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DNA-type results of Landrace sows for RYR1-gene and its association with productivity

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Recently the assessment of QTL genes has been a relevant focus of research. Among other genes, the RYR1 is one of the most important. Research on this gene for Landrace sows of Ukrainian selection has been insufficient. This fact confirms the need for our work. A total of 63 Landrace sows from different families were evaluated by reproductive qualities for two generations. A comprehensive assessment of the sows' reproductive ability was performed using the SIRQS-index. Determination of polymorphism in the RYR1 gene in pigs was performed by using DNA-typing of animals. Genetic potential was calculated between two generations "mother-daughter". By assessment of polymorphism of the RYR1 gene, it was found that 6.3% of the Landrace sows were the carriers for the mutant allele of the RYR1 gene. No animals with the RYR1nn genotype were detected. Accordingly, animals with the RYR1NN genotype accounted for 93.6%. The frequency of the N allele of the RYR1 gene was 0.97, the n frequency of the RYR1 gene allele was 0.03. Sows with RYR1NN genotype had a higher level of reproductive ability compared to the RYR1Nn genotype. RYR1NN genotype also had a higher level of genetic potential. The greatest progress was established between generations of Landrace sows which were carriers for the mutant allele. The highest values of this progress were by the NBA, the lowest – by the NW. On the contrary, there was regression between populations for part of the population (Landrace sows of Ukrainian selection of RYR1Nn genotype) by the all estimated indicators of reproductive ability. Sows that were free of the mutant allele of the RYR-1 gene had high SIRQS index. The phenotypic consolidation coefficients by the NBA were lower for sows free of the mutant allele than for its carrier. The advantage of sows free of the mutant allele of the RYR-1 gene over its carrier sows was established in almost all assessed indicators of reproductive ability. No significant differences in the level of consolidation of reproductive ability between sows with different allelic variants of the RYR-1 gene were established. The higher productive level of sows with RYR1NN genotype is reflected in the indicators of economic efficiency of production. It allows higher levels of profitability to be obtained and net profit to be increased by 1093 UAH compared to sows with RYR1Nn genotype. The further monitoring of the studied gene and the gradual elimination of carriers of the mutant allele is a promising direction in breeding work.

Keywords: pigs; reproductive qualities; genetic potential; Landrace breed; QTL.

Introduction

Providing the population with a full and sufficient quantity of food was and remains one of the most pressing problems of our time. The experience of advanced states shows that the elimination of the deficit of meat by more than 42.0% is solved by the pig industry (Schook et al., 2005; Kozyr et al., 2019). Pork is a relatively popular meat product in Ukraine and other countries. The total meat consumption in the world is continuously rising and on global average approaches 42 kg per person annually (Mlynek, 2020). In pork meat, colour, marbling and fat content are the most important quality attributes (Verbeke et al., 2005). The usefulness of this product in human nutrition should also be considered. In addition to provision of food, the requirement for valuable individual nutrients and biologically active substances in which certain products are rich is also important. In the case of pork, in particular, we are talking about polyunsaturated fatty acids. The importance of n-3 polyunsaturated fatty acids (PUFA) in a healthy human diet has been known for decades. In the human body, alpha-linolenic acid (ALA) is a major n-3 PUFA of importance because it is metabolized to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The dietary levels of n-3 PUFA in relation to n-6 PUFA (the omega-6:omega-3 ratio) are the key metric for determining the health of a diet (Simopoulos, 2000; Ma et al., 2016).

Today pig breeding is a high-tech livestock industry. Compared to the previous period, in pig breeding for the domestic market the number of piglets born alive has increased by almost one and a half times, while the period of fattening to favourable condition is decreased. Also the thickness of fatback has significantly decreased and the efficiency of feed use has increased.

In recent decades, a number of aspects of swine production have been improved: the introduction of the latest keeping technologies, improvement of feeding rations, and the increase in total head of pig. Further increase of production marketability in this industry in the domestic market requires a transition to a more intensive level of its management (Ivanov & Guk, 2019). More attention is paid to the maintenance conditions of pigs (Voloshchuk & Herasymchuk, 2017), their full feeding and rearing (Voloshchuk, 2018). The latest approaches to assessment of meat quality (Voitenko, 2018; Kramarenko et al., 2019) are used and modern DNA technologies are increasingly involved (Rudoman et al., 2017).

