# Effect of RYR1<sup>T</sup> Gene Polymorphism on the Initial Growth and Fattening and Slaughter Values of Polish Synthetic Line 990 Pigs Reared in Standardized Litters

A. PIETRUSZKA, A. SOSNOWSKA

Department of Pig Breeding, University of Agriculture in Szczecin, Szczecin, Poland

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#### Abstract

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The aim of this study was to determine the effect of the ryanodine receptor gene - RYR1<sup>T</sup> polymorphism on the initial growth and fattening and slaughter values of Polish Synthetic Line 990 pigs reared in standardized litters. The study was carried on 276 offspring of hyperprolific sows. The sows gave birth to at least 12 live-born piglets. On the first day after birth, litters were equalised to 12 piglets in litter. The body weight was examined on the 21st (21BW), 28th (28BW), 63rd (63BW) and 180th (180BW) days of life. During evaluation, the live average daily gain (LADG) from birth to day 180 of life and the average daily gain (ADG) from day 63 to day 180 of life were determined. The percentage meat content (PMC), backfat thickness (BFT) and loin eye thickness (LET) was determined using PIGLOG 105 ultrasound apparatus. Two alleles of the RYR1 gene (RYR1<sup>c</sup>, RYR1<sup>T</sup>) and three genotypes (C/C, C/T and T/T) were identified. The 21BW and 28BW of the T/T genotype were significantly lower than that of the C/C and C/T. The highest PMC was characteristic of the T/T genotype, whereas the lowest one of the C/C genotype  $(p \le 0.05)$ . The T/T genotype had a higher LET than the C/C genotype  $(p \le 0.05)$ . No significant differences with respect to LADG, ADG and BFT between RYR1 genotypes were found. It can be stated that early identification of homozygous animals with respect to the RYR1<sup>T</sup> gene may allow the prediction of the body weight of animals in the initial period of their rearing.

### RYR1 genotype, body weight, meatiness, gilts, barrows

As generally known, piglets that are heavier on the day of birth are also stronger and grow better when compared to lighter piglets from the same litter. Heavier piglets distinguish themselves by a higher survival rate than lighter piglets (Hermesch et al. 2001). Furthermore, heavier piglets are characterised by a higher growth rate than lighter ones (Roehe 1999). The tendency to keeping a higher growth rate is also maintained after piglets have been weaned from a sow. The body weight of piglets on the day of birth and in the subsequent rearing period depends on many factors. One of them is the litter size (Johansson 1981). The results of research by Milligan et al. (2002) and Quiniou et al. (2002) suggest that selection towards increasing the litter size results in the rise of the number of lighter piglets in litter. With the increase of litter size by one piglet, individual piglet body weight decreases by 35 (Quiniou et al. 2002) to 44 g (Roehe 1999).

Pig production traits can be shaped, to a certain degree, by genes and genetic markers. First of all, main genes (of large effects) with considerable influence on the level of production traits have taken the interest of many researchers. One of the main genes with proven influence on meat tissue development is the ryanodine receptor gene - RYR1<sup>T</sup> (Leach et al. 1996; Fisher et al. 2000). Due to its negative influence on the

quality of meat (Barton-Gade and Christensen 1998), breeding programmes provide for its elimination from pig populations of different breeds. The results of studies of some authors point out also the effect of the RYR1 genotype on sow reproduction performance results and piglet rearing (Stalder et al. 1997; XunPing et al. 1999).

Considering the foregoing, an examination was undertaken that aimed at determining the effect of the RYR1<sup>T</sup> gene polymorphism on the initial growth of reared gilts and barrows of the Polish Synthetic Line 990. It is assumed that homozygous individuals with respect to the RYR1<sup>T</sup> gene will distinguish themselves by lower body weight during the rearing period than heterozygous ones and those not loaded with the carrierstate of this gene.

#### **Materials and Methods**

The study was carried out at a farm of the Pig Hybridisation Centre at Pawłowice, Poland. In total, 276 animals were covered in the study, originating from the Polish Synthetic Line 990 sows. The study included piglets from sows that gave birth to at least 12 live-born piglets. On the first day after birth, sow litters were equalised with respect to their size. Each sow fed 12 piglets per litter; in this way the chances of piglet access to mother's milk were equalised. Piglets remained with their mothers for 28 days. During this time they were weighed twice (on day 21 and 28 after birth). On day 63 of life, all litters under examination were taken out for test evaluation, which lasted until day 180 of life. During this time, animals were also weighed (on day 63 and 180). All examined individuals were kept under uniform environmental conditions. During test evaluation, barrows and gilts were fed individually with the same feed mixture. On day 180 of life, a live evaluation of fattening and slaughter values was made.

