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GENE POLYMORPHISM IN A POPULATION OF CHAROLAIS BEEF CATTLE USING DNA-MARKERS

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Aim. To study the polymorphism of genes, associated with resistance to different diseases (*TLR1*, *TLR4, SLC11A1, TLR1*, *TLR4*, *IFNGR2*, *SLC11A1*, *TNFα,* and *MBL1*) in the population of Charolais beef cattle. **Methods.** The studies were conducted using the methods of polymerase chain reaction and the restriction fragment length polymorphism (PCR-RFLP); the artificially created restriction site-PCR (ACRS-PCR) was used to study the polymorphism of gene *MBL1***. Results.** In the population (n = 100) of Charolais cattle, genes *TLR1*, *IFNGR2*, *SLC11A1*, *TNFα,* and *MBL1* were polymorphic, while *TLR4* was found to be monomorphic by three mutant variants (8732G>A, 8834G>C, and 2021C>T). BclI-polymorphism of the first exon was used to determine the excess of heterozygous animals (81.8 %); there were no animals homozygous by allele A. By polymorphism of locus *SLC11A1* for SNP6 (7808A>T), we determined complete domination of the frequency of allele A over T (0.985 vs 0.015), no animals, homozygous by allele TT, were found. As for SNP5 7400C>G, there were no animals homozygous for allele G; the ratio of alleles C and G was 0.79 and 0.21, respectively. By locus *IFNGR2* (1008A>G), we determined the prevalence of the frequency of allele A over G (0.745 vs 0.255); the animals with genotype AA were the most common. As for gene *TNFα* (–824A>G), we determined a two-fold prevalence of the frequency of allele G over A due to a higher number of animals with genotype GG (39 %). Some 50 % of the animals were heterozygous for this gene.By locus *MBL1*,we found the prevalence of the frequency of allele G (*StyI*-) as compared to A (*StyI*+) due to a considerable number of homozygous animals GG (64 %). The percentage of homozygous animals was 37.8 %. **Conclusions.** General characteristics of genetic variability of the Charolais beef cattle population were determined for the loci *TLR1*, *TLR4*, *IFNGR2*, *SLC11A1*, *TNFα,* and *MBL1*. As for SNP6 7808A>T of locus *SLC11A1,* we have determined the final stage of allele A fixation, by the results of which the gene will become completely monomorphic by this mutation. The results of the analysis of the ratio between the frequencies of alleles and genotypes by the revealed polymorphic markers demonstrated that it is unreasonable to use animals with different allelic variants of genes *TLR1*, *IFNGR2*, *SLC11A1* in further studies on the search for associations with the manifestation of economically valuable traits in Charolais beef cows of the experimental population due to insufficient number of animals with different genotypes. For loci *TNFα* and *MBL1,* we found enough animals with different genotypes to conduct further association studies. The deviation from the genetic Hardy-Weinberg equilibrium was observed for the loci *TLR1*, *SLC11A1* (7400C>G), and *MBL1* in the population.

Key words: polymorphism, variability, allele, gene, population, resistance.

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INTRODUCTION

In modern animal breeding, the efficiency of using different breeding programs, first and foremost targeted at the maximization of the productive potential of animals, is often restricted by methodological approaches in conducting the breeding work. A significant number of practical issues can be solved by the applica-

tion of effective modern DNA-technologies, such as marker-assisted and genomic selection. In addition to classic methods of evaluating the traits of animals, DNA technologies provide information about the structural organization of the genome of animals (Johnsson, 2023).

Study of genome organization at the level of functional gene polymorphism allows for precise assessment of genetic variability in the hereditary material. This distinctively differentiates the methodological approaches of marker-assisted selection (MAS) from classic selection methods in animal breeding, which primarily rely on evaluation of the phenotype (Wakchaure et al, 2015; Sharma et al, 2024). The intensification of the domestic animal breeding industry, characterized by high indices of milk and meat cattle performance, is closely linked to the animals' high adaptive abilities, but should also be linked to possible adaptations/changes in resistance to diseases and unfavorable environmental factors. Therefore, studying microevolutionary changes occurring in the genetic structure of populations as they progress towards maximal realization of productive potential is a pressing issue, especially concerning the structural organization of the purebred nucleus within experimental breeds.