Efficient pork production is the result of ensuring a high level of all process components. An important role is played by the reproductive qualities of sows because they influence the further production capacity. The group of traits responsible for reproduction is no exception, because the reproductive ability of sows belongs to the group of traits that are largely reflected in the overall efficiency of pork production. (Melnik et al.,

2017; Kramarenko et al., 2018; Tsereniuk et al., 2018). An important issue for increasing the gross production of pork is solving the problems associated with the reproduction of the herd, increasing the level of fattening and meat qualities of young pigs, obtained using different breeding schemes. Achievement of this goal is impossible without the use of genetic-molecular techniques for the creation of populations of herds of pigs with a high and stable level of productivity. This is a prerequisite for the economically efficient operation of specialized pig farms (Kozyr et al., 2019). An important point for the expansion and development of methodological approaches to increasing pig productivity, which can be implemented in the future on a large herd, is the assessment of polymorphism, identification of individual patterns and establishment of existing relationships at the level of different breeds of pigs. The limitations of classical methods of selection motivate breeders to look for other methods, such as genetic markers. Accordingly, scientists and practitioners are looking for markers that would be the main genes or those that would be associated with them (Buczyński, 2006). Also in recent decades, methodological approaches to DNA evaluation permit significant acceleration of the pace of selection in pig breeding (Korwin-Kossakowska et al., 2003; Houston et al., 2004; Nanuwong & Bodhisuwan, 2014; Mankowska et al., 2015). At the same time, the most well-known candidate-genes in the direction of improving sows' reproductive qualities are the genes of estrogenic receptor (ESR) and prolactin. There are also some references to the presence of a link between the ryanodine receptor gene (RYR1) and the level of sows' reproductive qualities (Buczyński, 2006; Pestis et al., 2015). The ryanodine 1 receptor is a calcium ion channel of the sarcoplasmic reticulum of skeletal muscle. Several polymorphic loci have been identified in the human and animal RYR1 gene. Some of them were associated with specific phenotypes. However, data on the genetic variability of RYR1 in pigs are scarce (Dokāne et al., 2016). An important point of the assessment of the RYR1 gene is also the fact that this gene is largely reflected in product quality, particularly in marketable pork. It is well known that satisfaction of consumers is a crucial part of modern shopping behaviour. Thus, it is necessary to understand their needs and to know the factors that generate these demands (Font-i-Furnols & Guerrero, 2014). At the same time, the productivity of sows largely determines the economic performance of pig breeding. Thus the reproduction indicators are largely determined by environmental factors, which is due to the low values of the coefficient of heredity from 0.1 to 0.2. For maternal breeds of pigs, reproductive ability is considered particularly important (Buczyński, 2006). It should also be mentioned that in recent decades, the transition to a reduced weaning period, the widespread use of pre-starter feed-stuff and the regulation of the microclimate in farrowing facilities have made the high level of reproductive ability of sows even more important.

An important point in pig farming is the maximum realization of the genetic potential of animal productivity. After all, the genetic potential of animal productivity can be considered as the maximum productivity that can be obtained from a particular animal or group of animals by providing optimal and appropriate conditions for keeping, feeding, and so on. At the same time, the realization of genetic potential depends on the ability of animals to adapt. It is known that a high level of productivity in pigs can be obtained by creating appropriate conditions for maintenance and feeding, as well as the use of stress-resistant pigs with a high level of genetic potential. Besides, the early weaning of piglets from the sows, the formation of groups for rearing and fattening, the transportation of animals are extreme stimuli that do not correspond to the level of evolutionarily determined protective and adaptive reactions of the organism. As a result, there is a stressful situation, accompanied by growth retardation, dysfunction of reproductive ability, increased morbidity and mortality of pigs, and reduced quality of meat products (Ivanov & Guk, 2019). All these points must be taken into account when developing breeding programs for domestic pig populations.

Preserving the current diversity of the living material on Earth is fundamental for the survival of future generations. In the case of animals, in the recent past, more and more effective breeding programs have been implemented, and have led to an emphasis on a few specialized stocks. Consequently, breeds that are less suited to current needs tend to see their numbers decline and to be eventually lost. Conservation of variation, however, is necessary to meet future agricultural challenges and particularly

food needs, as well as to preserve the rich agricultural heritage of the various regions of the world (San Cristobal et al., 2003; Kramarenko et al., 2018). Until the twentieth century, Ukrainian pig production was conducted on a relatively small scale. Most pigs were imported from either Great Britain or China and were raised by small families or landlords (Trasпов et al., 2016). Although pigs in Ukraine originated from imported breeds, after their introduction, they adapted to the local climate, poor quality feed and resident pathogens, which led to the development of breeds with unique characteristics that differed from those of the founding stocks (Trasпов et al., 2016). In the twentieth century, the formation of the Ukrainian breeding base was realized in pig breeding. Domestic populations were formed from imported pig breeds and an active creative breeding process took place. In the 1970s, Ukrainian pig farming was reoriented to the industrial production and breeding of meat pigs. At the same time, meat breeds of pigs were imported and later they formed domestic populations.

At the beginning of the 21st century, work on the creation of Ukrainian meat breeds of pigs on a crossbred basis was completed. These breeds are the following: Ukrainian Meat, Poltava Meat and Red White-belt. However, in recent decades, a significant reduction in the number of animals of local breeds and the import of animals from abroad has taken place. Livestock populations have been subjected to a variety of evolutionary forces during their histories. The cumulative effect of foundation events, genetic drift and natural or artificial selection has led to the formation of distinct breeds (Kramarenko et al., 2018).

