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MOLECULAR-GENETIC ANALYSIS AS COMPONENT OF ORGANIZATION SELECTION PROCESS IN RABBIT BREEDING

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Presents results of genetic certification New Zealand White, Silver and Californian rabbits breeds by$(GA)_9C$, $(AG)_9C$, $(ACC)_6C$, and $(GAG)_6$CISSR- markers.Analysis of genetic structure New Zealand White breed rabbits is carried out by C34T gene polymorphism of myostatin gene and G2464A progesterone receptor. Set influence of rabbits genotype by MSTN and PGR gene to expression economically useful traits: average daily gain, fertility, differential adaptation to infectious diseases. Based on these studies suggested usy of integral genomic and BLUP evaluation of rabbits which is basis for determining the genetic potential of animals and forecast productive qualities offspring.

Key words: **rabbits, breed, DNA markers, genes polymorphism, economically important traits, genetic diversity**

МОЛЕКУЛЯРНО-ГЕНЕТИЧНИЙ АНАЛІЗ ЯК КОМПОНЕНТ ОРГАНІЗАЦІЇ СЕЛЕКЦІЙНОГО ПРОЦЕСУ В КРОЛІВНИЦТВІ

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Представлені результати генетичної паспортизації кролів новозеландської білої, сріблястої та каліфорнійської порід за (GA)9C, (AG)9C, (ACC)6C, (GAG)6C ISSR-маркерами. Проведений аналіз генетичної структури кролів новозеландської білої породи за поліморфізмами C34T гена міостатину та G2464A прогестеронового рецептора. Встановлений вплив генотипу кролів за генами MSTN та PGR на прояв господарськи корисних ознак: середньодобовий приріст, багатоплідність. На основі проведених досліджень запропоновано інтегральне використання геномної і BLUP оцінки кроликів, яке є основою для визначення генетичного потенціалу тварин і прогнозу продуктивних якостей потомства.

Ключові слова: **кролі, порода, ДНК-маркери, поліморфізм генів, господарськи корисні ознаки, генетичне різноманіття**

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЙ АНАЛИЗ КАК КОМПОНЕНТ ОРГАНИЗАЦИИ СЕЛЕКЦИОННОГО ПРОЦЕССА В КРОЛИКОВОДСТВЕ

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Представлены результаты генетической паспортизации кроликов новозеландской белой, серебристой и калифорнийськой пород по $(GA)_9C$, $(AG)_9C$, $(ACC)_6C$ и $(GAG)_6C$ ISSR-маркерам. Проведён анализ генетической структуры кроликов новозеландской белой породы по полиморфизмам C34T гена миостатина иG2464A прогестеронового рецептора. Установлено влияние генотипа кроликов по генам MSTN и PGR на проявление хозяйственно полезных признаков: среднесуточный привес, многоплодие. На основе проведенных исследований предложено интегральное использование геномной и BLUP оценки кроликов, которое является основой для определения генетического потенциала животных и прогноза продуктивных качеств потомства.

Ключевые слова: **кролики, порода, ДНК-маркеры, полиморфизм генов, хозяйственно полезные признаки, генетическое разнообразие**

Introduction. Rabbits husbandry – one of the most promising sectors of livestock, which is characterized by rapid reproduction cycle descendants [1].

Improving the efficiency of breeding rabbits largely due to the integrated assessment of genotype and important role was played by DNA markers [2].

By using molecular genetic markers investigate genetic heterogeneity of rabbit populations, establishe genetic relationship and divergence of rabbits populations from different breeds [3]. In order to enhance genetic evaluation of breeding material based on the genetic mechanisms that lead to differences animals on the basis of performance used quantitative traits loci of rabbits. This is achieved by analyzing of genotype sires by genotyping for different types of DNA markers [4]

A vital part of improving herd of rabbits both tribal and productive qualities is the creation and maintenance of genetic potential genealogical structures [5]. This approach provides selection of animals based on their genotype by DNA markers while maintaining the linear structure of the herd.

It should be noted that research in the area of rabbits molecular genetics in Ukraine were not conducted. Thus, obvious need to analyze genetic structure of rabbits from different breeds at the individual and at the population level by DNA markers for selection process intensification.

Materials and methods of research. Research conducted at the rabbit farm "Marchuk N.V." (v. Tashlyk, Smilyanskiy district, Cherkassy region), Department of Genetics of Institute Animal

Breeding and Genetics nd. a. M. V. Zybets NAAS and Cherkassy research station of bioresources NAAS during 2010-2014 years.

