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GENETIC STRUCTURE FEATURES OF CATTLE POPULATIONS OF UKRAINIAN SELECTION BY POLYMORPHISM OF LOCI, ASSOCIATED WITH MILK PRODUCTIVITY TRAITS

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Aim. To study the genetic structure of cattle populations of Ukrainian selection by polymorphism of functional genes (*PRL*, *PL*) and microsatellites (BM027, RM185). **Methods.** The study was conducted using the method of polymerase chain reaction (PCR) and restriction analysis in case of loci *PRL* and *PL,* and using classic PCR with subsequent electrophoresis in polyacrylamide gel to analyze microsatellite variability. **Results.** The results of the studies demonstrated that the locus of prolactin by RsaI-polymorphism in the fourth exon was polymorphic in both experimental populations (Ukrainian Black-and-White and Ukrainian Red-and-White dairy breeds of cattle). The mutation (Indel) was first determined in the fourth exon of prolactin gene, the variants of which correlated with some alleles of the locus by RsaI-polymorphism. The locus of placental lactogen by RsaI-polymorphism in the fifth exon was monomorphic in both experimental populations. Microsatellite locus RM185 was polymorphic in both groups of animals, whereas BM027 – only in the Black-and-White dairy breed. **Conclusions.** The specificities of the genetic structure of the Ukrainian Black-and-White and Red-and-White dairy breed populations by polymorphism of functional genes and microsatellite loci were determined. The locus of placental lactogen by RsaI-polymorphism in the fifth exon cannot be used in further studies due to the absence of alternative variants of the gene in both studied populations of animals. The analysis of the distribution of haplotype frequencies demonstrated the absence of deviation from the equilibrium state by linkage for each of the investigated markers which makes their use impossible in the breeding programs as a separate functional unit.

Key words: cattle, polymorphism, gene, allele, population, productivity, restriction, electrophoresis, genetic equilibrium, linkage.

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INTRODUCTION

Regardless of the outstanding success of genomic selection, based on the analysis of large bulks of data about the whole genome of animals, the application of MAS or selection using markers is still urgent [1]. It is especially fair regarding local breeds as well as the tasks of preserving gene fund. Therefore, the studies on the specificities of genetic structure of the populations of different breeds of cattle by the combination of different types of molecular and genetic markers are conducted in many countries regarding very different objects [2]. Microsatellite markers are used for issues of passportization of breeds and lines as well as for identification and comparative analysis (phylogenetics) [3, 4]. However, the markers, related to polymorphism of target genes are even more popular, as this method allows using the results of studies in different breeding programs with the purpose of obtaining more productive lines [5, 6]. In this direction, the most widespread marker systems include PCR-RFLP and Indel, the efficiency of using which in the breeding work is determined by a number of factors. First of all, one of the main constituents is related to the selection of the object of studies. In this case, a good target is function-

al genes, whose products may be related to the manifestation of economically valuable features of animals [7–9]. Rather many works were conducted in this direction, involving such genes as prolactin, growth hormone receptor, pituitary transcription factor 1, etc. [10–15]. Special attention in the practical genetics of cattle is paid to the issues of polymorphism of milk protein genes and the determination of desirable allelic variants, related to the parameters of milk quality [16–18]. In general, according to the common methodology of research work, following the analysis of genetic structure by the selected objects it is necessary to pass to the next phase, which is a key phase in many respects, – the analysis of the association between different allelic variants of polymorphic loci and the performance indices for animals. The studies demonstrate a picture of the most desirable complex genotypes by different polymorphic loci [19–21]. Similar work is also done regarding the issues of adaptive ability of animals and resistance to diseases [22, 23]. In this context, it is necessary to consider the breed-wise specificity of each marker, which leads to the impossibility of mere copying of results, obtained in one line/breed of animals, onto other lines. Thus, the accumulation of a large bulk of data about the association between the polymorphism of very different genes and the productive traits of animals from different breeds is an urgent and necessary task of agricultural biology.

In the presented work we highlight the study on different types of molecular and genetic markers, each of which is related in some way to the regulation of milk performance of cattle of Ukrainian selection. First of all, we shall analyze the polymorphism of prolactin gene and placental lactogen using PCR-RFLP. On the next stage, we shall conduct the analysis of microsatellite variability of experimental populations on two loci – RM185 and BM027, which are actually connected to the prolactin gene due to its location in the genome. The selection of microsatellite loci is determined by the need to conduct the linkage disequilibrium analysis as in case of the presence of stable haplotypes it is possible to change the general approach to conducting the breeding work in experimental cattle populations considerably.

