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GENETIC POTENTIAL OF POLTAVA SILVER RABBITS: G-BLUP EVALUATION BASED ON POLYMORPHISM OF MSTN AND PGR GENES

Shevchenko E. A., *candidate of agricultural sciences,*
Honchar O. F., *candidate of agricultural sciences, research associate,*
DzitsiukV. V. *doctor of agricultural sciences, professor*
Cherkassy Experimental Station of Bioresources of NAAS
bioresurs.ck@ukr.net

The article presents the results of the assessment of rabbits of different lines of the Poltava Silver breed using the G-BLUP method with the analysis of the influence of polymorphic variants of the myostatin (MSTN) genes on live weight and progesterone receptor (PGR) on reproductive ability.

The indicators of gene diversity in different lines of the Poltava Silver breed for these polymorphisms showed a positive value of the Wright fixation index (Fis), indicating the predominance of heterozygotes for the C and T alleles. Based on the obtained heterozygosity data, Wright fixation indices were calculated.

The inbreeding coefficient (Fis) for the genes – MSTN and PGR – showed different distributions between rabbit lines. A higher value of Fis for the MSTN gene indicates a greater deficit of heterozygosity, potentially indicating higher inbreeding effects. On the other hand, a lower value of Fis for the PGR gene means smaller inbreeding effects or that the population is closer to panmixia.

Analysis of the influence of the SNP C34T polymorphism in the MSTN gene on the average daily gain of rabbits revealed the following patterns. Heterozygous rabbits with the ST genotype demonstrated higher average daily gains. Their gains were 2.3% higher than those of homozygotes for the C allele (39.0 ± 0.3 g vs. 38.2 ± 0.2 g, $p<0.05$). At the same time, the average daily gains of homozygotes for the T allele were 2.6% lower than those of heterozygotes for the ST (38.2 ± 0.2 g vs. 39.0 ± 0.3 g, $p<0.05$).

It was found that line 1871817 is the most promising in terms of meat productivity, showing the highest values of BLUP-index (1358), estimated breeding value (EBV, 1.412) and reliability (REL, 1.827), which confirms its high genetic potential. Lines 1847213 and 1832221, on the contrary, have small negative EBV and low REL, which indicates their lower productivity. The G-BLUP method also confirmed the influence of the PGR gene on reproductive traits. Lines 1871817 and 1811231 showed the highest BLUP-index (5.57) for these indicators, which indicates their high potential for transmitting reproductive potential to offspring. In general, the line 1871817 demonstrates high genetic potential both in terms of live weight and the number of weaned rabbits, which makes it a priority in the selection of the best individuals for future breeding programs in rabbit breeding.

Keywords: *rabbits, G-BLUP, genes, myostatin, progesterone receptor, Poltavska Silver breed.*

Introduction. Enhancing the genetic potential of livestock populations, particularly rabbits, is a key objective in modern breeding programs. This is primarily achieved through the strategic selection and use of animals with high genetic value. However, accurately predicting the true genetic characteristics of quantitative and qualitative traits – especially those influenced by complex polygenic effects – remains a significant challenge for breeders [1, 2].

Traditionally, the Best Linear Unbiased Prediction (BLUP) method – often referred to as pedigree-BLUP – has been widely used to evaluate the breeding value of animals. This method utilizes genealogical data (pedigree information) to construct a relationship matrix [3]. Although pedigree-BLUP is effective, it has limitations, as it considers only the expected relatedness based on pedigree rather than the actual genetic similarity realized in the inherited genes.

Modern genetic evaluation is evolving with the introduction of the Genomic Best Linear Unbiased Prediction (G-BLUP) method [4]. Unlike traditional BLUP, G-BLUP uses a G-matrix, which is calculated based on DNA similarity between animals using a large number of genetic markers (e.g., SNPs). This provides a more accurate representation of actual genetic relatedness and allows for the estimation of breeding value at early stages of ontogeny, even before the traits are expressed [5]. Today, these methods are widely applied to assess various types of livestock species [6, 7].

In rabbit breeding, the G-BLUP method has recently been actively developed, particularly through the integration of information on the effects of quantitative trait loci (QTL) associated with economically important traits [8 – 16]. This comprehensive genetic evaluation aims to improve breeding efficiency by accounting for both QTL effects and additive polygenic effects [17]. In G-BLUP models, the parameters typically include the overall value of fixed effects (such as the influence of a genotype at a specific locus) and the random additive genetic effect from polygenic loci, enabling the consideration of marker-associated effects.