For research was formed group of rabbits Silver breed (40 goals), New Zeland White (250 goals) and Californian (50 голів). The original parent stock of New Zealand rabbits breed white males age accounted for tribal use – 2 years and females of all ages (after the first - fifth pregnancy). For feeding rabbits on the farm using granulated feed that contained a concentrated feed, grass meal, feed additives of animal origin, minerals and premixes. Indoor rabbit farm support optimal microclimate parameters (constant temperature, relative humidity, air velocity). Lighting was artificially with a duration of 16 hours.

Blood of rabbits for research selected from the ear vein disposable syringe or vacuum system Vacutainer type of EDTA or sodium citrate. DNA isolation from blood were performed using standard commercial set «DNA Sorb B».

Molecular genetic analysis of and progesterone receptor myostatin gene polymorphism conducted under Fontanessi L., 2008 and Argente M., 2010 [5, 6]. For event PCR-RFLP analysis we using primers of nucleotide motifs: $(AG)_9C$, $(GA)_9C$, $(ACC)_6G$, $(GAG)_6C$Electrophoretic separation of DNA fragments restryktnyh conducted in 2 % and 3 % agarose gel in Tris-borate electrophoresis buffer. Visualization of the results was performed on the transilyuminator (UV light) at a wavelength of 300 nm after ethidium bromide staining of the gel.

In the statistical analysis we used the assessment division each feature based on the Kolmogorov-Smirnov criterion of normality. To assess the degree of influence of genotype on the expression of economically useful traits using single-factor analysis of variance (ANOVA). In case of deviation from normal distribution features we used Kruskal-Wallis test [7].

For features that are not subjected to normal distribution, heritability coefficient was determined as twice the Spearman correlation coefficient breeding traits of parents and offspring.

To determine the genetic diversity of different breeds of rabbits we used cluster analysis.

The structure of BLUP evaluation of male rabbits we used mixed model of equations [8]

$$y = Xв + Zu + e,$$

where y - the vector of observations dimension N; a - vector of fixed effects of dimension p; u - vector randomized effects of dimension q; e - vector of random effects dimension N; X - matrix of the fixed effects coefficients; Z - matrix of the coefficients of randomized effects.

Statistical analysis of data was performed using Statistica v.10, GenStat v.11, BLUPF90. To calculate population-genetic parameters we used computer program Popgene v. 1.32 and specialized macross GenAlEx 6.5 to Microsoft Office Excel.

Results of research. Analysis of the rabbit genetic structure by ISSR-markers has the following features. Using marker $(AG)_9C$ heterozygosity (H) for species averaged 0.247. As a result of amplification of genomic DNA from rabbits by $(GA)_9C$ marker, H index value was slightly higher - 0.293, and the marker $(ACC)_6G$ - 0,295. It should be noted that with polymorphic loci for three breeds of rabbits were identified breed specific fragments that are important for genetic examination of the origin of breeding animals (table. 1). Most of breed specific fragments by ISSR-markers present in New Zealand White rabbits breed and the least – in the rabbits of Silver breed.

For the New Zealand White rabbit breed polymorphism share was the highest (87,6 %), for the Californian species – at 12,1% lower, while the Silver rabbits breed was 75 %. These data are consistent with the spanish and egyptian researchers who after molecular genetic analysis of rabbit breeds of New Zealand White, Californian and New Zealand Red by microsatellite markers were mentioned share polymorphism for these species: 88,1 %, 69,05 % та 71,7% [9].

1. Breed specific fragments of DNA rabbits for different types of ISSR-markers, bp

Breed	ISSR-marker			Total for ISSR-markers
	$(ACC)_6G$	$(GA)_9C$	$(AG)_9C$	
New Zeland White	1150, 1200	700	850, 1110	5
Californian	800, 890	500, 600	-	4
Silver	510	-	750	2

Overall, the features of polymorphism amplicon derived from PCR microsatellite sites between genomic DNA of various breeds of rabbits show highly informative of ISSR-markers, suggesting that the ability to identify the genotype of each animal and determine the phylogenetic relationship. Therefore, based on DNA polymorphism of different breeds of rabbits detected using ISSR-analysis was characterized genetic diversity and phylogenetic relationships of these animals, the results are displayed in a dendrogram (fig. 1)