Even though in several recent years, the attention of the scientific community has gradually been shifting towards investigating the variability on the level of the entire genome of animals, i.e. towards practical aspects of genomic selection, the analysis of polymorphism of specific genes and their complexes, as a foundation of MAS strategy, still remains very profitable and essential (Colombi et al, 2024). Especially in combination with the study of new functional genes, such as myostatin (Kostusiak et al, 2023), pyruvate kinase L/R (Du et al, 2022), heat shock proteins (HSPs) (Hariyono et al, 2022), adiponectin (Fonseca et al, 2015; Pandey et al, 2020).

Apart from ensuring maximum performance, resistance/susceptibility and sensitivity to diseases of different etiology should be studied and taken into account. There are cases where breeding for maximum performance of cattle has resulted in the deterioration of resistance indices of the animals, (Curone et al, 2018). Selection and breeding, which involves evaluating and selecting animals based on their general resistance to diseases, apart from considering adaptive traits, using modern molecular-genetic tools, is one of the most urgent and promising areas in cattle breeding (Hu et al, 2020). Despite the focus on achieving maximal values of productive traits, the issue of disease resistance is increasingly gaining attention, driven by its economic significance (Knap et al, 2020).

Breeding for high performance and disease resistance are usually conducted on different levels – from genomic selection to applying methodological approaches in genetic engineering (Islam et al, 2020; Gao et al, 2023). It involves using both commercial breeds of animals and native aborigine ones (Curone et al, 2018; Gogoi et al, 2021). From the standpoint of general MAS strategy, noteworthy are the studies on genes, the products of which are directly associated with the regulation of the immune system of the organism and, thus, with the indices of resistance/sensitivity to different diseases (Al-Sharif et al, 2023). As for cattle genetics, in the context of issues of resistance to diseases, some of the best-investigated genes relevant for the study are the *TLR*-family (the family of toll-like receptors) (Mazzone et al, 2023; Maljković 2023), *IFNGR2* (Muthusamy et al, 2019; Holder et al, 2020), *TNFα* (Sattar et al, 2019), and *MBL1* (Aksel et al, 2021; Kamaldeep et al, 2021; Moretti et al, 2021). For example, associations between different allelic variants of genes in the *TLR* gene family and the resistance of individuals from various cattle breeds to paratuberculosis, tuberculosis, and mastitis have been studied (Cinar et al, 2018; Bartens et al, 2021; Chen et al, 2022). Associations with resistance to mastitis have also been demonstrated for genes such as *MBL1* and *TNFα* (Aksel et al, 2021; Sattar et al, 2019).

This study is a continuation of our previous investigations which focused on polymorphism of genes *TLR1*, *TLR4*, and *SLC11A1* in populations of Ukrainian Black-and-White dairy breed, Ukrainian Red-and-White dairy breed, Ukrainian Gray cattle (Kulibaba et al, 2021; Ivashchenko & Kulibaba, 2022). In this case, our focus is on studying beef cattle to address the question: Do the observed patterns of allele and genotype frequencies at identified polymorphic loci align with general trends, or do they characterize cattle breeds based on their productivity direction (meat or dairy)? Thus, the aim of this publication is to investigate the polymorphism of *TLR1*, *TLR4*, *IFNGR2*, *SLC11A1*, *TNFα*, and *MBL1* genes in a Charolais beef cattle population.

MATERIALS AND METHODS

The study was conducted in the Laboratory of Molecular-Genetic Studies in Animal Science at the Livestock Farming Institute of NAAS of Ukraine, and in the Laboratory of Molecular-Genetic Studies

at the Department of Animal Biology of the National University of Life and Environmental Sciences of Ukraine.

The study involved a population of Charolais beef cattle (n = 100), kept at the agrocompany Pryvillia, Luhansk region, Ukraine.