The percentage meat content was determined using a PIGLOG 105 ultrasound apparatus. According to its operation manual, measurements were made in two points on the back on the right-hand side:

P2 - backfat thickness (P2) - between the 13<sup>th</sup> and the 14<sup>th</sup> thoracic vertebrae, 3 cm away from the middle line of the back:

P4 - backfat thickness at the level of the last thoracic vertebra (P4) and thickness of the longissimus dorsi muscle (P4M), 8 cm away from the middle line of the back.

The percentage meat content in the evaluated animals was automatically calculated based on the aforesaid measurements. The second indicator of live evaluation determined was the piglet live growth rate (daily live body weight gain). Furthermore, the growth rate was also determined during test evaluation, i.e. from day 63 to 180 of life (daily body weight gain).

In order to identify RYR1 (ryanodine receptor) genotypes, the blood collected from the jugular vein of animals under examination was used. The blood was sampled to vacuum test tubes, containing K3EDTA anticoagulant. The DNA isolation was made using the sampled blood by means of Master Pure Kit of Epicentre Technologies (Madison, WI, USA). For a direct analysis of genotypes, PCR-RFLP method was used. Using the polymerase chain reaction, DNA fragments with 134 base pairs were amplified, applying the following primer sequences (Brenig and Brem 1992):

forward primer: 5'-GTGCTGGATGTCCTGTGTTCCCT-3' reverse primer: 5'-CTGGTGACATAGTTGATGAGGTTTG-3'.

The polymerase chain reaction was made using a thermostable Taq polymerase. The reaction mixture of a final volume of 20 µl contained ca 100 ng genomic DNA, 10 pmol of each primer, 2 µl 2 mM dNTP mixture, 1.2 µl 25 mM MgCl., 0.5 U Taq DNA polymerase (MBI Fermentas Burlington, Ontario, Canada) and 2  $\mu$ l 10 × PCR buffer. The amplification reaction was carried out in a thermocycler (Whatman Biometra<sup>®</sup>, Göttingen, Germany) under the following conditions: denaturation at 94 °C for 5 min, and 35 cycles comprising denaturation DNA at 94 °C for 40 s, annealing at 59 °C for 40 s, complementary strand polymerisation at 72 °C for 40 s, and final extension at 72 °C for 5 min. The amplified PCR product was digested with 6 U of the restriction enzyme Hin6I (MBI Fermentas Burlington, Ontario, Canada) at 37 °C for 3 h. The digested PCR product was separated by electrophoresis on 4% agarose gel, stained with ethidine bromide (Sigma-Aldrich Chemie Gmbh P.O., Steinheim, Germany). After electrophoresis, gels were analysed with using UV.

Statistical analysis of the collected performance results was done with one-factor analysis of variance. The significance of differences between groups was calculated according to Duncan's multiple-range test. When making these calculations, a computer statistical software package Statistica 6.0 PL was used.

The following equation was used:

 $y_{ijk} = \mu + a_i + s_j + e_{ijk}$ where:  $y_{ijk}$  observed value,  $\mu$  - overall mean,  $a_i$  effect of i-th genotype (i = CC, CT, TT),  $s_j$  - effect of j-th sex, e<sub>iik</sub> - random residual effect.

### Results

The sizes of the restriction fragment were as follows:

- -134 bp RYR1<sup>TT</sup> (T/T) genotype,
- -84 and 50 bp RYR1<sup>CC</sup> (C/C) genotype,
- -134, 84 and 50 bp RYR1<sup>CT</sup> (C/T) genotype.

In the examined material, two alleles of the RYR1 gene were identified (dominant - RYR1<sup>C</sup>, and recessive - RYR1<sup>T</sup>) that determine the occurrence of three genotypes (C/C; C/T, and T/T) (Table 1). The RYR1<sup>C</sup> allele occurred at a frequency of 0.54, whereas the RYR1<sup>T</sup> allele at a frequency of 0.46. Participation of the C/T heterozygous genotype was the highest and amounted to 63%. The analysed experimental material was characterised by a relatively high percentage of the recessive homozygotes T/T - 14.5%. However, the dominant homozygotes C/C occurred at a frequency of 22.5%.