MATERIALS AND METHODS

The studies were conducted in the laboratory of molecular-genetic and physiological-biochemical research in animal science of the Institute of Animal Science, NAAS.

The populations of cattle of Ukrainian Black-and-White and Red-and-White dairy breeds (Hontarivka experimental farm, Vovchansk district, Kharkiv region) were used as an object of study. 100 animals per population were analyzed.

Hair follicles (individually from each animal) were used as a source of biological material. The commercial set of reagents DNA-Sorb-B was used to extract DNA according to the manufacturer's instructions.

The amplification of exon fragments of the genes of prolactin and placental lactogen was conducted with use of primers developed using the programs FastPCR v6.5.54 and PerlPrimer v1.1.21 based on the analysis of nucleotide sequences (NCBI, Ensembl): for the locus of prolactin: GTTCTTGCTTTATGTAACACCG and TAGGTCAATCACTCTGAGCA; for the locus of placental lactogen: TTTGGGTGCTTAGGTTCATCC and ATCATCACTAACCATCTCAGGAC.

PCR-RFLP was used to study RsaI-polymorphism of the fourth exon of prolactin gene (C/T transition in position 35106206) as well as RsaI-polymorphism of the fifth exon of the gene of placental lactogen (C/A transversion, missense-mutation in position 35071890).

In both cases the amplification products were processed using restriction endonuclease RsaI according to the manufacturer's instructions (ThermoScientific).

The genotyping of animals was conducted using the analysis of the distribution of restriction fragments after electrophoresis in agarose gel (1.5 %). Ethidium bromide was used for staining.

The size of amplified fragment by locus *PRL* is 416 b.p. Allele C is notable for the presence of one restriction site which leads to the formation of two restriction fragments of 56 and 360 b.p. In case of allele T, there are two restriction sites and thus three restriction fragments of 56, 165 and 195 b.p.

The size of amplified fragment by locus *PL* is 239 b.p. Allele C does not contain a restriction site for RsaI and thus is presented in the electrophoregram in the form of a single fragment of 239 b.p. In its turn, allele A contains one restriction site and is presented in the electrophoregram in the form of two fragments of 65 and 174 b.p.

Along with the study on the polymorphism of target genes, related to milk productivity traits for animals, there was a study on the genetic structure of experimental populations of animals by two microsatellite

loci – BM027 and RM185, directly connected to prolactin gene.

The microsatellite locus BM027 is located in 5′UTR of *PRL* gene; it is a dinucleotide motif (CA) nTA(CA)n. The following primers were used for amplification: GCCCTCTCTTCTACAATGAACAC and GGAAAGTGAACATGACTGTCTAG. The size of the amplified fragment is in the range of 160 b.p.

The microsatellite locus RM185 is located in 3'UTR of *PRL* gene; the dinucleotide motif is presented by the formula (CA)nGA(CA)n. The following primers were used for its amplification: TGGCTGCCTTATGCTTGCATC and GAGTTTCCTTTGCATGCCAGTC. The size of the amplified fragment is in the range of 106 b.p.

In case of microsatellite markers, the electrophoresis of amplification products was conducted with 7 % polyacrylamide gel. Ethidium bromide was used for staining.

The amplification of different experimental targets was conducted using programmed thermocycler AMPLY 4 using standard programs: 1 cycle – denaturation at 94 °C for 3 min, 35 cycles – denaturation at 94 °C for 45 sec, annealing for 45 sec (56 °C for *PRL*; 58 °C for *PL*; 61 °C for BM027; 62 °C for RM185), elongation at 72 °C for 45 sec; 1 cycle – final elongation at 72 °C for 10 min. The volume of the reaction mixture was 20 μL, the concentration of primers – 0.2 μM for each case respectively.

The frequencies of alleles were calculated using the formula of maximum likelihood according to E.K. Merkurieva (1977). The obtained data were used to evaluate the observed and expected distribution of genotypes, the compliance with Hardy-Weinberg equilibrium state by the method of χ^2, the indices of observed (H_o) and expected (H_e) heterozygosity, the total number of alleles in the locus (Na), the effective number of alleles (n_e) and Wright's fixation index (F_{is}) by standard methods using the computer program Popgen32 (https://sites.ualberta.ca/~fyeh/popgene_do-wnload.html). The frequencies of haplotypes were determined via the calculation of EM-algorithm using the program EH+ [24]. The index of standardized value of the measure of deviation by linkage (D′) was determined using the program MIDAS v.1 (Multiallelic Interallelic Disequilibrium Analysis Software) [http://www.genes.org.uk/software/midas].