Whole blood samples (4 ml) were used as a source of biological material. Samples were stored at –8 °C before examination and further testing. The commercial DNA-Sorb-B kit was used to isolate DNA according to the manufacturer's protocol (AmpliSense Biotechnologies, Russia).

To detect polymorphisms the following marker systems were used:

Bc1I-polymorphism of gene *TLR1* (chromosome 6, exon 1, 1596G>A, rs207882984, V>I, GTC>ATC);

BsiHKAI-polymorphism of two genes – *TLR4* (chromosome 8, exon 3, 2021C>T, rs8193069, 674Thr>Ile) and *IFNGR2* (chromosome 1, MN473461.1, exon 7, 1008A>G, rs109579937);

MspI-polymorphism (missense mutation 8731A>G, 8732G>A, 322Ser>Asp), and *RsaI*-polymorphism (synonymic mutation 8834G>C) of gene *TLR4* (chromosome 8, DQ839567.1, exon 3);

PstI-polymorphism of gene *SLC11A1* (chromosome 2, 2 SNP – exon 11 7400C>G, A>P and intron 12 7808A>T);

SacI-polymorphism of gene *TNFα* (chromosome 23, Z14137.1, promoter fragment, –824A/G);

StyI-polymorphism of gene *MBL1* (chromosome 28, MN473461.1, exon 2, 2651G>A, GTT(Val)>ATT (Ile), rs109492835).

The information about the primers, amplicon lengths and restriction endonucleases is shown in the **Table 1**.

The selected genome fragments were amplified using either a AMPLY-4 (Biocom, Russia) or a MiniAmp Thermal Cycler (Applied Biosystems, USA) using the following programs: 1 cycle – denaturation at 94 °C 5 min; 35 cycles – denaturation at 94 °C 45 s; annealing for 45 s (64 °C – for *TLR1*; 59 °C – for *TLR4*; 60 °C – for *SLC11A1*; 56 °C – for *IFNGR2*; 60 °C – for *TNFα*; 53 °C – for *MBL1*); elongation for 72 °C 45 s; 1 cycle – final elongation at 72 °C for 10 min. The volume of the final mixture was 20 μL that included 10 μL of PCR Master mix (Thermo Scientific DreamTaq PCR Master Mix 2X), 5 μL of sample and 5 ul of each primer (the final concentration of primers – 0.2 μM).

The genotyping of animals was conducted using PCR-RFLP and ACRS-PCR (the latter only for *MBL1*). The restriction procedure was conducted according to the manufacturer's protocols (New England Biolabs). The electrophoresis of the restriction products was performed using 1.5–3.0 % agarose gels. Ethidium bromide (0.5 μg/mL) concentration used was used to visualize fragments in the ultraviolet spectrum.

The sizes of DNA fragments were determined using the molecular mass markers: 100 bp Ladder

Table 1. Primer sequences and restriction endonucleases

SNP	Primer sequence	Amplicon length (bp)	Restriction enzyme	Reference
TLR1 (1596G>A)	tttagcagcctttccatact; cagatccaggtagatacagag	354	*Bc1I*	Arslan et al, 2018
TLR4 (8732G>A; 8834G>C)	gcctaaaccacctctccac; agaagggcttgtagacttct	682	*MspI* *RsaI*	Elmaghraby et al, 2018
TLR4 (2021C>T)	aacaggtagcccagacagcatttc; gcacgccctcctccaagttc	579	*BsiHKAI*	Beecher et al, 2010
SLC11A1 (7400C>G; 7808A>T)	tgtgcttcacatctccttccta; agcacattgagcaggtcgtt	936	*PstI*	Liu et al, 2017
IFNGR2 (1008A>G)	atcttagatgccctggac; cgactgaacgactttcac	306	*BsiHKAI*	Prakash et al, 2014
TNFα (-824A/G)	gagaaatgggacaacctcca; ccaggaactcgctgaaactc	249	*SacI*	Bojarojć-Nosowicz et al, 2015
MBL1 (2651G>A)	ggtggcaaatgttggcta; gtcttctgagcatcctcca	162	*StyI*	Wang et al, 2011

DNA Marker (MD1002, Simgen; Size Range:100 to 3000 bp), 50 bp Ladder DNA Marker (MD1001, Simgen; Size Range: 50 to 500 bp), and GeneRuler 50 bp DNA Ladder (SM0371, Thermo Fisher Scientific; Size Range: 50 to 1000 bp).

