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GENOTYPING OF NEW ZEALAND WHITE RABBITS BY PCR-RFLP MARKERS

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Aim. To investigate the genetic structure of New Zealand white rabbits population by different types of DNA-markers. **Methods**. The individual genotypes of animals were identified using the polymerase chain reaction with further determination of the restriction fragment length polymorphism (RFLP-analysis). **Results.** The data on the distribution of allele variants of molecular markers in the population of rabbits were obtained; the impact of the genotype factor on meat production, prolificacy and milk production traits was determined. The relationship between genotypes by polymorphic DNA-markers of myostatin and progesterone receptor of animals and the values of meat productivity traits and reproduction capability was established. It was demonstrated that TT homozygotes excel other animals in the indices of average daily growth, while GG homozygotes excel others in prolificacy. **Conclusions.** The "desired" genotypes by myostatin gene (CC) and myostatin of rabbits were revealed. They may be used for targeted selection with the purpose of increasing the indices of meat production.

Key words: New Zealand white rabbit, myostatin, progesterone receptor, production.

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INTRODUCTION

Most economically valuable traits of farm animals, which are of interest for selection, are quantitative. At present there are several key genes, identified in rabbit breeding, which determine the formation of quantitative traits [1, 2]. The significance of their contribution is regulated by the environment. Myostatin (MSTN) was defined among individual genes, the polymorphism of which determines the impact on the manifestation of phenotypic traits, related to meat production and reproduction, noteworthy for reproductive capability of rabbits are progesterone receptor (PGR), uteroglobulin (SCGB1A1), insulin-like growth factor 1 (IGF1) and tissue inhibitor of metalloproteinase 1 (TIMP1) [3–5].) Myostatin and progesterone receptor are the most wide-spread among the quantitative trait loci (QTL).

Myostatin, also known as GDF8, is a member of the family of the transforming growth factor (TGF)-β, participating in the inhibition of the development of skeletal muscles of animals [4–7]. The mutations in the myostatin gene are known for cattle; they cause sharp muscle gains of the livestock. A similar mechanism of double muscling was also established for mice and dogs [8]. In rabbits the length of the nucleotide sequence of myostatin gene is 4,912 base pairs (bp). located on chromosome 7. The mutation, manifested in the transition of C→T in intron 2, was first described by Fontanessi's group [4].

Progesterone receptor is a member of the superfamily of intracellular receptors; its physiological role being the binding of steroid hormones [5, 9, 10]. PGR gene is 297,639 bp and is located on chromosome 11. It is remarkable for polymorphism in two alleles: G and A (position 2464) in the promotor region [3].

It should be noted that the issues of determining the allelofund, estimating the genetic situation – the level of consolidation and differentiation of different rabbit breeds are solved on the population level. The population genetic monitoring also provides information on the distribution and flow of hereditary material in generations, genetic regularities in micropopulations. RAPD (random amplified polymorphic DNA) as well as ISSR DNA-markers are used in the study of genetic diversity and classification of rabbits [11−14].

The application of the mentioned molecular markers in the analysis and selection of valuable genotypes of animals is relevant for the intensification of the selective process in rabbit breeding.

MATERIALS AND METHODS

The study was conducted using New Zealand white rabbits (n = 130), bred at a rabbit farm of the agricultural private enterprise Marchuk N. V. (Tashlyk village, Smila District, Cherkasy Region). Blood samples were taken from the auricular vein and placed into 1 ml polyethylene test-tubes (Eppendorf, Germany), containing 200 µl of 3.8 % sodium citrate solution. The genomic DNA was isolated from blood using the standard method [5] with the DNA-sorb B set (Amplisense, RF) according to the manufacturer's recommendations.

The nucleotide sequences and restrictases, indicated in Table, were used to conduct the polymerase chain reaction in order to determine the polymorphism of myostatin and progesterone receptor.

The mixture for PCR of the genes of myostatin and progesterone receptor contained 1 × buffer for DNA-polymerase, 200 µmol of the mixture of triphosphates (Amplisense), 0.5 µmol of the corresponding primer, 0.6 u. a. of DNA-polymerase (Fermentas, Lithuania). Genomic DNA was added in the amount of 50 ng. The total volume of PCR-volume was 25 µl. The genomic DNA of rabbits with primers to myostatin was amplified using the programmed four-channel thermocycle Tertsik (DNA-technology, RF) in the following conditions: 180 s − denaturation at 95 °C ("hot start"); 30 s − denaturation (95 °C); 30 s − annealing of primers (60 °C); 30 s − elongation (72 °C); 10 min − synthesis (72 °C) − 40 cycles. DNA with primers to the progesterone receptor were amplified in the following conditions: 5 min − denaturation (95 °C) ("hot start"); 30 s − denaturation (95 °C) − 34 cycles; 30 s – annealing of primers (66 °C); 60 s – elongation (72 °C); 5 min – pre-synthesis (72 °C). The amplification products of genes *MSTN* and *PGR* were cleaved using endonucleases Eco31I and AluI respectively (Fermentas) at 37 °C for 12−16 h. The restriction fragments were separated in 2 % agarose gel (Helicon, RF) and stained with ethidium bromide.

