changes in backfat thickness or carcass meatiness (Hamilton et al., 2000; Mucha et al., 2007; Škrlep et al., 2010; Rybarczyk et al., 2010). On the other hand, animals with the *T* allele had a significantly higher content of meat, especially in the ham, which in principle explains why animals of *CT* genotype are kept in some breeding programmes (Orzechowska et al., 2004; Škrlep et al., 2010).

In our study, we found a significant effect of the analysed polymorphism only in gilts on increasing backfat thickness ( $CC < TT$ ). Rybarczyk et al. (2010) demonstrated that the mean backfat thickness of pigs is influenced mainly by their meatiness ( $P \leq 0.01$ ), followed by an interaction between meatiness and *RYRI* genotype ( $P \leq 0.05$ ). The same authors showed that animals with less than 54% meatiness, which had the *T* allele in their genotype, exhibited significantly thicker backfat compared to *CC* animals, which is consistent with our findings. In addition, backfat thickness is affected by several other genes that may also reduce the effect of polymorphism in the *RYRI* gene. This is also the same regarding pig meat quality, where the presence of the *RR-II* genotypes of the *PRKAG3* gene offset the negative effect of the *T* allele of the *RYRI* gene on meat pH and drip loss (Škrlep et al., 2010).

The effect of polymorphism in the *RYRI* gene on the reproductive performance of sows has not been conclusively established. Research conducted in the 1990s showed that polymorphism in the *RYRI* gene adversely affects the number of piglets born alive per litter (Kurył and Wróblewski, 1992; Reiner et al., 1993). However, such results were not obtained for Landrace sows, in which this polymorphism had no significant effect on the number of piglets born and weaned, and for Large White and White Meaty sows, in which the *T* (*n*) allele had a significantly beneficial effect on increasing the number of piglets born per litter (Omelka et al., 2006; Mucha et al., 2007). In the case of a local Złotnicka White breed, Buczyński et al. (2006) showed that animals of the *CT* genotype, compared to *CC* and *TT* sows, were characterised by a significantly higher number of piglets born and weaned per litter. Furthermore, Złotnicka White sows with *TT* genotype lost significantly more piglets during rearing compared to the other females.

Opposite results were obtained by Nienartowicz-Zdrojewska et al. (2017), who reported a lack of statistically significant effect of the polymorphism in the *RYRI* gene on the number of piglets born and weaned per litter in both the Złotnicka White and Złotnicka Spotted breeds. In the case of piglet weaning losses, sows with *TT* genotype, compared to the other genotypes, were characterised by significantly lowest piglet mortality – 11.89% in the Złotnicka White and 2.63% in the Złotnicka Spotted breed. Likewise, our study demonstrated that Puławska sows of *RYRI TT* genotype, compared to sows of *RYRI CC* genotype, lost fewer piglets during rearing, as a result of which they reared significantly more piglets to 21 days of age throughout their productive life.

The inconsistent effect of the polymorphism in the *RYR1* gene on the number of piglets born or weaned by sows of different breeds is indicative of the presence of genetic interbreed variation but, above all, suggests the effect of environmental factors, including stress-induced ones. Babicz et al. (2016) showed that susceptibility of *CT* genotype to stress factors is more conspicuous in the highly productive Polish Landrace breed than in the local Puławska breed. As a result of stress factors (temperature, noise) during early pregnancy, Puławska sows with *CT* genotype, compared to Polish Landrace sows with *CT* genotype, gave birth to significantly fewer live piglets, which were characterised by significantly lower daily gains to 7 days of age. At the same time, the above results obtained for the Puławska sows with *CT* genotype were similar to the results of Puławska sows with *CC* genotype subjected to no stressors.

### Conclusions

The presented results show that gilts with *TT* genotype were only characterised by greater backfat thickness ( $P \leq 0.05$ ) compared to the other females. In the boars of *CC* and *CT* genotypes, no significant differences were noted between the values of analysed traits. For reproductive traits of the sows kept in the family farms, it was observed that the polymorphism had a beneficial effect on the number of piglets weaned to 21 days of age ( $CC < TT$ ,  $P \leq 0.05$ ). Although the analysed polymorphism had no negative effect on reproductive traits of the sows in the studied population, it is suggested that boars carrying the *T* allele should be eliminated first from breeding due to the possible increase in the number of stress-susceptible pigs in the population and the incidence of malignant hyperthermia (*TT*), and thus the increased risk of PSE meat defects in fattening pigs.