Table 1. The frequency of RYR1 genotypes and alleles

|               | RYR1     |     |      |        |      |  |
|---------------|----------|-----|------|--------|------|--|
|               | Genotype |     |      | Allele |      |  |
|               | C/C      | C/T | T/T  | С      | Т    |  |
| Number        | 62       | 174 | 40   |        |      |  |
| Frequency (%) | 22.5     | 63  | 14.5 | 0.54   | 0.46 |  |

| Trait                       |        | Genotype          |                   |                    |
|-----------------------------|--------|-------------------|-------------------|--------------------|
|                             |        | C/C<br>n = 62     | C/T<br>n = 174    | T/T<br>n = 40      |
| Body weight on day 21 (kg)  | Mean.  | 5.86ª             | 5.84 <sup>b</sup> | 5.51 <sup>ab</sup> |
|                             | ± S.D. | 0.97              | 0.93              | 0.90               |
| Body weight on day 28 (kg)  | Mean   | 7.89 <sup>A</sup> | 7.70ª             | 7.18 <sup>Aa</sup> |
|                             | ± S.D. | 1.40              | 1.32              | 1.16               |
| Body weight on day 63 (kg)  | Mean   | 20.30             | 19.90             | 19.70              |
|                             | ± S.D. | 2.65              | 3.05              | 2.66               |
| Body weight on day 180 (kg) | Mean.  | 108.01            | 106.08            | 105.10             |
|                             | ± S.D. | 8.80              | 7.73              | 9.71               |

Table 2. Body weight depending on RYR1 genotype

Mean values with the same lower case superscripts differ significantly ( $p \le 0.05$ ) Mean values with the same upper case superscripts differ significantly ( $p \le 0.01$ )

The results presented in Table 2 suggest that an important source of information on the body weight of animals in the initial period of their rearing can be their RYR1 genotype. In this table, mean values for the body weight of the examined animals in respective rearing stages are presented according to the RYR1 genotype. On day 21 of life, mean body weight of recessive homozygotes (T/T) was considerably lower than that of the dominant homozygotes (C/C) and heterozygotes (C/T). The obtained differences were confirmed statistically ( $p \le 0.05$ ). The differences with respect to body weight were also maintained in the later period of rearing. On day 28 of life, animals with the T/T genotype also distinguished themselves by a lower body weight when compared to piglets with the C/C and C/T genotypes. In this case, the observed differences were also confirmed statistically (at  $p \le 0.05$ , respectively). It was observed that the body weight of heterozygotes on day 28 of life, lower body weight was still observed in animals with the

T/T genotype. However, this difference was not confirmed statistically. Similar situation was observed on day 180 of life.

| Trait                             |        | Genotype |         |        |
|-----------------------------------|--------|----------|---------|--------|
|                                   |        | C/C      | C/T     | T/T    |
|                                   |        | n = 62   | n = 174 | n = 40 |
| Daily live body weight gain (g)   | Mean   | 600.00   | 592.00  | 589.00 |
| Durly nive body weight gain (g)   | ± S.D. | 48.70    | 42.46   | 49.81  |
| Daily body weight gain (g)        | Mean   | 753.00   | 743.00  | 743.00 |
| Dany body weight gam (g)          | ± S.D. | 71.99    | 67.16   | 76.27  |
| Feed conversion (kg/kg)           | Mean   | 2.86     | 2.88    | 2.92   |
| r eeu conversion (kg/kg)          | ± S.D. | 0.22     | 0.25    | 0.23   |
| Backfat thickness at P2 (mm)      | Mean   | 11.00    | 10.76   | 10.80  |
| Dackiat unekness at 12 (mm)       | ± S.D. | 2.42     | 2.00    | 2.20   |
| Backfat thickness at P4 (mm)      | Mean   | 9.90     | 9.31    | 9.45   |
| Backlat unekness at 14 (mm)       | ± S.D. | 2.58     | 1.93    | 2.26   |
| Average backfat thickness (mm)    | Mean   | 10.45    | 10.05   | 10.13  |
| Average backfat theckiess (hill)  | ± S.D. | 2.21     | 1.67    | 1.98   |
| Thickness of loin eye muscle (mm) | Mean   | 51.47 ª  | 51.83   | 53.80ª |
| Thekness of foll eye musele (min) | ± S.D. | 6.10     | 5.65    | 6.98   |
| Meat content (%)                  | Mean   | 58.50 ª  | 58.98   | 59.36ª |
| Weat content (70)                 | ± S.D. | 2.07     | 1.75    | 2.02   |
| Index                             | Mean   | 123.00   | 124.00  | 124.00 |
| Index                             | ± S.D. | 9.06     | 9.13    | 9.89   |

Table 3. Fattening and slaughter values depending on RYR1 genotype

Mean values with the same lower case superscripts differ significantly ( $p \le 0.05$ )

The results for the fattening and slaughter traits of the examined material are presented in Table 3. The highest daily live body weight gains and the gains during test evaluation (i.e. from day 63 to day 180 of life) were obtained by dominant homozygotes, whereas the lowest ones by recessive homozygotes. Heterozygotes distinguished themselves by the intermediate values of that trait between homozygotes. Despite obtaining the lowest body weight gains by animals with the T/T genotype, which is also reflected by a lower body weight of these animals in the respective rearing stages, these differences were not confirmed statistically. Most likely, this was caused by body weight equalisation in the examined animals in the later stage of rearing.