The integration of gene polymorphism dataparticularly for genes associated with key economic traitssignificantly enhances the capabilities of G-BLUP evaluation. This approach enables a more comprehensive assessment of animals by taking into account not only genetic polymorphism and gene expression, but also non-genetic factors such as environmental or paratypic influences.

In the context of improving rabbit productivity, particular attention is given to genes influencing meat and reproductive traits, specifically the myostatin and progesterone receptor genes.

The myostatin gene (MSTN) plays a key role in shaping meat productivity traits in rabbits. It is located on chromosome 7, belongs to the transforming growth factor beta (β-TGF) family, and is involved in the acceleration of skeletal muscle development and differentiation [18].

The progesterone receptor gene (PGR) is important for the reproductive capacity of rabbits. This gene encodes a protein that is part of the superfamily of intracellular receptors and plays a crucial role in mediating the effects of steroid hormones, particularly progesterone. The rabbit PGR gene is located on chromosome 1 and spans 71,875 base pairs (GenBank NC_013669). A substitution

of A→G in the promoter region of the PGR gene (position 2464) has been identified and is associated with reproductive traits in female rabbits [19].

Considering the limited number of studies in Ukrainian rabbit breeding on G-BLUP evaluations using molecular genetic markers, the aim of our research is to assess different lines of Poltava Silver rabbits with an analysis of the influence of polymorphic variants of the myostatin (MSTN) and progesterone receptor (PGR) genes on economically important traits.

Materials and methods. For the study, blood samples were collected from the ear vein of Poltava Silver rabbits (n=500). EDTA or sodium citrate were used as anticoagulants. DNA extraction was performed using the commercial DNA-sorb-B nucleic acid extraction kit (AmpliSens).

Genotyping of individuals was carried out using the PCR-RFLP method (polymerase chain reaction with restriction fragment length polymorphism analysis).

For amplification, specific primers were used for each locus (table 1).

Table 1. Sequences of used primers for genotyped rabbits by PCR-RFLP

Locus	Primer	Nucleotide structure	Source
MSTN (C34T)	F	AATTTTGCTTGCCATTACTGA	Fontanesi et al., 2008 [15]
	R	TCAGCAGAACTGTTGACATACAC	
PGR (G2464A)	F	GAAGCAGGTCATGTCGATTGGAG	Peiró et al.,2010 [14]
	R	CGCCTCTGGTGCCAAGTCTC	

Amplification was carried out using protocols specific to each locus. The following amplification protocol was used: one initial cycle of denaturation at 95°C for 5 minutes; 34 cycles of denaturation at 95°C for 30 seconds, annealing at 66°C for 60 seconds, and elongation at 60°C for 60 seconds; followed by a final elongation step at 72°C for 5 minutes.The total reaction volume for amplification of both genes was 20 µL, with a primer concentration of 0.2 µM.

Restriction of the MSTN gene fragments was performed using the AluI restriction endonuclease (Fermentas, Lithuania), while for the PGR gene, Eco31I (Thermo Fisher Scientific, USA) was used. The restriction products were separated in a 2.5% agarose gel stained with ethidium bromide at 110 V for 60 minutes. Visualization of DNA fragments in the gel was carried out under ultraviolet light. To determine the size of the restriction fragments, the GeneRuler 100 bp molecular weight marker (Thermo Fisher Scientific) was used. The structure of the BLUP evaluation for male rabbits was based on the following mixed model equation:

$$y=Xb+Zu+e$$

y – vector of observations of dimension *N*;
b – vector of fixed effects of dimension *p*;
u – vector of random effects of dimension *q*;
e – vector of residual (random) errors of dimension *N*;
X – design matrix for fixed effects;
Z – design matrix for random effects.

The reliability (REL) of the BLUP estimates was calculated using the formula:

$$\text{REL} = \frac{n}{n + \frac{4}{h^2} - 1}$$

where:
n – number of offspring of the male rabbit;
h² – heritability coefficient.

To assess the genetic potential of the animals, Estimated Breeding Values (EBV) were used. EBVs were calculated using the BLUP method, taking into account pedigree and phenotypic data for traits such as live weight and the number of weaned kits.

Statistical analysis of the data was performed using Statistica v.10, GenStat v.11, and the BLUPF90 software package [18, 19]. Population-genetic parameters were calculated using the Popgene v.1.32 software [20].