### References

- Babicz M., Kurył J., Walkiewicz A. (2003). Evaluation of the genetic profile of the Puławska breed. *J. Appl. Genet.*, 44: 497–508.
- Babicz M., Szyndler-Nędzia M., Skrzypczak E., Kasprzyk A. (2016). Reproductive performance of native Puławska and high productivity Polish Landrace sows in the context of stress during the period of early pregnancy. *Reprod. Domest. Anim.*, 51: 91–97.
- Buczyński J.T., Panek A., Kempisty B., Skrzypczak E., Luciński P. (2006). Reproductive performance of Złotnicka White pigs as related to *RYR1* gene. *Anim. Sci. Pap. Rep.*, 24: 43–50.
- Eckert R., Szyndler-Nędzia M. (2017). Results of performance-tested boars (in Polish). Report on pig breeding in Poland. Kraków, IZ PIB, XXXV: 19–33.
- Eckert R., Żak G., Bereta A. (2017). Results of performance-tested gilts (in Polish). Report on pig breeding in Poland. Kraków, IZ PIB, XXXV: 34–48.
- Fujii J., Otsu K., Zorzato F., De Leon S., Khanna V.K., Weiler J.E., O'Brien P.J., MacLennan D.H. (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science*, 253: 448–451.
- Guàrdia M.D., Estany J., Balasch S., Oliver M.A., Gispert M., Diestre A. (2004). Risk assessment of PSE condition due to pre-slaughter conditions and *RYR1* gene in pigs. *Meat Sci.*, 67: 471–478.



- Hamilton D.N., Ellis M., Miller K.D., McKeith F.K., Parrett D.F. (2000). The effect of the Halothane and Rendement Napole genes on carcass and meat quality characteristics of pigs. *J. Anim. Sci.*, 78: 2862–2867.
- Janik A., Pieszka M., Migdał W. (2006). Effect of *RYRI* genotype on carcass and meat quality in Polish Landrace pigs. *Anim. Sci.*, 1: 70–71.
- Kasprzyk A., Babcz M., Kamyk-Kamiński P., Lechowski J. (2013). Slaughter value and meat quality of Pulawska and Polish Landrace breeds fatteners. *Ann. UMCS, Sectio EE*, 31: 1–9.
- Kurył J., Wróblewski P. (1992). The effect of halothane-sensitivity gene (HALn) in pigs on litter size, piglets live weight and rate of piglets survival to the age of 9–11 weeks. *Anim. Sci. Pap. Rep.*, 9: 47–52.
- Marini S.J., Vanzetti L.S., Borelli V.S., Villareal A.O., Denegri G.D., Cottura G.A., Panichelli D., Silva P., Campagna D., Spinner N., Brunori J.C., Franco R. (2012). *RYRI* gene variability and effect on meat pH in Argentinean hybrids swines. *In. Vet.*, 14: 19–23.
- Mucha A., Różycki M., Blicharski T. (2007). Fattening, slaughter and reproductive performance of Polish Landrace breeding sows according to *RYRI* genotype (in Polish). *Rocz. Nauk. Zoot.*, 34: 151–156.
- Murray A.C., Johnson C. P. (1998). Impact of the halothane gene on muscle quality and pre-slaughter deaths in Western Canadian pigs. *Can. J. Anim. Sci.*, 78: 543–548.
- Nienartowicz-Zdrojewska A., Sobek Z., Buczyński J.T., Konieczka A., Różańska-Zawieja J. (2017). Productivity of pigs of conservation breeds in terms of selected gene polymorphisms. *Med. Weter.*, 73: 352–356.
- Omelka R., Peškovičová D., Martiniaková M., Bauer M., Bauerova M. (2006). Effect of the estrogen receptor (ESR) and ryanodine receptor (*RYRI*) genes on reproductive traits of Slovak Large White, White Meaty and Landrace pigs. *Arch. Tierz., Dummerstorf*, 49: 357–362.
- Orzechowska B., Różycki M., Tyra M., Kamyczek M. (2004). Effect of the stress-sensitivity *RYRI* gene on muscling and fatness of carcasses and cuts and meat quality in a Polish Large White, Duroc and Pietrain population. *Anim. Sci. Pap. Rep.*, 22: 51–59.
- Reiner G., Hecht W., Herzog A., Dzapo V. (1993). The reproductive performance of sows with different malignant hyperthermia syndrome status. *Deut. Tierarztl. Woch.*, 4: 144–146.
- Ropka-Molik K., Piórkowska K., Oczkiewicz M., Szyndler-Nędza M., Blicharski T. (2017). The occurrence of the unfavorable allele of the *RYRI* gene in the population of Pulawska breed (in Polish). *Wiad. Zoot.*, 5: 35–38.
- Rybarczyk A., Pietruszka A., Jacyno E., Dvořák J., Karamucki T., Jakubowska M. (2010). Association of *RYRI* and *MYOG* genotype with carcass and meat quality traits in grower-finisher pigs. *Acta Vet. Brno*, 79: 243–248.
- Škrlep M., Kavarič T., Čandek-Potokar M. (2010). Comparison of *PRKAG3* and *RYRI* gene effect on carcass traits and meat quality in Slovenian commercial pigs. *Czech J. Anim. Sci.*, 55: 149–159.

Received: 8 VI 2018

Accepted: 20 II 2019