As expected, the highest percentage of meat content was found in animals with the T/T genotype, whereas the lowest one in animals with the C/C genotype. The differences found were confirmed statistically ( $p \le 0.05$ ). Animals with the T/T genotype, being an experimental material in the present study, had the thickest loin muscle. Significant differences ( $p \le 0.05$ ) with regard to this trait were demonstrated between the T/T and C/C genotypes.

## Discussion

In an earlier study (Pietruszka et al. 2001) with the Polish Synthetic Line 990 pigs, allele frequency distribution was as follows: RYR1<sup>C</sup> - 0.68, and RYR1<sup>T</sup> - 0.32. Similar distribution of allele frequency in the population of Polish Synthetic Line 990 pigs was also found by Janik (1999). According to these authors, frequency of the RYR1<sup>C</sup> allele amounted to 0.70, whereas that of the RYR1<sup>T</sup> allele was 0.30.

Janik (1999) and Pietruszka et al. (2001) found also the highest participation of the C/T genotype, 54.5 and 51.4%, respectively, although it was lower when compared to the results presented in this paper. The analysed material was characterised by a higher percentage (14.5%) of the T/T genotype when compared to the results of the authors mentioned above (from 2.9 to 6.3%). In the herds of the Polish Synthetic Line 990 pigs analysed earlier by the authors, frequency of the C/C genotype amounted from 42.3 to 42.6%, whereas in the present study it was 22.5%.

The frequency of respective alleles differs among different pig breeds. Among them, Pietrain pigs are considered as the most RYR1<sup>T</sup> gene-loaded pig population in Europe (Sellier 1998). On the other hand, Duroc pigs are characterised by low frequency of that gene (Houde et al. 1993; Dovč et al. 1996; Urban et al. 2002; Zhang et al. 2007).

The use of different methods in order to obtain heavier piglets in the initial period of rearing is reasonable. According to Gondret et al. (2006), piglets lighter on the day of birth were also lighter on the day of weaning. Furthermore, they distinguished themselves by a slower growth rate during the rearing period. As a result, they reached the final body weight 12 days later than animals that were heavier on the day of birth and on the day of weaning. Also Wolter et al. (2002) are of similar opinion. These authors report that higher mortality was also observed in the group of lighter piglets. Moreover, they suggest that a higher body weight on the day of birth has a stronger effect on the rearing results of piglets after weaning than administering supplemental milk replacers to them during lactation. Another effective method for increasing the body weight of piglets can be the administration of porcine somatotropin (pST) to sows during pregnancy (Gatford et al. 2004).

Unfavourable effect of the RYR1<sup>T</sup> allele on the body weight of piglets was also found by Kurył and Wróblewski (1991). Piglets of the Polish Landrace 21 line with the T/T genotype from primiparous sows distinguished themselves by a lower body weight on day 21 of life (6.01 kg) when compared to those with the C/C genotype (6.16 kg). These authors, basing on the research, showed that heterozygotes with respect to the RYR1<sup>T</sup> gene were the heaviest (6.46 kg). In the next stage of this study, the authors also included litters from multiparous sows. Also in this case it was shown that the lowest body weight was characteristic of piglets with the T/T genotype. Higher body weight was characteristic of heterozygotes, and the highest was found this time in dominant homozygotes, which is a confirmation of the results of the present study.

Many researchers demonstrated a positive effect of the RYR1<sup>T</sup> allele on the proportion of meat in the carcass (Simpson et al. 1986; Sather et al. 1991; Leach et al. 1996; De Smet et al. 1998; Fisher et al. 2000).