Results of research. The analysis of the population-genetic structure of the Poltava Silver rabbit herd based on polymorphic variants of the MSTN and PGR genes revealed the following.Genetic diversity indicators across different lines of the Poltava Silver breed for these polymorphisms showed a positive value of Wright's fixation index (Fis), indicating a predominance of heterozygotes for the C and T alleles (Table 2).

Table 2. Gene diversity indicators of rabbits from different lines of the Poltava Silver breed for polymorphisms C34T `of the MSTN gene and G2464A of the PGR gene (n=500)

Gene	Line							
	Indicator	1871817	1847213	1811231	1894136	1832221	1811231	1941524
MSTN	Ho	0,481	0,472	0,474	0,480	0,473	0,481	0,476
	He	0,519	0,528	0,526	0,520	0,527	0,519	0,524
PGR	Ho	0,495	0,494	0,489	0,495	0,494	0,490	0,493
	He	0,505	0,506	0,511	0,505	0,506	0,510	0,507
Count of animals		120	89	94	104	114	117	108

Based on the obtained heterozygosity data, Wright's fixation indices were calculated. Their distribution in the Poltava Silver rabbit population for the MSTN and PGR genes is presented below (Figure 1).

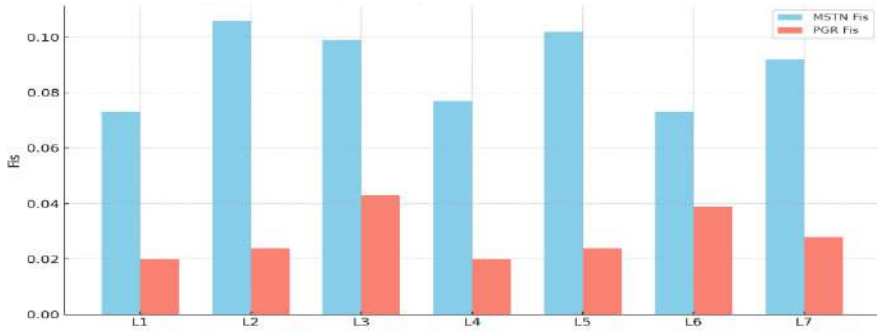

Fig. 1. Distribution of Wright's fixation index based on heterozygosity indicators of polymorphic variants of the MSTN and PGR genes in Poltava Silver rabbits

The inbreeding coefficient (Fis) for both genes – *MSTN* and *PGR* – showed different distributions across rabbit lines. A higher Fis value for the *MSTN* gene indicates a greater deficiency of heterozygosity, potentially pointing to stronger inbreeding effects. In contrast, a lower Fis value for the *PGR* gene suggests weaker inbreeding effects or that the population is closer to panmixia.

The analysis of the impact of SNP C34T polymorphism in the *MSTN* gene on the average daily gain in rabbits revealed the following patterns. Heterozygous rabbits with the CT genotype exhibited higher average daily gain values. Their gains were 2.3% greater than those of homozygous individuals for the C allele (39.0 ± 0.3 g vs. 38.2 ± 0.2 g, $p<0.05$). Meanwhile, the average daily gain in homozygous individuals for the T allele was 2.6% lower compared to heterozygotes (38.2 ± 0.2 g vs. 39.0 ± 0.3 g, $p<0.05$).

Subsequently, in the G-BLUP evaluation, polymorphism data for rabbits were taken into account for SNP C34T of the *MSTN* gene (allelic variants CT, CC, TT) and SNP G2464A of the *PGR* gene (allelic variants GA, GG, AA).

It was established that, according to the G-BLUP evaluation for the *MSTN* gene, the rabbit line 1871817 had the highest BLUP index value (1358), EBV (1.412), and REL (1.827), indicating its high breeding value and accuracy of estimation (Table 3). In contrast, the rabbit lines 1847213 and 1832221 showed negative EBV values and low REL, suggesting lower productivity and less reliable evaluations. Lines 1811231 and 1894136 demonstrated variable results, which may indicate within-line variability or reflect repeated evaluations of different individuals within the same lineage.

According to the G-BLUP evaluation of Poltava Silver rabbits based on polymorphic variants of the progesterone receptor gene and the number of weaned kits, lines 1871817 and 1811231 demonstrated the highest BLUP index (5.57), indicating potentially high reproductive efficiency (Table 4).