DNA, obtained during the PCR or as a result of amplification product restriction, were measured using the molecular mass marker O'GeneRuler™ DNA Ladder Mix (Fermentas), 3,000 bp long.

The electrophoregrams were visualized using the transilluminator in the ultraviolet light. After the electrophoresis the gel was photographed using the professional digital camera. The size of the amplified fragments was defined using TotalLab 2.01 software.

The meat production and reproductive capability of rabbits were revealed using the data of zootechnical accounting according to the Instruction on evaluation of rabbits.

The statistical processing of the data was performed using standard methods and MS Excel, GenAlEx 6.0, STATISTICA 6.0 software.

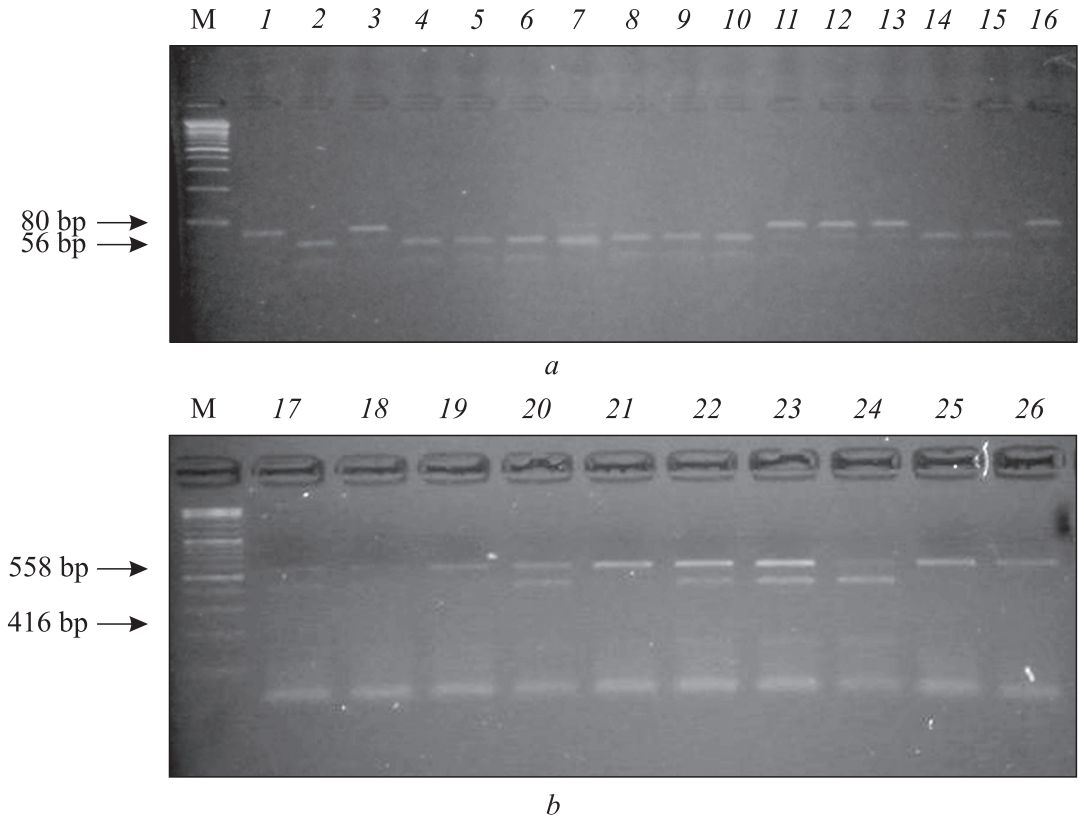
RESULTS AND DISCUSSION

The DNA-fragments of polymorphic variants of the genes of myostatin and progesterone receptor of New Zealand white rabbits are presented in Figure.