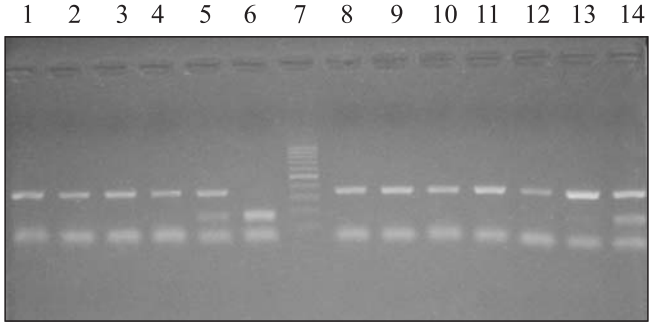

Fig. 1. The electrophoregram of restriction products of the fourth exon of prolactin gene: 1–14 – sample numbers; 1–4, 8–13 – genotype CC; 5, 14 – C/T; 6 – T/T; 7 – marker of molecular masses M-100

RESULTS AND DISCUSSION

The results of the conducted studies demonstrated that prolactin locus by RsaI-polymorphism of the fourth exon was found to be polymorphic in both experimental populations of cows. The animals of three possible genotypes – CC, CT and TT – were found in each population.

The electrophoregram of restriction products of the fourth exon *PRL* was presented in Fig. 1.

As seen in the presented electrophoregram, different genotypes correspond to expected restriction patterns by the locus of prolactin hormone completely.

Table 1. The genetic structure of the experimental populations by locus PRL

Genotype	Ukrainian Red-and-White			Ukrainian Black-and-White		
	O	E	χ^2	O	E	χ^2
CC	38	28.62		76	76.56	
CT	31	49.76	14.21	23	21.88	0.26
TT	31	21.62		1	1.56	
Allele	Allele frequencies					
C	0.535			0.875		
T	0.465			0.125		

Note: O – observed number of animals with this genotype; E – expected (estimated) number of animals with this genotype.

The study results were used to conduct the analysis of specificities of the genetic structure of experimental cattle populations (Table 1).

Regardless of the presence of all the possible variants by prolactin locus in both populations, the groups of animals are significantly different in the ratio of frequencies of alleles and genotypes. Therefore, the population of Ukrainian Black-and-White dairy breed is characterized by a considerable advantage (two-fold) regarding the number of animals with genotype CC compared to the Ukrainian Red-and-White dairy breed. In its turn, this regularity was not noted in the population of Ukrainian Red-and-White dairy breed – the frequencies of different genotypes are close to each other in their frequencies (Table 1). At the same time, this population is remarkable for higher indices of observed (H_o) and expected (H_e) heterozygosity – 0.31 and 0.498 against 0.23 and 0.219 in the second experimental group respectively. Regardless of prevalence in the value for the indicator of observed heterozygosity almost by 50 %, the population of the Red-and-White breed by the value of the Wright's fixation index (F_{is} = 0.37) demonstrates evident lack of heterozygotes, i.e. inbreeding. There is an opposite situation with the Black-and-White breed – the value of F_{is} was –0.05; which demonstrated an insignificant excess of heterozygotes. Here the first group had a deviation from Hardy-Weinberg genetic equilibrium state (first of all, due to the excess of homozygous animals). The populations also had significant differences by the index of effective number of alleles (n_e). For Ukrainian Red-and-White dairy breed, the index n_e reaches almost maximal value for two-allele systems (1.99); in its turn, its value for the Black-and-White breed is considerably lower – 1.28.

While conducting additional studies on more accurate determination of the sizes of amplicon in the experimental fragment of prolactin gene using electrophoresis in polyacrylamide gel, it was demonstrated that the presence/absence of the polymorphic restriction site for RsaI correlated with the presence/absence of insertion, which we managed to reveal during the studies. The electrophoregram is presented in Fig. 2.

The results of studies demonstrated that the presence of insertion corresponded to allele C (RsaI–), whereas its absence (deletion) – to allele T (RsaI+). In other words, the deletion in this fragment correlated with the presence of the restriction site for RsaI, while the insertion – with its absence (as seen in Fig. 2). It may be assumed that the insertion of the fragment is either related directly to the restriction site (changing its nucleotide sequence), or is connected to it via linkage.

