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Original Paper

Evaluation of the Genotypes of Pigs of Different Origins According to the RYR1 and LEP Genes

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Using the polymerase chain reaction method in real-time, we investigated the polymorphism of the RYR1 and LEP genes in sows of the sizeable white breed of different origins. Following the purpose of the work, we conducted a study of the genetic structure of the above-mentioned genes in animals of French and Belgian breeds and their crossbreeds. No RYR1 gene polymorphism was detected. All experimental animals had the homozygous NN genotype. According to the genetic structure of the LEP gene, a significant difference has been established between animals of different origins. Animals of French breeding had a high frequency of the TT genotype, while in animals of other origins, this genotype did not occur at all. Accordingly, French breeding animals are characterized by a high frequency of the T allele and Belgian and crossbred animals are characterized by the A allele. In French breeding animals, there is a violation of the distribution of genotypes, according to Hardy-Weinberg. Obtaining animals of the desired genotypes among the experimental herd is possible due to the use of breeders with the appropriate genotypes for the studied genes.

Keywords: sow, breed, genetic polymorphism, leptin, selection, coefficient, genotype

1 Introduction

To increase the productive traits of pigs, the analysis of QTL gene polymorphisms is increasingly used (Zhukorskyi et al., 2022). The basis of the introduction of marker selection in pig breeding is the determination of polymorphism of genes associated with economically advantageous traits of pigs. Among such candidate genes, whose impact is recognized on meat quality fattening qualities, the genes RYR1 and, CTSF, CTSD are of particular value (de Oliveira et al., 2006). Thus, the ryanodine receptor gene (RYR1) is localized in the 6th chromosome and controls the manifestation of PSS (Porcine Stress Syndrome) stress sensitivity in pigs, the component of which is pale, pork soft exudative (PSE) (Akkari et al., 2016). The presence of a dominant allele N and recessive n was established. Animals with the NN genotype have less lean meat than animals with the nn genotype. It is believed that the latter animals are characterized by a considerably deteriorated quality of beef in comparison with the former. Animals with a heterozygous genotype have an intermediate value regarding meat quality. A high frequency of the recessive allele n is found in pigs of the Pietren breed and Belgian and German Landrace breeds. A lower frequency of this allele is characteristic of other populations of the landrace breed, and a low frequency is characteristic of the Great White and Durok. Recently, breeding programs for most breeds have been aimed at reducing the frequency of the recessive allele, which is related to its impact on the quality of meat and the preservation of young animals. In general, the effect

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of this allele on the pig industry is considered negative (Carolino et al., 2007).

The leptin gene (LEP) also significantly influences the fattening qualities of pigs and the quality of meat. Depending on the genotype of this gene, the moisture content of fat, the moisture-holding capacity of meat, the content of intramuscular fat, and the energy value of meat may change in animals. Several single-nucleotide polymorphisms were found in this gene. It should be noted that there are almost no studies on the polymorphism of this gene in the domestic pig population. Studies conducted on the population of the large blue breed indicate that the frequency of the T allele is 0.85 and the A allele is 0.15, respectively (Matiiuk et al., 2020). According to the results of other studies, the frequency of allele A in animals of the large white breed is 0.93, and allele T – 0.07, in animals of the landrace breed – 0.60 and 0.40, durok – 0.83 and 0.17, p'etren – 0.55 and 0.45 (Tatsiy & Susol, 2023).

The objective of our study – is to study the genetic structure of a herd of large white pigs based on single-nucleotide polymorphisms of the RYR1 and LEP genes, depending on the origin.

2 Material and Methods

Genetic research on DNA typing of pigs based on RYR1 and LEP gene polymorphisms was performed in the Genetics Laboratory of the Institute of Pig Breeding and Agro-Industrial Production of the National Academy of Agrarian Sciences of Ukraine in the period from February 22, 2024, to May 26, 2024. Selection of biomaterial samples (bristle) from large white sows of the breed was carried out in the breeding breeder for breeding animals of the specified breed of the State Enterprise "Experimental Farm of the Institute of Agriculture of the Northeast of the National Academy of Agrarian Sciences of Ukraine. The total number of sows in which RYR1 and LEP gene polymorphisms were studied is 178 heads, including French breeding – 105 heads, Belgian breeding – 38 heads, and sows combining 1/2 French breeding × 1/2 Belgian breeding – 35 heads.