At present there are more than 730 breeds or lines of pigs throughout the world and more than two thirds are found in China and Europe. Many of these (possibly more than 270 breeds) are now in danger of extinction and others are threatened by inefficient use or loss due to cross breeding. To evaluate genetic uniqueness and breed diversity of pigs and assist in rationalizing breed conservation programs, microsatellites have been and remain efficient markers (Nidup & Moran, 2011; Kramarenko et al., 2018). Today, pedigree pig-breeding in Ukraine is represented by the following breeds: Large White, Duroc, Landrace, Pietren, Poltava Meat, Welsh, Ukrainian Meat, Ukrainian White Steppe, Ukrainian Spotted Steppe, Red White-belt. The Landrace was the leader by the number of pigs in breeding herds. During 2011–2019, the number of sows increased by 2–3 times for the Large White breed and Landrace, and by 8–12 times for Poltava Meat and Ukrainian Meat breeds. Currently, only pigs of Large White and Landrace breeds can be competitive by numerical strength. The remaining breeds should be classified as not numerous (Voitenko et al., 2019). In this context, at present the condition of animal genetic resources in the sphere of food production and agriculture is the subject of monitoring by relevant institutions, including the Food and Agriculture Organization of the United Nations Organization. Since 2000 the breeds with specified molecular and genetic characteristics have been added into the Global Databank of Farm Animal Genetic Resources. It may be explained by the fact that sometimes it is rather hard to have a clear distinction between breeds, as some of them have similar features, which are of special economic value (Pocherniyayev, 2016). At the breed level, the situation is even more complicated. As for the Landrace breed, there are currently populations of this breed in Ukraine originating from Great Britain, France, Denmark, Hungary and other countries. The Landrace population of domestic origin (Ukrainian landrace or ULN) has been constantly declining recently. This premise points to the high relevance and urgency of research that can help to increase the competitiveness of domestic pig populations, in particular, the largest population – Landrace of Ukrainian selection.

The aim of the research which is presented in this work is the assessment of the economic efficiency of pork production based on identifying differences in the reproductive ability of sows with different allelic variants of the RYR1 gene.

Materials and Methods

The research was carried out on the farm "Shubske", Bohodukhiv district, Kharkiv region, Ukraine. The research was conducted against the background of keeping animals on a breeding farm with a regulated microclimate and feeding of different sex and age groups with full-fledged rations. A total of 63 (for each of the generations) Landrace sows of Ukrainian selection of different families were evaluated, in accordance

with the general principles of research organization. Reproductive qualities of sows were evaluated by the following two generations. Indicators such as number of piglets born alive (NBA), number at weaning (NW) on the 28th day and litter weight at weaning (LWT) on 28th day (kg) (recalculation on the 60th day with a coefficient of 2.88, according to the rules of pedigree accounting of Ukraine) were taken into consideration. A comprehensive assessment of the sows' reproductive ability was performed using the selection index of reproductive qualities of sows (I) (Tserenyuk, 2015). The value of the index of 100 points characterizes the reproductive ability of sows of the elite class in accordance with the current Breeding Instructions in Ukraine: values greater than 100 points are desirable. The index is determined by the following formula (1):

$$I = 6X_1 + 9.34(X_2/X_3) \quad (1)$$

where I – selection index of reproductive ability (reproductive qualities) of sows; X_1 – NBA, heads; X_2 – LWT/kg; X_3 – days of weaning, days.

Determination of polymorphism in the RYR1 gene in pigs was performed by using DNA-typing of animals on the basis of the Laboratory of Molecular-genetic, Physiological and Biochemical Investigations in Animal Husbandry at the Institute of Animal Science NAAS (Kharkiv) and the Institute of Pig Breeding and Agro-industrial Production NAAS (Poltava). The presence of genes was determined by PCR-RFLP (polymerase chain reaction, restrictive fragment length polymorphism). Hair follicles were used as biological material. DNA isolation was performed using a commercial DNA-SORB-B set according to the manufacturer's instruction. The study of the genes of quantitative trait was performed as follows: for locus-specific amplification of a fragment of the RYR1 gene by the method (Brem & Brening, 1993) using primers:

$$\begin{aligned} \text{F: } & 5\text{'-GTGCTGGATGTCCTGTGTTCCCT-3'} \\ \text{R: } & 5\text{'-CTGGTGACATAGTTGATGAGGTTTG-3'} \end{aligned}$$

Temperature mode of PCR: DNA denaturation at 94 °C – 4 min; then 35 cycles according to the scheme: DNA denaturation at 94 °C – 60 s, annealing of primers at 69 °C – 60 s, synthesis of DNA chains at 72 °C – 60 s (for final cycle in 4 min).

Restriction mixture: H_2O – 1 μ L, 10 x buffer solution for the enzyme – 2 μ L, restriction enzyme – 1 μ L, amplification – 10 μ L. The disintegration results of the PCR products by restriction enzyme were evaluated by electrophoretic method with 8% polyacrylamide gel stained with ethidium bromide. Restriction patterns: genotype nn – 134 n. p.; genotype Nn – 134, 81, 53 n. p.; genotype NN – 81, 53 n. p.