The correspondence of insertion/deletion to specific alleles was confirmed in each case of the whole bulk of the analyzed data (100 animals from each breed). The results of studies allow using the analysis in polyacrylamide gel for the presence of insertion/deletion in the fourth intron of prolactin gene as an alternative of PCR-RFLP method which allows reducing the expenses for restriction. There is also a possibility of required further studies, related to the determination of the nucleotide sequence of the experimental fragment of the gene (sequencing) with the purpose of specifying the structure of insertion.

In any case, this example may serve as a wonderful illustration of the phenomenon of "transformation, inversion" of different types of molecular-genetic markers one into another (PCR-RFLP in Indel).

The experimental groups of cattle of Ukrainian selection are similar to the prevailing majority of other, both commercial and local, breeds in the specificities of the ratio of allelic frequencies by the prolactin locus (the prevalence of allele C frequency) [13]. In our opinion, the observed picture is caused by the associative connection between prolactin alleles and the indices of milk productivity, which was already noted in many publications [25, 26]. One of the factors, impacting the differences in the values of milk productivity indices in the experimental groups of cows (6133 ± 136 kg in 305 days of lactation against 4811 ± 131 kg), may be the fact of two-fold prevalence in the frequency of homozygous animals by allele C in the population of Ukrainian Black-and-White dairy breed.

The presence of promising genotypes by prolactin locus of expressed phenotypic manifestation led to a great effect of selection while conducting the breeding work. However, it is practically impossible to reach the monomorphic character of the locus in the absence of the methods of molecular-genetic typing of alleles due to the inability of revealing heterozygous animals in the populations. Therefore, the observed phenomenon serves as a wonderful example of the need to have a complex approach to the work with animals, comprising the methods of both marker-associated and classic breeding.

The situation regarding the locus of placental lactogen is completely different from the aforementioned. The gene of placental lactogen was found to be mono-

morphic in each of the experimental populations of animals. Only the animals with CC genotype (RsaI–/RsaI–) were revealed in both groups. The fact of monomorphic nature of this locus leads to the impossibility of its further application in breeding, as the absence of allelic variants allow neither studying their associative connections with the performance indices of animals, nor evaluating the main genetic-populational parameters of the experimental groups.

Further on, the studies were conducted on microsatellite variability of the experimental populations by loci BM027 and RM185, which are potential markers of different alleles (in case of linkage disequilibrium) of prolactin gene, as they are located in flanking regions 5' and 3' of the locus.

The results of the studies in the population of cows of Black-and-White dairy breed demonstrated 2 alleles by locus BM027 and 7 by RM185; of Red-and-White breed – 1 and 6 respectively. In the population of Black-and-White dairy breed by locus BM027, the allele frequency of 164 b.p. was 0.115; 168 b.p. – 0.885. In its turn, by locus RM185 the allele frequency of 104 b.p. was 0.025; 106 b.p. – 0.1, 108 b.p. – 0.4, 110 b.p. – 0.2, 112 b.p. – 0.125, 114 b.p. – 0.125, 116 b.p. – 0.025.

At the same time, in the population of Red-and-White breed locus BM027 was found to be monomorphic. There were only animals with genotype 168/168 b.p. present, which makes this population of cows considerably different from the abovementioned. By locus RM185 the allele frequency of 104 b.p. was 0.06; 106 b.p. – 0.365, 108 b.p. – 0.325, 110 b.p. – 0.135, 112 b.p. – 0.095, 114 b.p. – 0.02.

The main genetic-populational parameters of the experimental breeds of cattle are presented in Table 2.

According to the results of the presented studies, the experimental populations of cattle have considerable differences. For instance, as it has already been aforementioned, the Black-and-White population is polymorphic by locus BM027, whereas the Red-and-White population is monomorphic. The analysis of frequency distribution demonstrates that allele of 168 b.p. is expressively prevalent in this locus and is present in the only variant in the second group of animals. The values of the observed and estimated heterozygosity are rather close (0.18 against 0.21) which demonstrates some excess in homozygotes according to the indices of Wright's fixation index, which still does not lead to the deviation from the Hardy-Weinberg's equilibrium state of the population.