Isolation of DNA from biomaterial samples was performed using Chelex-100 ion exchange resin (Walsh et al.,1991). DNA typing was performed using the PCR-RFLP technique (Glazko, 2001) at the loci of the Leptin gene (LEP) (Kennes et al., 2001), RYR1 (Short & Rothschild, 1997). The structure of primers, PCR conditions, and corresponding restriction fragments of alternative alleles for each locus are shown in Table 1.

The analysis was performed per the "Instructions on genomic typing in pig breeding for genetic research using DNA markers" approved by the Technical Council of MATI of Ukraine.

To conduct PCR-RFLP analysis, a set of reagents for amplification from TAPOTYLY and Helicon was used. DNA restriction was performed using Fermentas enzymes according to the manufacturer's recommendations. Restraint fragments were analyzed using electrophoresis in an 8% polyacrylamide gel and a 2% agarose gel. Visualization was performed by staining the polyacrylamide gel with ethidium bromide, followed by viewing in ultraviolet light on a transilluminator. Photo documentation was carried out with a Canon digital camera. The frequency of alleles was determined according to the generally accepted method using the formula:

$$P(A) = \frac{2N1 + N2}{2n} \quad (1)$$

where: N_1 and N_2 – number of homozygotes and heterozygotes for the studied allele, respectively; n – sample number

In order to assess the statistical reliability of the discrepancy between the distribution of the obtained results the Pearson criterion was used:

$$\chi^2 = \frac{\sum (A-T)^2}{T} \quad (2)$$

where: A – actual number of genotypes; T – theoretical number of genotypes (Ladyka et al., 2023)

The actual (available) heterozygosity was determined by direct calculation using the following formula:

$$H_O = \frac{N2}{n} \quad (3)$$

Table 1 PCR amplification conditions, PCR-RFLP fragments of gene alleles

Genes	The structure of primers for PCR	PCR1	PCR-RFLP fragments of different alleles
Leptin (LEP)	F: 5'-TTGGCGAGCCTGGAGCAGT-3' R: 5'-GCAGCCTCCATCCCTAAGTGGG-3'	242/55/2,0	PCR-RFLP (Xball): alleles g.2845A, 242 p. n.; alleles c. g.2845T, 170 + 72 p.n.
RYR*	F: 5'-GTGCTGGATGTCCTGTGTTCCCT-3' R: 5'-CTGGTGACATAGTTGATGAGGTTTG-3'	134/68/2,0	PCR-RFLP (Hha I): allele g. 1843T (n) 134 bp; allele g. 1843C (N) 84 + 50 p.n.

* PCR product size (n. p.)/annealing temperature (°C)/[$MgCl_2$ (mM)]

The expected heterozygosity was determined using the following formula:

$$H_E = 1 - \sum_{i=1}^{n} p_i^2 \quad (4)$$

where: $p_1, p_2 \ldots p_n$ – frequency of alleles

The fixation index was calculated using the following formula:

$$F_{is} = \frac{H_E - H_O}{H_E} \quad (5)$$

The coefficient of genetic similarity was calculated according to the formula:

$$r = \frac{\sum x \cdot y}{\sqrt{\sum x^2 \cdot y^2}} \quad (6)$$

where: x and y – the frequencies of one allele in different groups of animals (Merkuryeva, 1977)

All statistical calculations were performed according to generally accepted methods (Kovalenko et al., 2010).

3 Results and Discussion

Figure 1 shows the results of the study of the RYR 1 gene polymorphism. According to the results, it was established that the polymorphism of this gene is absent in the animals of the studied populations. All animals have the homozygous NN genotype.

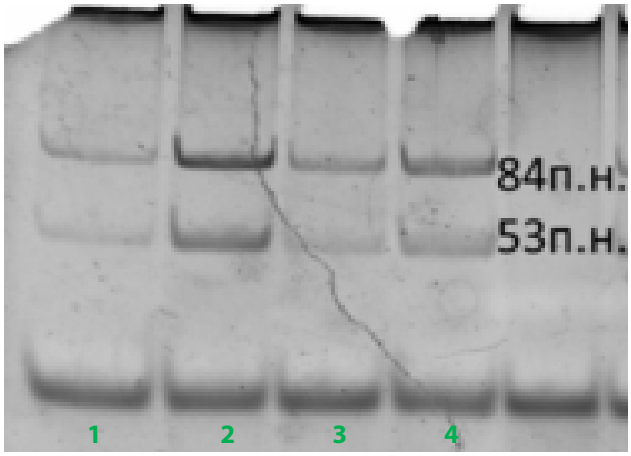

Figure 1 Electrophoresis in 8% polyacrylamide gel of RYR1 gene restrictions
Track: 1–4 genotype NN RYR1 – gene

Studying the polymorphism of the leptin gene (Figure 2), we established that among the livestock of the experimental groups, there are animals of different genotypes, depending on the origin.