The allele frequencies of the RYR1 gene were calculated according to Hardy-Weinberg's law (2):

$$p^2 + 2pq + q^2 = 1 \quad (2)$$

where p^2 – proportion of homozygotes by the N allele; p – frequency of N allele; q^2 – proportion of homozygotes by the n allele; q – frequency of the n allele; $2pq$ – proportion of heterozygotes.

The frequencies of the N allele and the n allele were determined by taking into account the frequencies of the genotypes $f(NN)$, $f(Nn)$, $f(nn)$ according to formulae (3–4):

$$p = f(RYR1_{NN}) + \frac{1}{2} f(RYR1_{Nn}) \quad (3)$$

$$q = f(RYR1_{nn}) + \frac{1}{2} f(RYR1_{Nn}) \quad (4)$$

where p – frequency of the N allele; q – frequency of the n allele; $f(RYR1_{NN})$ – proportion of animals with RYR1_{NN} genotype; $f(RYR1_{Nn})$ – proportion of animals with RYR1_{Nn} genotype; $f(RYR1_{nn})$ – proportion of animals with RYR1_{nn} genotype.

The genetic potential (GP) of various traits of productivity (in the study of trait of sows' productivity – their reproductive ability) was determined by the method (Basovskiy, 1991), in the performance (Kovalenko & Nezhlukchenko, 2008) based on the of relative heredity share of the original breed for all estimated indicators of sows' reproductive ability: the number of piglets born alive, heads; the number of piglets at weaning on the 28th day, heads; and litter weight at weaning on 28th day, kg.

The genetic potential (GP) is determined according to the following formula (5):

$$GP_A = B - \left(\frac{B - F_n}{n_A} \right) \quad (5)$$

where GP_A – genetic potential of productivity by the evaluated group of animals; B – productivity of improving breed (best), or animals generation of the producing line or family; n_A – the relative heredity share by the animals of the previous generation of the producing line or family (B) respectively; F_n – productivity of offspring of the corresponding relative heredity share by the improving breed (B).

Genetic potential was calculated between two generations "mother-daughter" as follows: separately for the entire estimated population, for part of the population with the RYR1_{NN} genotype, and for the part of population with the RYR1_{Nn} genotype. GP at evaluation of productivity within the generations of "mother-daughter" was determined only for those groups in which the indicators for daughter generation exceeded those one of the mother generation.

Progression and regression in generations were calculated separately for the entire estimated population and for parts of the population: sows of RYR1_{NN} genotype and sows of RYR1_{Nn} genotype. The calculations were carried out in relative equations. Existence of an advantage of the daughter generation or its lack was determined in percent compared to the mother generation. According to the same principle, progression and regression in the generations were calculated by the comprehensive assessment of sows' reproductive ability (SIRQS-index).

The selection differential (Sd) was evaluated by the difference between the arithmetic mean of the indicators (NBA, NW, LWT and SIRQS-index) of the entire evaluated population and the arithmetic mean of the same indicators of parts of the population (Landrace sows of Ukrainian selection of RYR1_{NN} genotype and Landrace sows of Ukrainian selection of RYR1_{Nn} genotype).

According to the obtained absolute indicators, the coefficients of phenotypic consolidation were determined both by the standard deviation (K_1) and by the coefficient of variation (K_2) by (Polupan, 2001). The coefficients were calculated by the following formulae (6–7):

$$K_1 = 1 - \frac{\sigma_g}{\sigma_z} \quad (6)$$

$$K_2 = 1 - \frac{Cv_g}{Cv_z} \quad (7)$$

where K_1 , K_2 – the degree of phenotypic consolidation of the evaluated group; σ_g та Cv_g – the standard deviation and the coefficient of variation of the evaluated group of animals by an individual feature; σ_z та Cv_z – the same indicators of the parent population.

A one-way analysis of variance by indicators of sow productivity (NBA, NW & LWT) was also performed. The economic efficiency of results was calculated according to comparative evaluation of the efficiency of pork production between the animals with different allelic variants of the RYR1 gene expressed through cost-effectiveness ratio (kvp) (Olyadnichuk, 2008) according to the following formula (7):

$$k_{vp} = \frac{P_z}{P_o} \div \frac{Z_z}{Z_o} \quad (7)$$

where k_{vp} – cost-effectiveness ratio; P_o and Z_o – productivity and production costs per sow in the basic version; P_z and Z_z – productivity and production costs per sow in the experimental version.

The obtained indicators were processed by the method of variation statistics. The results were processed by standard methods with the calculation of \bar{x} – the mean value, SD – the standard deviation. Differences between the values of the between sows with different alleles and the evaluated population were determined using ANOVA. Differences were found to be statistically significant at $P < 0.05$.

Results

By estimation of the RYR1 gene polymorphism, it was found that 6.4% of sows within the evaluated population of Landrace pigs of Ukrainian selection were carriers of the mutant allele of the RYR1 gene (Table 1). No animals with the RYR1_{nn} genotype were detected. Accordingly, animals with the RYR1_{NN} genotype accounted for 93.6%. The frequency of the N allele of the RYR1 gene was 0.97. Accordingly, the n frequency of the RYR1 gene allele was insignificant and was at the level of 0.03. This frequency of the mutant allele of this gene indicates the presence of single animals of its carriers in the population.