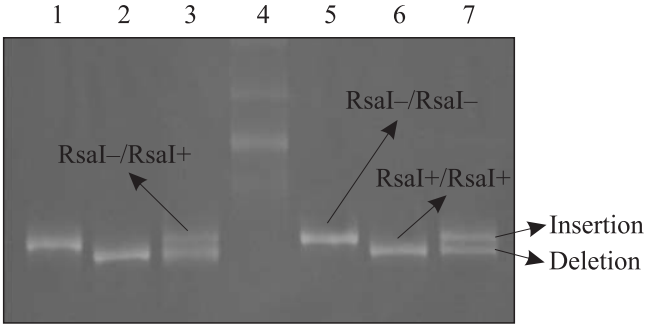

Fig. 2. The electrophoregram of amplification products of the fourth exon of prolactin gene (PAAG, 6 %)

The situation is somewhat different by locus RM185. Higher number of alleles (7 against 6) was found in the Black-and-White population along with higher values of the indices of observed and estimated heterozygosity compared to the second group of animals. At the same time, a considerable excess of homozygotes, inbreeding (43 %) was determined in the Red-and-White population. Wright's fixation index was 0.43.

Both groups are rather similar in the value of effective number of alleles, the differences are defined by a different number of alleles in the locus, first of all. Both populations demonstrated the deviation from the state of genetic equilibrium.

It was noted during the comparison of the study results against the data of other authors that the monomorphic character of locus BM027 is also revealed in the population of the Red Gorbatov breed, whereas the Black-and-White breed of Russian breeding and the Ayrshire breed were noted for the parameters of heterozygosity, similar to the ones, presented in this study (0.13 and 0.10 respectively) [3]. It should be noted that

Table 2. The main genetic-populational parameters of the experimental groups of animals by loci BM027 and RM185

Breed	Na	n_e	H_o	H_e	F_{is}
Ukrainian Black-and-White	BM027				
	2	1.27	0.18	0.21	0.15
	RM185				
	7	4.14	0.65	0.76	0.14
Ukrainian Red-and-White	BM027				
	1	–	–	–	–
	RM185				
	6	3.73	0.42	0.73	0.42

in the context of the analysis of microsatellite variability of different breeds and lines of animals in different laboratories there should be consideration of the variation factor of allele frequency values in case of using electrophoresis in polyacrylamide gel, which may lead to the discrepancy in the absolute values of the sizes of detected alleles. However, it does not reflect on the main parameters of genetic variability of the populations (number of alleles, heterozygosity indices, etc.). A promising direction in exercising the control over the origin of animals with the purpose of maximally accurate identification of the sizes of alleles for passportization of cattle specimen is genotyping using modern automatic analyzers.

The check of the level of deviation from the equilibrium state between loci BM027 and prolactin (*PRL*), which potentially are in the same group of linkage due to close location within a gene, the highest frequency of incidence was revealed for haplotype $BM027^{168}$/PRL^{C} (0.73) in the Red-and-White population. However, it was not sufficient for the impairment of genetic equilibrium ($\chi^2 = 0.93$), which reflected on the value of the standardized measure of the deviation ($D' = 0.21$). Therefore, the prevalence of the frequency of haplotype $BM027^{168}$/PRL^{C} in the experimental population is derivative from the accidental distribution of variability of the occurrence of the two analyzed alleles.

In a similar way, the analysis of distribution of haplotype frequencies *PRL* and RM185 demonstrated the absence of allelic combinations, which are inherited together with high probability in the form of a single functional structure ($D' < 0.33$).

A similar picture is also observed in the Red-and-White population. The analysis of distribution of haplotype frequencies of loci *PRL* and RM185, as well as *PRL* and BM027 in the experimental population demonstrates the absence of the impairment of equilibrium state. The values of the standardized measure of deviation D' (in the range of 0.25–0.30) indicated that the observed allelic combinations (forming different haplotypes) were conditioned by accidental factors.

Therefore, the fact of deviation from the equilibrium state by linkage was not revealed in both studied populations of cattle, which does not allow considering different haplotypes in the form of a unified functional structure in the context of breeding tasks.

CONCLUSIONS

The analysis of genetic structure of cattle populations of Ukrainian Black-and-White and Red-and-White dairy breeds was conducted by loci of prolactin (*PRL*), placental lactogen (*PL*), microsatellite markers BM027 and RM185. It was demonstrated that the locus of prolactin by RsaI-polymorphism in the fourth exon was polymorphic in all the experimental populations of animals. The mutation (Indel) was first revealed in the fourth exon of prolactin gene, here the presence/absence of the polymorphic site of restriction for RsaI correlated with the presence/absence of insertion in the experimental fragment *PRL*. The locus of placental lactogen by RsaI-polymorphism in the fifth exon was monomorphic in both experimental populations of animals. The microsatellite locus RM185 was polymorphic in both experimental populations, while BM027 was polymorphic only in the Ukrainian Black-and-White population. The analysis of distribution of haplotype frequencies revealed the absence of deviation from the equilibrium state by linkage for each of the studied markers.