The following distribution of alleles C34 and 34T of gene *MSTN* (frequencies p and q) was obtained: for

The genotyping of New Zealand white rabbits by the genes of myostatin and progesterone receptor using the PCR-RFLP method

Gene	Primer	Restrictase
Myostatin [4]	Forward: 5′-TAACTGAAAAGAACCCTCTAGTAGC-3′ Reverse: 5′-TCGGTAGTTGTTTCCCACTTT-3′	AluI
Progesterone receptor [3]	Forward: 5′-GAAGCAGGTCATGTCGATTGG AG-3′ Reverse: 5′-CGCCTCTGGTGCCAAGTCTC-3′	Eco31I



The electrophoregram of restriction products of the amplified fragments of the genes of myostatin (*a*) and progesterone receptor (*b*) of New Zealand white rabbits. Gel electrophoresis of PCR products, hydrolyzed with restriction endonucleases AluI (*a*) and Eco31I (*b*), in 3 %- and 2 % agarose gel, respectively; M – molecular marker O'geneRuler^{TM} DNA Ladder Mix, ready-to-use (Fermentas); *2*, *4–10*, *14–16* – homozygotes by allele T; *1*, *3, 11–13* – heterozygotes CT; *17–20*, *22–24* – heterozygotes GA; *21*, *25*, *26* – homozygotes by allele A

males of New Zealand white rabbits p^{MSTN} – 0.500; q^{MSTN} – 0.500; for females p^{MSTN} – 0.457; q^{MSTN} – 0.543. The distribution of alleles G2464 and 2464A of gene *PGR* was somewhat different: for males p^{PGR} – 0.500; q^{PGR} – 0.500; for females p^{PGR} – 0.433; q^{PGR} – 0.567. There was no statistically relevant difference revealed in the distribution of alleles of myostatin gene between males and females of New Zealand white rabbits ($df = 1$; $\chi_{st}^2 = 3.84$; $\chi_f^2 = 0.007$; $p < 0.05$).

The data obtained are somewhat different from the results, presented by Markowska A. *et al*. [7]. Polish researchers established that the frequency of allele C for the New Zealand white rabbits was 0.618, and the frequency of allele T – 0.382. While genotyping the Polish Hermelin by the polymorphic variants of myostatin gene they had the following values: the frequency for allele C was 0.558, and that for allele T – 0.442.

The analysis of the actual and estimated distribution of the genotypes of New Zealand white rabbits by polymorphic variants of myostatin gene demonstrated that the population structure corresponds to Hardy-Winberg equation ($df = 2$; $\chi_{st}^2 = 5.99$; $\chi_f^2 = 0.034$; $p > 0.05$). It was established that the structure of the population by the polymorphic variant G2464A of progesterone receptor gene also corresponds to Hardy-Weinberg equation ($df = 2$; $\chi_{st}^2 = 5.99$; $\chi_f^2 = 0.018$; $p > 0.05$).

Taking into account the fact that polymorphic variants of the investigated genes are not bound, the equilibrium of the analyzed selection may be expected for all the independent combinations. However, the data of the analysis of the distribution series for genotypes of New Zealand white rabbit females allowed determining statistically relevant difference between estimated and actual frequencies ($df = 8$; $\chi_{st}^2 = 20.09$; $\chi_f^2 = 20.65$; $p > 0.01$). The selection of animals demonstrates the deviation from equilibrium by the combinations of alleles of the analyzed genes. Such deviations may testify to the fact that the carriers of some genotypes are of selective advantage, while those of others – vice versa, of decreased adaptivity and viability and thus are due to the selection impact.

The study of the effect of eimeriosis epidemic on the distribution of genotypes in the population of rabbits demonstrated that the ratio of genotypes of animals has a statistically relevant deviation from the estimated one ($df = 2$; $\chi_{st}^2 = 13.82$; $\chi_f^2 = 79.05$; $p > 0.001$). The share of animals-carriers of allele T, conditioning the impairment of the functional activity of myostatin, decreased almost thrice.

It is known that in the course of inflammatory processes in liver the rabbits, affected by eimeriosis, also have other symptoms, including paralysis of limbs and cervical muscles, and convulsions [15]. The animals-carriers of allele T, which have inhibited growth and differentiation of muscles, may have less resistance to infectious diseases with this pathogenesis. From this standpoint allele C and genotype CC have a selective advantage compared to allele T and genotypes CT and TT.

The results of the single-factor analysis of variance demonstrated the impact of the genotype of New Zealand white rabbit by myostatin gene on the manifestation of average daily growth ($\eta^2 = 0.45$; $p > 0.05$) and on the weight of a fresh animal ($\eta^2 = 0.35$; $p > 0.05$).

The share of the impact of the genotype of New Zealand white rabbit by *MSTN* gene on the manifestation of a phenotypic feature of feed-gain relationship relative to 1 kg of gain for the period of 60–120 days was 24 %, but it was proven unreliable ($p < 0.05$).