Table 1
Reproductive ability of Landrace sows of Ukrainian selection by two generations ($\bar{x} \pm SD$)

Indicator	mother	daughter	P	GP
Evaluated population (n = 63)				
Number of piglets born alive, piglets	13.1 ± 1.1	13.4 ± 1.3	> 0.05	13.7
Number at weaning, piglets	11.6 ± 0.8	11.6 ± 1.3	> 0.05	11.6
Litter weight at weaning, kg	203.2 ± 3.5	204.0 ± 5.2	> 0.05	204.7
Part of the population with genotype RYR1 _{NN} (n = 59)				
Number of piglets born alive, piglets	13.1 ± 1.1	13.5 ± 1.3	> 0.05	13.8
Number at weaning, piglets	11.6 ± 0.8	11.7 ± 1.3	0.011	11.9
Litter weight at weaning, kg	202.8 ± 3.5	205.7 ± 5.2	0.011	208.5
Part of the population with genotype RYR1 _{Nh} (n = 4)				
Number of piglets born alive, piglets	12.9 ± 0.8	12.3 ± 1.6	> 0.05	13.1
Number at weaning, piglets	11.9 ± 0.8	10.2 ± 0.9	0.012	10.4
Litter weight at weaning, kg	207.8 ± 3.3	178.1 ± 3.9	0.012	181.4

Comparing the reproductive abilities of Landrace sows of Ukrainian selection free of the mutant allele and its carrier sows, no significant evidence of the advantage of the former animals over the latter by all estimated indicators of reproductive ability were found. The progress of the population from mother to daughter generation was established according to all estimated indicators of sows' reproductive ability.

Animals with the RYR1_{NN} genotype had a higher level of sows' reproductive ability compared to the RYR1_{Nh} genotype. RYR1_{NN} genotype also had a higher level of genetic productivity potential by all estimated indicators of sows' reproductive ability. At the same time, the greatest progress was established between generations of Landrace sows which were carriers for the mutant allele.

An important element of selection work in pig breeding is the achievement of gradual progress over the generations by all the traits that are selected. Evaluation of progress in the population (within two evaluated generations of Landrace sows of Ukrainian selection) by the indicators of reproductive ability both for the entire evaluated population and for Landrace sows of Ukrainian selection with different genotypes is shown in Figure 1.

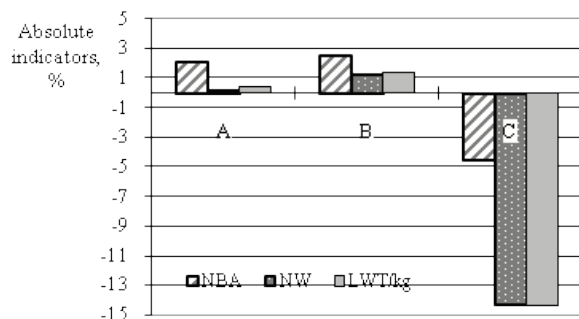


Fig. 1. Evaluation by absolute indicators in the generation for Landrace sows of Ukrainian selection: A – evaluated population (n = 63 heads); B – part of the population with genotype RYR1_{NN} (n = 59 heads); C – part of the population with genotype RYR1_{Nh} (n = 4 heads); NBA – number of piglets born alive; NW – number at weaning; LWT – litter weight at weaning

The obtained results reveal good progress in generations for the entire evaluated population (A) by all traits of reproductive ability which were identified in our studies. At the same time, there was progress between populations for the part of the population (Landrace sows of Ukrainian selection of RYR1_{NN} genotype) by the all estimated indicators of sows' reproductive ability. The highest values of this progress were by the NBA, the lowest – by the NW. On the contrary, there was regression between populations for part of the population (Landrace sows of Ukrainian selection of RYR1_{Nh} genotype) by the all estimated indicators of sows' reproductive ability. The most pronounced regression values were for LWT/kg and NW. The lowest regression values were by the NBA.

Landrace of Ukrainian selection is used in Ukraine pig breeding for industrial crossbreeding or breed-linear hybridization as a maternal or intermediate paternal form (by the reciprocal combinations with a Large White or Welsh). Complex selection is used in the population. The repro-

ductive ability of sows is estimated comprehensively using the SIRQS-index. Therefore, it is necessary to calculate the value of the SIRQS-index to compare sows with different genotypes by RYR1 gene (Fig. 2).