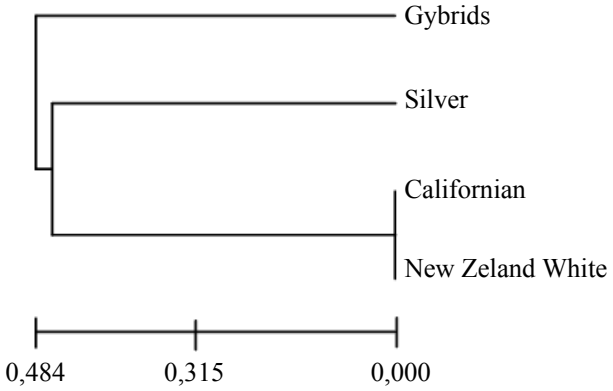

Fig. 1**. Dendrohram of genetic relationship of different rabbits breeds and their hybrids by ISSR-markers**

The range of genetic distances between breeds of rabbits varied in the range of 0,000 to 0,484. The structure was a single cluster dendrogram which was formed by two branches. Moreover, clustering species dependent on fragment microsatellite sequences. The first part ofwas formed of rabbits Silver breed, the second formed subcluster, mapped array of animal species Californian and New Zealand White. Separated from a single branch cluster formed interbreed hybrids of rabbits.

Based on the data of rabbit interbreed genetic differentiation of the peculiarities variation index polymorphic information content. For $(ACC)_6G$ ISSR-marker PIC = 0,3, for $(AG)_9C$ ISSR-marker PIC = 0,17 and for $(GA)_9C$ – ISSR-marker PIC = 0,2.

We have analyzed the genetic structure of the New Zealand White rabbits breed for C34T myostatin (MSTN) gene polymorphism that is associated with characteristics of meat animals. Also, data from gene polymorphism G2464A progesterone receptor rabbits (PGR), which is associated with reproductive and maternal characteristics of females. The distribution of genotypes of the New Zealand White rabbits breed for polymorphic variants of studied genes is presented in fig. 2.

Fig. 2. **The distribution of genotypes of the New Zealand White rabbits breed by polymorphic gene variants of myostatin and progesterone receptor**.

For myostatin gene animals accounted for the largest number of CT genotype carriers. Rabbits homozygous by the allele C was 2 times less, and homozygotes by allele T - almost 1,6 fewer. The frequencies of alleles C and T were 0,471 and 0,529. By polymorphic variants of progesterone receptor gene females of New Zealand White rabbit breed were distributed as follows. The largest number were heterozygotes GA, number of homozygotes for allele G and A were by 31,7% and 18,3% less then other animals. The highest frequency was allele A - 0,567. G allele frequency was lower in 23,6 % (0,433).

Using ANOVA was set significant effect of genotype New Zealand White breed rabbits for myostatin gene expression on average daily increments (η^2 = 0,45) and carcass weight pair (η^2=0,35).

The analysis of differentiation interline New Zealand White rabbits breed by MSTN gene polymorphism C34T and PGR gene G2464A the following results. The highest frequency of allele C of the myostatin gene had male descendants of Imperator (0,551), the frequency of allele T was 0,449. For rabbits of line Bilash was observed higher values for G allele gene progesterone receptor (0,488) due to the benefits of homozygous animals. To male sires for breeding by genetic index was attributed Nazar (I= 9,9±0,8, p<0,05), White (I= 9,8±0,7, p<0,05) and Bilash (9,5±0,3, p<0,01).

To determine the breeding value of male New Zealand White rabbits breed we used the method of the Best Linear Unbesian prediction BLUP. The results of the evaluation factors for animal genotype (homozygotes for allele C, T and CT heterozygotes for myostatin gene), average daily gain and birth year revealed the following patterns. The highest BLUP index were males Nazar (0,199), Baikal (0,357) and Casper (0,046). They had Relative Breeding Values: 101,0% and 100,5%, respectively. BLUP breeding value estimation indexes of males New Zealand White rabbits breed flogged for reproductive characteristic of their daughters (number of separate rabbits at 35 days) were distributed similarly. The highest BLUP index characterized by Nazar (0,140), Baikal (0,087) and Casper (0,045). They had Relative Breeding Value: 102,3%; 101,5% and 100,7%, respectively. Based on the values of the index Relative Breeding Value (RBV), the technique males fruitful rabbits of New Zealand White breed (table. 2).

According to the analysis of ranking male rabbits to the categories BLUP index (average daily gain) we had following results. Nazar, Baikal and Casper were attributed to the improvers rabbits, White, Caesar - neutral and Graf, Imperator - worses.