Some authors (Schlenkler et al. 1984; Gregor and Hardge 1995) suggest that presence of the "meatiness gene" (RYR1<sup>T</sup>) also leads to limitation of boar reproductive capabilities. With reference to sow reproduction performance traits and piglet rearing indices, these results are not so straightforward. Reports within this scope of research are focused first of all on determining the association of the RYR1 genotype of sows with the rearing results of piglets from these sows (Stalder et al. 1997, 1998; XunPing et al. 1999). On the other hand, there are not many studies that refer to the effect of the RYR1 genotype of piglets on their body weight during the rearing period. Stalder et al. (1997) analysed the rearing indices of piglets from sows with a different RYR1 genotype. They did not find significant differences with respect to the number of born piglets, the number of piglets on day 21, the litter weight on day 21 and the survival rate until day 21 of life between C/C and C/T genotypes. Next studies of these authors provided information relating to the fact that litters of sows with the C/T genotype were heavier on day 21 than those of sows with the T/T genotype (Stalder et al. 1998), According to XunPing et al. (1999), not only piglets from C/T sows were characterised by a higher growth rate but also those from T/T sows when compared with the dams with C/C genotype.

Similar results with respect to daily body weight gains were obtained by Jensen and Barton-Gade (1985). Also Leach et al. (1996) did not find significant differences with respect to the growth rate between RYR1 genotypes either, although they demonstrated that C/T animals had slightly higher gains than animals with the C/C genotype. Non-significant differences with respect to the growth rate between RYR1 genotypes were also demonstrated by Pommier et al. (1992).

Regarding the percentage of meat content, similar results were obtained by Fisher et al. (2000). Moreover, these authors demonstrated significant differences between T/T and C/T genotypes. The results of research works carried out in Scandinavia (Nyström and Andersson 1993) also confirmed the superiority of animals with the T/T genotype over those with the C/T genotype with regard to meatiness. The highest carcass meat content in recessive homozygotes with respect to the RYR1<sup>T</sup> gene was also found by De Smet et al. (1996). The results of the present study confirm the effect of RYR1 genotypes on the size of the loin eye area. Also McPhee and Trout (1995) and Piedrafita et al. (2001) when analysing animals with three different genotypes demonstrated that the largest area of loin eye muscle were found in animals (T/T) that were susceptible to stress.

In conclusion, the application of the DNA test for the RYR1<sup>T</sup> gene identification in pigs of the Polish Synthetic Line 990 showed that the pig population of that line is considerably loaded with this gene. Furthermore, a negative effect of the RYR1<sup>T</sup> gene on the body weight in the initial period of pig growth was demonstrated, which confirmed the hypothesis framed earlier. Based on the obtained results, it can be stated that early identification of homozygous animals with respect to the RYR1<sup>T</sup> gene may allow for prediction of the body weight of animals in the initial period of their rearing. Should these results be also confirmed in pig populations of other breeds, this would be an argument supporting the necessity of eliminating the allele T from parental pig herds.

## Vliv polymorfismu genu RYR1<sup>T</sup> na počáteční přírůstky, výkrm a jateční užitkovost prasat polské syntetické linie 990 odchovaných ve standardních vrzích

Cílem této studie bylo určit vliv polymorfismu genu ryadinového receptoru RYR1<sup>T</sup> na počáteční přírůstky, výkrm a jateční užitkovost prasat polské syntetické linie 990 odchovaných ve standardních vrzích. Studie byla provedena na 276 selatech prasnic s vysokou reprodukční užitkovostí. Každá prasnice porodila nejméně 12 selat. První den po narození byly vrhy standardizovány na počet 12 selat. Selata byla vážena 21., 28., 63. a 180. den po narození. Byl stanoven průměrný denní přírůstek v období od narození do 180. dne (LADG) a průměrný denní přírůstek za období od 63. do 180. dne života (ADG). Průměrná zmasilost (PMC), výška hřbetního tuku (BFT) a tloušťka pečeně (LET) byla stanovena pomoci ultrazvukového přístroje PIGLOG 105. Byly identifikovány dvě alely genu *RYR1 (RYR1<sup>C</sup>, RYR1<sup>T</sup>)* a tři genotypy (C/C, C/T a T/T). U genotypu T/T byla 21. a 28. den po narození významně nižší živá hmotnost oproti ostatním dvěma genotypům. Nejvyšší PMC byl u genotypu T/T a nejnižší u genotypu C/C (P ≤ 0,05). Genotyp T/T vykazoval vyšší LET než genotyp C/C (P ≤ 0,05). Mezi genotypy RIR1 nebyly zaznamenány významné rozdíly v rámci LADG, ADG a BFT. Z výsledků pokusu vyplývá, že včasná identifikace homozygotů RYR1<sup>T</sup> genu umožňuje předvídat živou hmotnost v rámci první fáze odchovu.

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