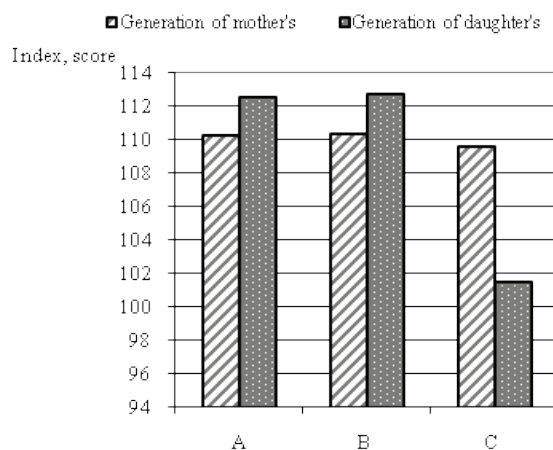


Fig. 2. The value of the Selection index of reproductive qualities of sows (I) Landrace sows of Ukrainian selection: A – evaluated population (n = 63 heads); B – part of the population with genotype RYR1_{NN} (n = 59 heads); C – part of the population with genotype RYR1_{Nh} (n = 4 heads)

Determination of the SIRQS-index by the complex estimate of reproductive qualities precisely distributed Landrace sows of Ukrainian selection into two types: Landrace sows of Ukrainian selection, free of the RYR1 mutant allele gene and heterozygous Landrace sows of Ukrainian selection by this allele.

Besides, the differences by the indicators of the mother generation between the sows of RYR1_{NN} genotype and the sows of RYR1_{Nh} genotype were less significant compared to the sows of the of the daughter generation. Similar to the evaluation by absolute indicators, the evaluation of progress in the population by the SIRQS-index (Fig. 3) also showed progress for the entire evaluated population and regression for part of the population (sows of RYR1_{Nh} genotype). As well as by the absolute indicators, the progress for the entire evaluated population and part of the population (sows of RYR1_{NN} genotype) was insignificant (2.0% and 2.2%), but the regression by the Selection index of reproductive qualities of sows (I) for part of the population (sows of RYR1_{Nh} genotype) had a significant level (-7.4%).

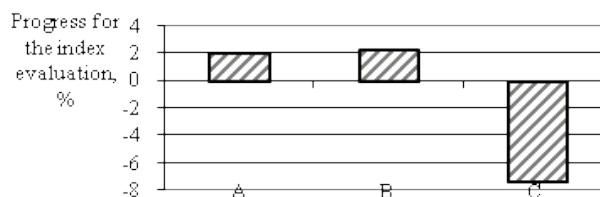


Fig. 3. Progress for the index evaluation (selection index of reproductive qualities of sows) in generations for Landrace sows of Ukrainian selection: A – evaluated population (n = 63 heads); B – part of the population with genotype RYR1_{NN} (n = 59 heads); C – part of the population with genotype RYR1_{Nh} (n = 4 heads)

Realization of short-term forecast of progress or regression in the population by a certain group of traits is possible in particular with use of values of a selection differential.

Evaluation of the selection differential (Table 2) showed that its highest values were obtained for the part of the population (sows of RYR1_{NN} genotype) by the LWT and Selection index of reproductive qualities of sows (I). The lowest values of the selection differential were obtained by the same indicators for part of the population (sows of RYR1_{Nh} genotype).

In general, the scope of selection differential between parts of the populations (sows of RYR1_{NN} and RYR1_{Nh} genotypes) was the following: NBA – 1.23; NW – 1.65; LWT – 29.4; SIRQS index – 11.56. A no less important characteristic of the population is the uniformity and similarity of individual animals in the group. On the basis of signs with a low

degree of heredity, this indicator allows us to estimate the technological suitability of a group or population of animals.

Table 2
Selection differential by the reproductive ability for parts of the population of Landrace sows of Ukrainian selection

Indicator	Sd for parts of the population with genotype	
	RYR1 _{NN}	RYR1 _{Nn}
Number of piglets born alive, piglets	+0.07	-1.16
Number at weaning, piglets	+0.10	-1.55
Litter weight at weaning, kg	+1.76	-27.64
Selection index of reproductive qualities of sows (I)	+0.27	-11.29

As well, the coefficient of phenotypic consolidation for both investigated groups of sows was estimated in comparison with the evaluated populations on the whole (Table 3). It was found that Landrace sows of Ukrainian selection of RYR1_{NN} genotype of daughter generation by the estimated indicators were not significantly more consolidated with respect to the sows of the mother generation. There are no significant differences among the indicators for Landrace sows of Ukrainian selection of RYR1_{Nn} genotype.

Table 3
Coefficient of phenotypic consolidation by the reproductive ability of Landrace sows of Ukrainian selection for two generations

Indicator	Mother generation		Daughter generation	
	K1	K2	K1	K2
Part of the population with the RYR1 _{NN} genotype (n = 59)				
Number of piglets born alive, piglets	-0.024	-0.023	+0.029	+0.034
Litter weight at weaning, kg	-0.008	-0.009	+0.006	+0.015
Part of the population with the RYR1 _{Nn} (n = 4)				
Number of piglets born alive, piglets	+0.421	+0.411	-0.377	-0.499
Litter weight at weaning, kg	+0.088	+0.108	+0.436	+0.354

At the same time, one-way analysis of variance did not reveal significant differences between sows of RYR1_{NN} genotype and sows of RYR1_{Nn} genotype. According to the NBA, the value of F was at the level of 0.163 at a critical value of $F_{0.05} = 3.998$. According to the NW indicator, the value of F was at the level of 0.608 at the critical value of $F_{0.05} = 3.998$. According to LWT/kg, the value of F was at the level of 3.946 at the critical value of $F_{0.05} = 3.998$. It should be borne in mind that the small number of animals of the domestic population of Landrace pigs in Ukraine does not allow us to make reliable conclusions about the impact of individual alleles of genes.