All the applicable international, national and/or institutional principles of care for and use of animals were complied with.

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Особливості генетичної структури популяцій великої рогатої худоби української селекції за поліморфізмом локусів, пов'язаних з молочною продуктивністю

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Мета. Дослідження генетичної структури популяцій великої рогатої худоби української селекції за поліморфізмом функціональних генів (*PRL*, *PL*) та мікросателітів (BM027, RM185). **Методи.** Дослідження проведено з використанням методу полімеразної ланцюгової реакції та рестрикційного аналізу у випадку з локусами *PRL* та *PL*, та з використанням класичної ПЛР з наступним електрофорезом у поліакриламідному гелі для аналізу мікросателітної мінливості. **Результати.** За результатами досліджень встановлено, що локус пролактину за RsaI-поліморфізмом у четвертому екзоні є поліморфним

в обох дослідних популяціях тварин (українська чорно-ряба та українська червоно-ряба молочні породи великої рогатої худоби). Вперше виявлено мутацію (Indel) у четвертому екзоні гену пролактину, варіанти якої корелюють з певними алелями локусу за RsaI-поліморфімом. Локус плацентарного лактогену за RsaI-поліморфізмом у п'ятому екзоні є мономорфним в обох дослідних популяціях. Мікросателітний локус RM185 є поліморфним в обох групах тварин, в той час як BM027 – лише у популяції чорно-рябої молочної породи. **Висновки.** З'ясовано особливості генетичної структури популяцій великої рогатої худоби українських чорно-рябої та червоно-рябої молочних порід за поліморфізмом функціональних генів та мікросателітних локусів. Локус плацентарного лактогену за RsaI-поліморфізмом у п'ятому екзоні не можна використовувати у подальших дослідженнях внаслідок відсутності альтернативних варіантів гену в обох досліджених популяціях тварин. Аналіз розподілу частот гаплотипів виявив відсутність відхилення від рівноважного стану за зчепленням за кожним з досліджених маркерів, що унеможливлює їх використання у селекційних програмах у якості окремої функціональної одиниці.

Ключові слова: велика рогата худоба, поліморфізм, ген, алель, популяція, продуктивність, рестрикція, електрофорез, генетична рівновага, зчеплення.

Особенности генетической структуры популяций крупного рогатого скота украинской селекции по полиморфизму локусов, связанных с молочной продуктивностью

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Цель. Исследование генетической структуры популяций крупного рогатого скота по полиморфизму функциональных генов (*PRL*, *PL*) и микросателлитов (BM027, RM185). **Методы.** Исследования проведены с использованием метода полимеразной цепной реакции и рестрикционного анализа в случае с локусами *PRL* и *PL*, и с использованием классической ПЦР с последующим электрофорезом в полиакриламидном геле для анализа микросателлитной изменчивости. **Результаты.** По результатам исследований выяснено, что локус пролактина по RsaI-полиморфизму в четвертом экзоне является полиморфным в обеих опытных популяциях животных (украинская черно-пестрая и украинская красно-пестрая молочные породы крупного рогатого скота). Впервые выявлена мутация (Indel) в четвертом экзоне гена пролактина, варианты которой коррелируют с соответствующими аллелями локуса по RsaI-полиморфизму. Локус плацентарного лактогена по RsaI-полиморфизму в пятом экзоне является мономорфным в обеих опытных популяциях. Микросателлитный локус RM185 является полиморфным в обеих группах животных, в то время как BM027 – только в популяции украинской черно-пестрой молочной породы. **Выводы.** Определены особенности генетической структуры популяций крупного рогатого скота украинских черно-пестрой и красно-пестрой молочных пород по полиморфизму функциональных генов и микросателлитных локусов. Локус плацентарного лактогена по RsaI-полиморфизму в пятом экзоне нельзя использовать в дальнейших исследованиях вследствие отсутствия альтернативных вариантов гена в обеих опытных популяциях животных. Анализ распределения частот гаплотипов выявил отсутствие отклонения от равновесного состояния по сцеплению по каждому из изученных маркеров, что делает невозможным их использование в селекционных программах в качестве отдельной функциональной единицы.

Ключевые слова: крупный рогатый скот, полиморфизм, ген, аллель, популяция, продуктивность, рестрикция, электрофорез, генетическое равновесие, сцепление.

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