Thus, among animals of French breeding, the TT genotypes were more frequent, which were not found at all in representatives of Belgian breeding and sows of a combination of 1/2 French breeding × 1/2 Belgian breeding. A reliable difference was established between the studied groups of animals by origin in terms of AA genotypes. Accordingly, the frequency of the T allele was 6 times higher than the A allele in animals of French selection. In contrast, in animals of other experimental groups, the frequency of the A genotype prevailed.

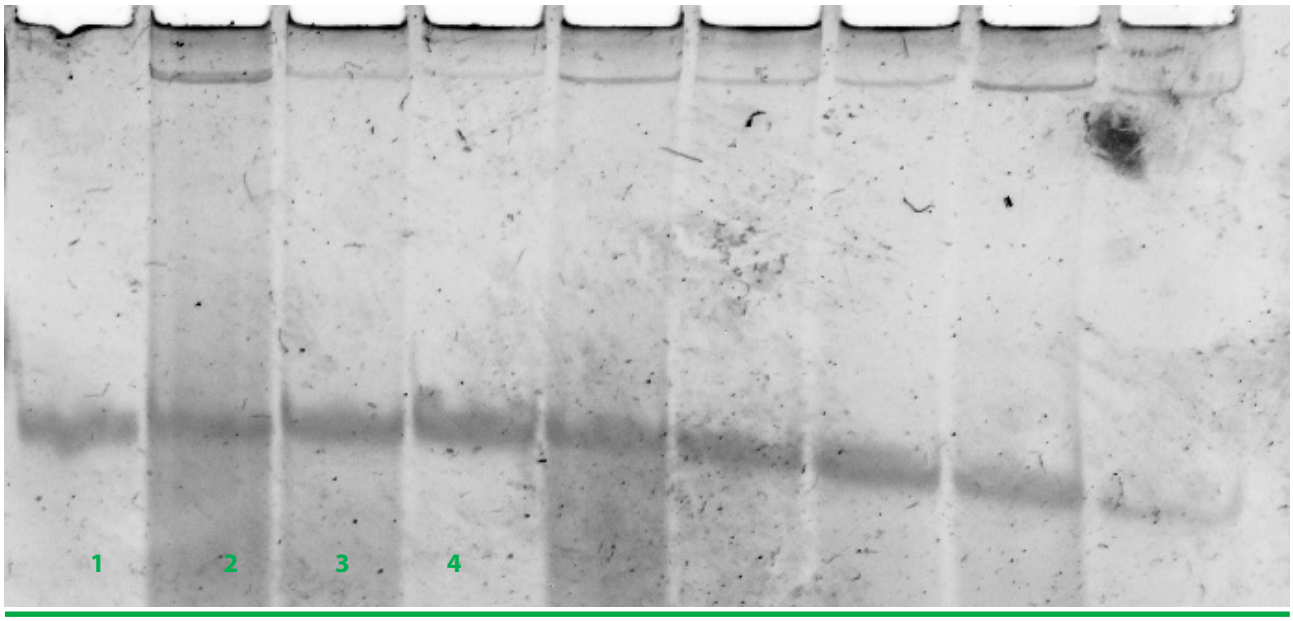

Figure 2 Electrophoresis in 8% polyacrylamide gel of LEP gene restrictions
Track: 1–8 genotype AA Leptin (LEP) – gene

Table 2 Study of frequencies of genotypes and alleles of the lepin gene locus

Distribution	Genotypes			Alleles, un.		χ^2
	AA	AT	TT	A $\pm m_A$	T $\pm m_T$	
	%	%	%			
French breed						
Factual	10	8	82	0.143 ±0.024	0.857 ±0.024	49.83
Expected	2	25	73			
Belgian selection						
Factual	94	6	0	0.972 ±0.019	0.028 ±0.019	0.03
Expected	95	5	0			
French/Belgian crossbreeds						
Factual	97a***b**	3	0	0.987 ±0.127	0.013 ±0.013	0.01
Expected	97	3	0			

p – level of significance according to Fisher's test: ** – *P* <0.01; a – to the French selection; b – to the Belgian selection

The frequency of alleles was probable. Only in animals of French breeding is there a violation of the distribution of genotypes according to Hardy-Weinberg, as evidenced by the value of the χ^2 criterion. According to the obtained data, we can note a lack of heterozygous genotypes and an excess of homozygous genotypes in them (Table 2).