Given that any animal productivity in the final result should be evaluated in economic equivalent, economic indicators were calculated and the cost-effectiveness ratio was determined (Table 4).

Table 4
Economic efficiency of sows' production by the different allelic variants of the RYR1 gene

Indicator	Evaluated population	Part of the population with genotype	
		RYR1 _{NN}	RYR1 _{Nn}
Litters	63	59	4
Net profit from the sale of one litter of pigs, UAH	1988	2059	966
Profitability of raising of the piglets (per litter), %	32.0	33.2	15.6
Cost-effectiveness ratio	-	1.01	0.88

On the strength of the results of the economic efficiency estimate, sows of RYR1_{NN} genotype allow the pig breeder to make more profit per litter compared to the entire evaluated population and to sows of RYR1_{Nn} genotype by 70.50 UAH and 1092.75 UAH respectively. Accordingly this is reflected in profitability. The profitability of the main production is higher by 2.13 times for RYR1_{NN} sows than it is for RYR1_{Nn} sows. The cost-effectiveness ratio is also higher.

Discussion

Recently, the number of studies on small, local and endangered breeds of pigs in Ukraine has significantly decreased. This situation is caused, first of all, by the increase in interest in the industrial production of

pork, which is due to market interests. The influence of imported selection is gradually increasing, the number of Large White and Landrace pigs of foreign selection is growing, and the number of Ukrainian breeds of both directions (meat-fat and meat) of production is decreasing. The downside is the decline in the number of breeds that are adapted to local keeping and feeding conditions and are carriers of valuable genetic information. Accordingly, it is important to assess the genetic variability of such populations, but there is little research in this area. A compromise solution for a partial assessment of the genetic variability of small populations may be the estimation of QTL gene polymorphism. The results of that assessment can be used in further selection work. This direction will help accelerate the rate of increase of productive potential of animals in small populations, accordingly reduce the distance in productivity from industrial breeds and further increase the chances of survival of a number of local populations. Our research was conducted according to this calculation.

Epigenetic variability may be the result of the selection of complex traits associated with metabolic processes or revealed in the process of adaptation to form a response to exogenous stressors. In addition, epigenetic marks of certain loci, particularly the state of DNA methylation, are driven by genetic variability (Ponsuksili et al., 2019). In our research, genetic polymorphism was detected by the ryanodine receptor gene. It was found that 6.4% of sows within the evaluated population of Landrace pigs of Ukrainian selection were carriers of the mutant allele of the RYR1 gene. However, only genotypes without the mutant allele and genotypes with the mutant allele of the RYR1 gene in heterozygous form were detected. It may be due to the fact that animals carrying the mutant allele in homozygous form are more prone to stress. They are inferior to other genotypes from an early age and, consequently, less likely to continue to be used as breeding animals. At the same time, the lack of continuous monitoring of the RYR1 gene leaves a chance for further entry into both the maternal and paternal components of heterozygous animals for this gene. This in turn, can cause the appearance of homozygous animals for the mutant allele RYR1 gene in the herd.

Studies of the effect of the RYR1 gene on growth, fattening and slaughter of pigs (Pietruszka & Sosnowska, 2008) concluded that early identification of animals by this gene will predict the body weight of animals in the initial period. The presence of differences in the sows' reproductive ability (it was found in our studies) suggests that early identification of animals by this gene will allow selection of pigs that will have higher levels of reproductive ability in the future.

In the study of Landrace, Large White and crossbreed animals, RYR1 genotypes partially influenced the physicochemical parameters of meat due to differences in the structural characteristics of the two types of muscle fibers. The role of RYR1 in the management of economic indicators has been noted. It may contribute to a greater economic response and growth of economic profits in pig breeding (Khoa & Huynh, 2013). Our studies also noted the effect of RYR1 on the further economic efficiency of pig breeding. It should also be borne in mind that in the prevailing opinion that the mutant allele of the RYR1 gene in the heterozygous state is not manifested (Ivanov & Guk, 2019) there is also evidence that a heterozygous genotype may also increase the number of animals with PSE meat (Ile et al., 2018).

In comparing our data with the results of research by other scientists conducted on other breeds of pigs, one should note the lack of consensus on the level of reproductive ability in animals with different genotypes of the RYR1 gene. Thus, a number of scientists point to the absence of probable differences in this group of indicators between animals with different allelic variants of this gene (Matoušek et al., 2003; Buczyński et al., 2006; Omelka et al., 2006), obtained opposite data from two different farms, where the predominance of different genotypes of the RYR1 gene was established.