Table 3. BLUP evaluation of male rabbits of different lineages based on polymorphic variants of the myostatin gene and live weight (n=330)

Line	BLUP index	EBV	REL
1871817	1358	1.412	1.827
1847213	1282	-0.827	-1.070
1811231	1210	0.398	0.514
1894136	1210	0.645	0.834
1832221	1282	-0.629	-0.814
1811231	1356	-0.369	-0.477
1941524	1282	-0.629	-0.814

Table 4. BLUP evaluation of female rabbits of different lineages based on polymorphic variants of the progesterone receptor gene and number of weaned kits (n = 170).

Line	BLUP index	EBV	REL
1871817	5.57	0.0042	0.588
1847213	4.55	-0.015	-2.157
1811231	3.57	0.0042	0.588
1894136	3.57	0.645	0.588
1832221	4.58	0.0042	0.588
1811231	5.57	0.0042	0.588
1941524	4.56	-0.0056	-0.78

EBV values for most rabbit lines showed low variability, which may indicate a weak or unstable correlation between genotype and litter size. REL for the majority of lines was 0.588, representing a moderate level of reliability. At the same time, lines 1847213 and 1941524 exhibited negative REL values, likely due to the small number of animals studied.

Conclusions. A population-genetic analysis of molecular polymorphisms in the *MSTN* (C34T) and *PGR* (G2464A) genes was conducted in rabbits from different lines of the Poltava Silver breed. The application of the G-BLUP method demonstrates genetic variability among lines for traits such as live weight and the number of weaned kits. This creates opportunities for selecting animals with superior genetic characteristics for further breeding.

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ГЕНЕТИЧНИЙ ПОТЕНЦІАЛ КРОЛІВ ПОЛТАВСЬКЕ СРІБЛО: G-BLUP ОЦІНКА НА ОСНОВІ ПОЛІМОРФІЗМУ ГЕНІВ *MSTN* ТА *PGR*

Шевченко Є. А., Гончар О. Ф., Дзіцюк В. В.
Черкаська дослідна станція біоресурсів НААН

У статті представлені результати оцінки кролів різних ліній породи полтавське срібло методом G-BLUP з аналізом впливу поліморфних варіантів генів міостатину (MSTN) на живу масу та прогестеронового рецептора (PGR) на відтворну здатність.

Показники генного різноманіття в різних лініях породи полтавське срібло за цими поліморфізмами показали позитивне значення індексу фіксації Райта (Fis), що вказує на переважання гетерозигот за алелями C і T. На основі отриманих даних гетерозиготності розраховані індекси фіксації Райта.

Коефіцієнт інбридингу (Fis) для генів – MSTN та PGR – показав різні розподіли між лініями кролів. Вище значення Fis для гена MSTN свідчить про більший дефіцит гетерозиготності, потенційно вказуючи на вищі ефекти інбридингу. Натомість, нижче значення Fis для гена PGR означає менші ефекти інбридингу або те, що популяція ближча до панміксії.

Аналіз впливу поліморфізму SNP C34T у гені MSTN на середньодобовий приріст кролів виявив такі закономірності. Гетерозиготні кролі з генотипом CT демонстрували вищі показники середньодобових приростів. Їхні прирости були на 2,3% більшими, ніж у гомозигот за алелем C (39,0 ± 0,3 г проти 38,2 ± 0,2 г, $p<0,05$). Водночас, значення середньодобових приростів у гомозигот за алелем T було на 2,6% нижчим, ніж у гетерозигот CT (38,2 ± 0,2 г проти 39,0 ± 0,3 г, $p<0,05$).

Встановлено, що лінія 1871817 є найперспективнішою за м'ясною продуктивністю, показуючи найвищі значення BLUP-індексу (1358), оціненої племінної цінності (EBV, 1.412) та надійності (REL, 1.827), що підтверджує

її високий генетичний потенціал. Лінії 1847213 та 1832221, навпаки, мали негативні EBV та низький REL, що свідчить про їхню нижчу продуктивність. Методом G-BLUP також підтверджено вплив гена PGR на репродуктивні ознаки. Лінії 1871817 та 1811231 показали найвищий BLUP-індекс (5.57) для цих показників, що свідчить про їхній високий потенціал передачі потомкам потенціалу репродуктивної здатності. Загалом, лінія 1871817 демонструє високий генетичний потенціал як за живою масою, так і за кількістю відлучених кроленят, що робить її пріоритетною у добору кращих особин для майбутніх селекційних програм у кролівництві.

Ключові слова: *кролі, G-BLUP, гени, міостатин, прогестероновий рецептор, порода полтавське срібло.*