Among animals of other origins, the actual proportions of genotypes coincided with the theoretically calculated ones.

A difference in actual and theoretical heterozygosity was also established between animals of different origins (Table 3).

We can characterize the advantage of theoretical homozygosity over actual sows of French origin (Table 4).

In contrast, in animals of Belgian origin and sows of a combination of 1/2 French selection × 1/2 Belgian selection, a slight advantage of actual homozygosity was observed. Accordingly, in animals of French breeding, the fixation index has a positive value, and in animals of other origins, it has a negative value.

Table 3 Values of the main indicators of variability by the LEP gene

Sows	H_O	H_E	F_{is}
French origin	0.076	0.245	0.689
Belgian origin	0.056	0.054	-0.029
Combination of 1/2 French selection × 1/2 Belgian selection	0.026	0.025	-0.013

H_O – actual heterozygosity, H_E – expected heterozygosity, F_{is} – fixation index

Table 4 Genetic structure of a herd of cattle by the LEP locus

Indicators	Sows					
	French origin		Belgian origin		Combination of 1/2 French selection × 1/2 Belgian selection	
	actual	theoretical	actual	theoretical	actual	theoretical
Heterozygotes	8	26	2	2	1	1
Homozygotes	97	79	34	34	38	38
Hetero/homozygote ratio	0.082	0.329	0.059	0.059	0.026	0.026
Heterozygosity test	-0.242	–	0.002	–	0.000	–
Heterozygosity degree, Ca (%)	75.5	–	94.6	–	97.5	–
Polymorphism level, Na	1.32	–	1.05	–	1.03	–
Excess coefficient D	-0.688	–	0.029	–	0.013	–
Proportion of homozygotes, %	92.4	–	94	–	97.4	–