TLR1 (*Bc1I*-polymorphism): allele A (*Bc1I*+) is presented on the electrophoregram by fragments of 261, 72, and 21 bp; allele G (*Bc1I*-) – 333 and 21 bp (Arslan et al, 2018).

TLR4 (*BsiHKAI*-polymorphism): allele C (*BsiHKAI*+) is presented by fragments of 485 and 84 bp; allele T (*BsiHKAI*-) – 579 bp (The size of restriction fragments was calculated based on analysis of the flanking fragment of the *TLR4* gene, AH013178.2).

TLR4 (*MspI*-polymorphism): allele A (*MspI*-) is presented by a fragment of 682 bp, allele B (*MspI*+) – 490, 110, and 82 bp (Elmaghraby et al, 2018).

TLR4 (*RsaI*-polymorphism): allele A (*RsaI*-) – 682 bp; allele G (*RsaI*+) – 365, 211, and 106 bp.

SLC11A1 (*PstI*-polymorphism). There are two SNPs (SNP5 and SNP6); SNP5 is presented by variants C (*PstI*-) and G (*PstI*+), SNP6 – A (*PstI*+) and T (*PstI*-). Both SNP are analyzed simultaneously; standard genotypes correspond to haplotype patterns. Haplotype CA is presented by fragments of 709 and 227 bp, GT – 633 and 303 bp, GA – 406, 303, and 227 bp, CT – 936 bp (Liu et al, 2017).

IFNGR2 (*BsiHKAI*-polymorphism): allele A (*BsiHKAI*-) is presented by a fragment of 306 bp; allele G (*BsiHKAI*+) – 200 and 106 bp (The size of restriction fragments was calculated based on analysis of the flanking fragment of the *IFNGR2* gene, XM_005201116.3).

TNFα (*SacI*-polymorphism): allele A (*SacI*+) is presented by fragments of 168 and 81 bp; allele G (*SacI*-) – 249 bp (Bojarojć-Nosowicz et al, 2011).

MBL1 (*StyI*-polymorphism): allele A (*StyI*+) is presented by fragments of 141 and 21 bp; allele G (*StyI*-) – 162 bp (Wang et al, 2011).

The results of the studies on each determined polymorphic locus were used to estimate the frequencies of genotypes and alleles, observed and expected distribution of genotypes, the correspondence of genetic Hardy-Weinberg equilibrium using χ^2, the parameters of expected (H_e) and observed (H_o) heterozygosity, the effective number of alleles (n_e) and Wright's fixation index (F_{is}). For statistical calculations Popgen32 software was used (https://sites.ualberta.ca/~fyeh/popgene_download.html).

RESULTS

It was found that loci *TLR1*, *IFNGR2*, *SLC11A1*, *TNFα,* and *MBL1* were polymorphic, and *TLR4* monomorphic. The detailed analysis of the obtained results by each specific polymorphism is as follows (also summarized in Table 1).

Restriction analysis using *Bc1I* showed polymorphism of locus *TLR1*, first exon (1596G>A) see **Fig. 1**.

Two genotypes, AG and GG, out of three possible ones, were observed. The mutant allele *Bc1I*+ (the presence of restriction site in position 1596) was found in 45 % of animals. No animals with genotype *Bc1I*+/*Bc1I*+ were found.