2.The distribution of the categories of males New Zealand White rabbits breed by Relative Vreeding value, RBV

RBV	Categoty
RBV>ARBV + 2*SDRBV	++ (likely improvers)
ARBV + 2*SDRBV≥RBV>ARBV + 0,75*SDRBV	+ (improvers)
ARBV + 0,75*SDRBV≥RBV>ARBV – 0,75*SDRBV	0 (neutral)
ARBV - 0,75*SDRBV≥RBV>ARBV - 2*SDRBV	- (worses)
ARBV – 2*SDRBV>RBV	-- (likely worses)

Note. *ARBV - average relative value of all breeding rabbits; SDRBV - standard deviation of relative breeding value rabbits*

By ranking animals under BLUP-index associated with reproductive qualities, show a somewhat different situation. Thus, the probable improvers were classified Nazar, Baikal and Caesar. Neutral sires were Graf, Imperator and Casper and to worces - White.

To assess the effective BLUP correlation coefficients were calculated breeding values of males New Zealand White rabbits breed from selection and genetic phenotypic index and average productivity of their daughters. However, the closer figure nearer to unity, the higher the efficiency of the method BLUP.

Results of correlation analysis, calculated among males of New Zealand White rabbits breed are presented in table 3.

3. Correlation of BLUP-index of males New Zealand White rabbits breed, average performance and reproductive capacity of their daughters

BLUP-index	Productivity, reproductive capacity of daughters	Selection-genetic-index
Productive qualities	+0,71*	+0,82*
Reproductive qualities	+0,78*	+0,67

Note*: significant at p<0,05*

Between BLUP indices of males New Zealand White rabbits breed, genetic and phenotypic parameters, index of their daughters set high and mostly accurate correlation.

Conclusions. The investigated population of rabbits New Zealand White breed have high levels of polymorphism. Thus, using ISSR-marker $(ACC)_6G$ found the greatest number of polymorphic amplicon. Also we found breed specific fragments.

In the New Zealand White rabbits breed often met by CT genotypes myostatin gene (47,1 %) and GA for progesterone receptor gene (50 %).

The information was obtained of distribution allele frequencies rabbits of New Zealand White rabbits breed from different linear facilities on genes of myostatin and progesterone receptor. Thus, the highest frequency of allele C were descendants of Imperator (0,551), Casper (0,510), White (0,531), allele T – Nazar (0,566). Meanwhile, for rabbits of line Bilash observed highest value of frequency allele G (0,488), and for generations Imperator - allele A (0,580).

Beween genotypes of New Zealand White rabbits breed by myostatin and progesterone receptor genes in phenotypic manifestation of signs are significant differences: 2,5 % ($p < 0.05$) on average daily increments 4 % ($p < 0,05$) by fertility

According to comprehensive study – using of evaluation index, BLUP-method and molecular-genetic evaluation of rabbits by ISSR and RFLP markers - promising males of New Zealand White as sires - Nazar, Casper and Baikal.

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ПОЛІМОРФІЗМ МІКРОСАТЕЛІТНИХ ЛОКУСІВ ДНК У РІЗНИХ ВИДІВ СІЛЬСЬКОГОСПОДАРСЬКИХ ТВАРИН

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Проведені дослідження генетичної структури трьох порід коней та двох порід великої рогатої худоби за мікросателітними локусами ДНК. Отримані результати вказують на те, що розподіл алельних варіантів генів та основні показники генетичної мінливості визначаються особливостями формування генофонду кожної породи відповідно до напрямку продуктивності та історії створення. Розподіл алельних варіантів та генотипів тварин за дослідженими молекулярно-генетичними маркерами можна розглядати як додаткові характеристики порід.

Ключові слова: **коні, велика рогата худоба, мікросателітні локуси, ДНК, мінливість**

POLYMORPHISM OF MICROSATELLITE DNA LOCI IN DIFFERENT SPECIES OF FARM ANIMALS

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The study of the genetic structure of three breeds of horses and two cattle by DNA microsatellite loci. The results obtained indicate that the distribution of alleles of the gene pool and parameters of genetic variation is determined by the peculiarities of formation of each breed according to the direction of productivity and creation stories. The distribution of genotypes and allelic variants of the animals studied by molecular genetic markers can be seen as additional characteristics of breeds.

Key words: **horses , cattle , microsatellite loci, DNA, variation**