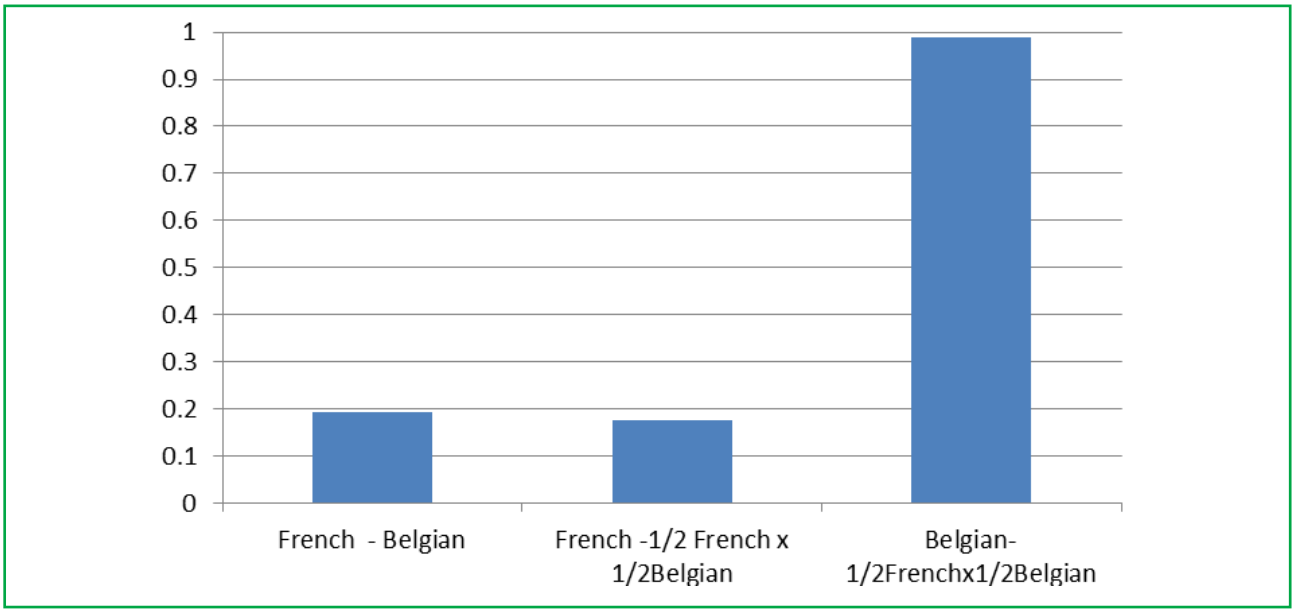

Figure 3 Coefficient of genetic similarity between animals of different origins

Analyzing the genetic structure of the studied groups of animals according to the leptin gene, we confirmed the previously voiced claims that there is a high frequency of homozygous genotypes. The ratio of heterozygotes to homozygotes in all groups was significantly less than one, and the proportion of homozygotes was 92.4–97.4%. Animals of Belgian breeding and sows of a combination of 1/2 French breeding × 1/2 Belgian breeding prevailed by the value of Ca, which indicates a more significant consolidation of this gene in these animals. Accordingly, they also had a lower value of polymorphism (Na). The kurtosis coefficient had a negative value in animals of French origin.

We calculated the coefficient of genetic similarity. Based on the results of the calculations, we can conclude that animals of Belgian breeding and sows of a combination of 1/2 French breeding × 1/2 Belgian breeding have a high value of this coefficient – 0.999, which indicates their similarity with the genetic structure of the leptin gene. On the contrary, the low value of the coefficient of genetic similarity between animals of French and Belgian selection (0.193) and between French selection and sows of a combination of 1/2 French selection × 1/2 Belgian selection (0.177) indicates the existence of a significant difference in the genetic structure of these animals (Figure 3).

The results indicate a significant difference in the genetic structure of the leptin gene between animals of different origins. In 2024, two breeder boars of a large white breed are used in the pig breeding stock: one is of French, and the other is of Belgian selection (Table 5).

According to the RYR1 gene, their genotype is NN, which allows you to have offspring only with this genotype

Table 5 Evaluation of breeding boars of the large white breed by gene LEP

Origin	Genotypes		
	AA	AT	TT
France	1	–	–
Belgium	–	1	–

Table 6 Possible variants of offspring genotypes when breeding large white pigs of French and Belgian breeding

Sow genotype	Genotype of breeder boars	
	AA	AT
AA	AA – 100%	AA – 50%, AT – 50%
AT	AA – 50%, AT – 50%	AA – 25%, AT – 50%, TT – 25%
TT	AT – 100%	–

(100% of the mothers have this genotype). Fertilizers have different genotypes with the leptin gene

Using a breeder of French breeding with a homozygous genotype AA will increase the share of allele A among pigs of French breeding and genotypes AA and AT. Accordingly, the use of breeder boars of the large white breed of Belgian selection in the breeding stock of this selection will allow for obtaining a small proportion of animals with the TT genotype, which is connected with the absence of sows with the TT genotype (Table 6).

The results of our research on the LEP gene polymorphism partially coincide with the results of other scientists. Thus, the frequency of alleles A and T in pigs of French selection is A – 0.15; T – 0.85; which almost completely corresponds to the results of other researchers (respectively A – 0.14; T – 0.86) (Matiiuk et al., 2020). Other researchers also write about the high frequency of the T allele in animals of the Large White breed (Tatsiy & Susol, 2023). However, according to the results of our research, pigs of Belgian breeding and crosses of French and Belgian breeding, on the contrary, have higher frequencies of allele A and lower frequencies of allele T (0.972 and 0.987; 0.028 and 0.013, respectively). According to the polymorphism of the RYR 1 gene, our results confirmed the absence of a mutant allele in animals of the Large White breed (n), which corresponds to the results of other researchers (Matiiuk et al., 2020). At the same time, some authors indicate a low frequency of the mutant allele in animals of this breed – 0.04 (Carolino et al. 2007).

4 Conclusions

Following the purpose of our work, we conducted studies to determine the frequency of alleles and genotypes at the RYR1 and LEP loci. It was established that all sows of the large white breed of different origins have the genotype at the first locus NN. That is, there is no RYR1 gene polymorphism in sows and breeding boars of the controlled population. The use of French and Belgian breeding boars will allow offspring to be obtained only with this genotype since breeding boars also have the homozygous NN genotype. According to the genetic structure of the LEP locus, pigs of different origins differ significantly. The data of the calculation of the coefficient of gene similarity confirms this. Genetic balance was found in our studies only in animals of Belgian breeding and sows of a combination of 1/2 French breeding × 1/2 Belgian breeding. The values of actual and theoretical heterozygosity confirm this. The use of breeding boars, which are attached to the breeding stock of the herd, will allow an increase in the frequency of the A allele among animals of French selection. We propose systematically evaluating sows and breeding boars in further breeding work according to the studied loci.

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