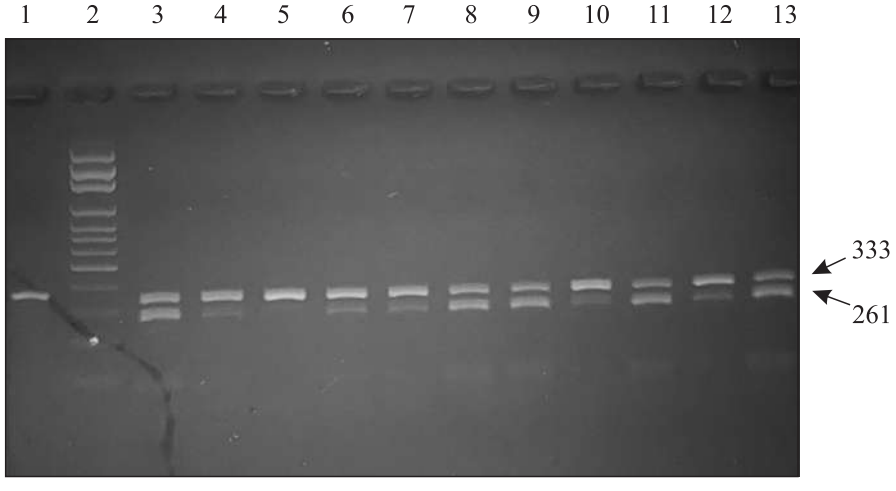

Fig. 1. The electrophoregram of Bc1I-restriction products of the first exon of gene *TLR1* (1596G>A).; 1, 5 – genotype GG; 2 – 100 bp Ladder DNA Marker; 3, 4, 6–13 – genotype AG

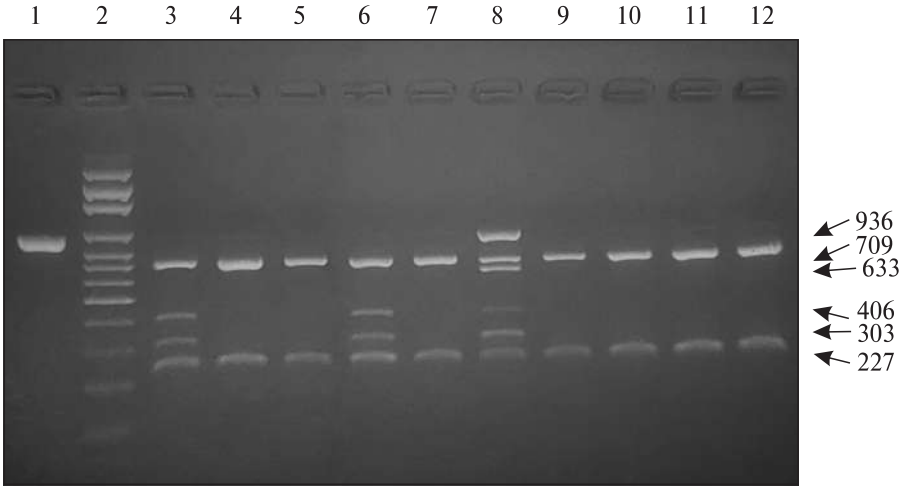

Fig. 2. The electrophoregram of restriction products of the fragments of gene *SLC11A1* (SNP5 and SNP6). 1–12 – numbers of samples; 1 – amplicon; 2 – 100 bp Ladder DNA Marker; 3, 6 – genotypes CG and AA; 4, 5, 7, 9–12 – genotypes CC and AA; 8 – genotypes CG and AT