The data of the analysis of average daily growth of males and females of New Zealand white rabbit testify to the regularities of the distribution of these traits among the animals with different genotypes. For instance, by myostatin gene the heterozygous males with genotype CT exceeded the homozygote by allele C in the average daily growth by 2.3 % (39.0 ± 0.3 against 38.2 ± 0.2 g; $t_f = 2.3$; $t_{st} = 2.09$; $df = 20$; $p < 0.05$). The values of the average daily growth for males with genotype TT was lower by 2.6 % than that for heterozygous animals (38.0 ± 0.3 against 39.0 ± 0.3 g; $t_f = 2.33$; $t_{st} = 2.13$; $df = 15$; $p < 0.05$).

There was a similar regularity in the phenotypic manifestation of the average daily growth among females of New Zealand white rabbit by the polymorphic variants of gene *PGR*. Breeding female rabbits with genotype CT had higher indices of average daily growth compared to homozygotes by allele C by 7.3 % (38.6 ± 0.3 against 35.8 ± 0.2 g; $t_f = 2.53$; $t_{st} = 1.99$; $df = 97$; $p < 0.05$). The animals with genotype TT were characterized by lower average daily growth compared to heterozygotes CT (37.2 ± 0.3 against 38.6 ± 0.3 g; $t_f = 3.23$; $t_{st} = 2.66$; $df = 78$; $p < 0.01$).

The results of the single-factor dispersion analysis testify to the impact of the genotype of breeding female rabbits by gene of the progesterone receptor on their milk production, but there was no statistically relevant difference between the groups of animals with different genotypes ($n = 60$; $\eta^2 = 0.35–0.43$; $p < 0.05$). Contrary to the results of our studies, Peiro R. *et al*. [5] established that the expression of polymorphic variants of the progesterone receptor gene, which conditions the formation of different isoforms of the receptor protein, has its impact on the development of rabbit embryos (94–100 %, $p < 0.05$ with the consideration of Monte-Carlo method).

CONCLUSIONS

Genotypes CT by myostatin gene (47.1 %) and GA by progesterone receptor gene (50 %) were most frequently observed for the New Zealand white rabbits.

There are reliable differences between the genotypes of New Zealand white rabbits by myostatin gene regarding the manifestation of phenotypic traits: 2.5 % ($p < 0.05$) of average daily growth.

The genotyping of rabbits by polymorphic variants C34T of myostatin gene and G2464A – the progesterone receptor gene serves as a foundation for the introduction of molecular and genetic expertise in rabbit breeding with the purpose of further selection of animals of different genotypes with maximal realization of their genetic potential.

Генотипування кролів новозеландської білої породи за ПЛР-ПДРФ маркерами

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Мета. Дослідження генетичної структури популяції кролів новозеландської білої породи за різними типами ДНК-маркерів. **Методи.** Індивідуальні генотипи тварин ідентифікували методом полімеразно-ланцюгової реакції з подальшим визначенням поліморфізму довжин рестриктних фрагментів (ПДРФ-аналіз). **Результати.** Отримано дані з розподілу алельних варіантів молекулярних маркерів у популяції кролів та визначено вплив фактора

генотипу на показники м'ясної продуктивності, багатоплідності і молочності. Встановлено взаємозв'язок між генотипами за поліморфними ДНК-маркерами міостатину і прогестеронового рецептора тварин та значеннями показників м'ясної продуктивності і відтворювальної здатності. Показано, що гомозиготи ТТ випереджають інших тварин за показниками середньодобових приростів, а гомозиготи GG – за багатоплідністю. **Висновки.** Виявлено «бажані» генотипи за геном міостатину кролів (СС), які можуть бути використані за цілеспрямованої селекції для підвищення показників м'ясної продуктивності.

Ключові слова: новозеландська біла порода кролів, міостатин, прогестероновий рецептор, продуктивність.

Генотипирование кролей новозеландской белой породы за ПЦР-ПДРФ маркерами

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Цель. Исследование генетической структуры популяции кролей новозеландской белой породы по разным типам ДНК-маркеров. **Методы.** Индивидуальные генотипы животных идентифицировали методом полимеразной цепной реакции с последующим определением полиморфизма длин рестрикционных фрагментов (ПДРФ-анализ). **Результаты.** Получены данные по распределению аллельных вариантов молекулярных маркеров в популяции кролей и определено влияние фактора генотипа на показатели мясной продуктивности, многоплодия и молочности. Установлена взаимосвязь между генотипами по полиморфным ДНК-маркерам миостатина и прогестеронового рецептора животных и значениями показателей мясной продуктивности и воспроизводительной способности. Показано, что гомозиготы ТТ опережают других животных по показателям среднесуточных привесов, а гомозиготы GG − по многоплодию. **Выводы.** Обнаружены «желаемые» генотипы по гену миостатина кролей (СС), которые можно использовать при проведении целенаправленной селекционной работы для повышения показателей мясной продуктивности.

Ключевые слова: новозеландская белая порода кролей, миостатин, прогестероновый рецептор, продуктивность.

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