There are also results similar to ours (Ju et al., 2006). Other researchers emphasize the difficulty of conducting a comparative estimation of the productivity of animals with different genotypes. It is due to the small percentage of carrier sows for the mutant allele (Kmieć et al., 2000; Buczyński et al., 2006; Omelka et al., 2006). This has also been the case in our research. In general, our studies confirmed the dependence of the level of reproductive ability of Landrace sows on the presence of a mutant allele of the RYR1 gene in the genotype. Our results indicate that the carrier

sows for the mutant allele of the RYR1 gene had worse reproductive performance compared to sows deprived of this allele.

Regarding the differences in the mechanism of realization of reproductive ability of the sows with different allelic variants of the RYR1 gene, it should be noted that the carriers of the mutant allele of this gene are more prone to stress. The mutant allele of this gene in the homozygous genotype has a significant effect on pig muscle tissue and leads to the production of PSE pork (Ile et al., 2018).

At the same time, sows kept on industrial farms are in a state of temperature stress both during farrowing and during lactation. A sharp rise in ambient temperature above the allowable values disturbs the thermal balance of the animal, causing hyperthermia and, consequently, rapid breathing, excessive urination and hypersalivation.

Decreased reproductive parameters may be observed in overheated sows (Muns et al., 2016; Schou & Malmkvist, 2018). If the carrier sows for the mutant allele are more prone to stress, it may be a prerequisite for reducing their reproductive ability. Regarding the susceptibility of certain breeds of pigs, Pietren, Polish-Chinese pigs, Landrace, Hampshire and Yorkshire are considered to be quite prone to stress while Chester White, Duroc, Large White and Berkshire are considered stress-resistant (Ivanov & Guk, 2019; Sipahelut et al., 2019; Rodríguez et al., 2020). In our studies, only 6.4% of animals carrying the mutant allele of the RYR1 gene were detected, while homozygous animals with the mutant allele of this gene were not detected at all. According to evaluation by the RYR1 gene, Landrace pigs of Ukrainian selection can be attributed to stress-resistant animals.

The results of our studies are similar to those previously obtained by other authors on other breeds of pigs. Thus, homozygous females for the mutant allele of the RYR1 gene were also not detected in the studies of population of Zlotnicka Spotted sows. Also, it is very difficult for authors to draw conclusions about the effect of mutations in the RYR1 gene on the reproductive ability of Zlotnicka Spotted sows due to the small proportion of heterozygotes for the RYR1 gene (Buczyński, 2006).

Given that animals with the RYR1_{nn} genotype were not detected in the evaluated population, and that the frequency of allele n of the RYR1 gene was insignificant and was at the level of 0.03 (it indicates the presence of single carrier animals in the population), there is a need to direct selection work in the herd towards elimination of carriers of the mutant allele of the RYR1 gene.

Eliminating such animals from the population will not only increase the level of sows' reproductive ability in the herd, but also focus further selection work on assessment of the polymorphism of other QTL genes in order to increase the productivity of Landrace pigs of Ukrainian selection.

As other scientists emphasize, there are already populations of pigs free of the mutant allele today. Thus the, unfavourable allele is not present in Latvian White pigs. The rs323041392 in the Latvian White pig has lost variability on the farms over the past nine years (Dokāne et al., 2016). At the same time, these researchers emphasize that the Latvian and European livestock industries need to keep in mind that intensification of reproduction can reduce genetic diversity, specific performance characteristics and adaptability to environmental problems in local breeds in small populations (Dokāne et al., 2016).

Besides, it should be borne in mind that the population of Landrace pigs of domestic selection is limited in number and will require the involvement of genetic material in the form of animals, sperm, etc. to maintain a sufficient heterozygous level. That, in turn, can lead to appearance of carrier sows for the mutant allele of the RYR1 gene. Thus, in Romania, it was introduced by crossing the breed of Mangalitsa pigs (this breed originates from Serbia) with other breeds of pigs by reason of the absence of a mutant allele in the local population of pigs from Central and Eastern Europe (Ile et al., 2018).

Conclusions

The polymorphism of the ryanodine receptor gene of Landrace sows of Ukrainian selection was assessed. The high level of reproductive qualities of the evaluated animals with different allelic variants for this gene was defined with the significant advantage of part of the population with RYR1_{NN} genotype.

The advantage of sows that were free of the mutant allele of the RYR1 gene over its carrier Landrace sows of Ukrainian selection was found by all the evaluated indicators of reproductive ability ($P < 0.05$), except for the number of piglets born alive by the daughter generation (there is no significant difference for NBA of the daughter generation). The advantage of sows free of the mutant allele of the RYR1 gene was also evident by the results of the SIROS-index. At the same time, there were no significant differences in the consolidation level of sows' reproductive ability between sows with different allelic variants in the RYR1 gene. The higher productive level of Landrace sows of Ukrainian selection with RYR1_{NN} genotype is reflected in the indicators of economic efficiency of production. It allows higher levels of profitability to be obtained and net profit to be increased by 1092.75 UAH compared to sows with RYR1_{Nn} genotype.

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