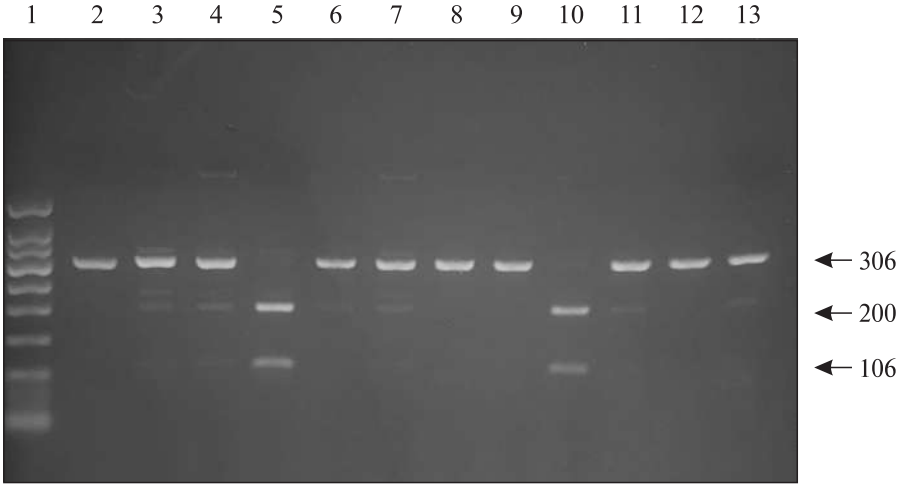

Fig. 3. The electrophoregram of restriction products of the seventh exon of gene *IFNGR2* (1008A>G). 1–13 – numbers of samples; 1 – 50 bp Ladder DNA Marker; 2, 3, 6–9, 11–13 – genotype AA (*BsiHKAI*-/*BsiHKAI*-); 4 – genotype AG (*BsiHKAI*+/*BsiHKAI*-); 5, 10 – genotype GG (*BsiHKAI*+/*BsiHKAI*+)

Locus *TLR4* was studied in three mutant variants following restriction, namely *MspI* (8732G>A), *RsaI* (8834G>C), and *BsiHKAI* (2021C>T). This locus proved monomorphic with all three restriction enzymes. *MspI* restriction yielded only genotypes BB (*MspI*+/*MspI*+). Only homozygotes GG (*RsaI*+/*RsaI*+) were obtained for *RsaI*. As for *BsiHKAI*-polymorphism, there were restrictive fragments of 485 and 94 bp, which indicated the animals with genotype CC (*BsiHKAI*+/*BsiHKAI*+), respectively.

PstI was used to study the polymorphism of locus *SLC11A1* (**Fig. 2**).

Four complex genotypes by two restriction sites were observed: $7400^{CC}/7808^{AA}$, $7400^{CG}/7808^{AT}$, $7400^{CG}/7808^{AA}$ and $7400^{GG}/7808^{AA}$. The most prevalent complex genotype was $7400^{CC}/7808^{AA}$, up to 75 % of the total number of animals in the population. As it was discovered in our previous studies (Kulibaba et al, 2021), incomplete activity of *PstI* may lead to possible mistakes in the genotyping of animals indicating the need for strict compliance with the restriction procedure (primarily due to the large number of fragments).

The polymorphism of the gene of interferon gamma-receptor 2 (*IFNGR2*) was determined by using *BsiHKAI* for the SNP mutation 1008A>G in the seventh exon of the gene, viz. A (*BsiHKAI*-) and G (*BsiHKAI*+). All possible genotypes were found in the 100 animals studied (**Fig. 3**).

Table 2. The genetic structure of Charolais cattle (n = 100) after analysis of 6 polymorphic loci

Genotypes						Alleles		χ^2
TLR1 (1596G>A)								
AA		AG		GG		A	G	66.95
O	E	O	E	O	E	0.450	0.550	
0	20.25	90	49.5	10	30.25			
SLC11A1 (SNP5 7400C>G)								
CC		CG		GG		C	G	7.067
O	E	O	E	O	E	0.790	0.210	
58	62.41	42	33.18	0	4.41			
SLC11A1 (SNP6 7808A>T)								
AA		AT		TT		A	T	0.021
O	E	O	E	O	E	0.985	0.015	
97	97.02	3	2.96	0	0.02			
IFNGR2 (1008A>G)								
AA		AG		GG		A	G	0.069
O	E	O	E	O	E	0.745	0.255	
55	55.50	39	38.00	6	6.50			
TNFα (-824A>G)								
AA		AG		GG		A	G	0.723
O	E	O	E	O	E	0.360	0.640	
11	12.96	50	46.08	39	40.96			
MBL1 (2651G>A)								
AA (*StyI+/StyI+*)		AG (*StyI+/StyI-*)		GG (*StyI-/StyI-*)		A	G	13.710
O	E	O	E	O	E	0.245	0.755	
13	6.00	23	36.00	64	57.00			

Note: O – observed number of animals of a particular genotype; E – expected number of animals of a particular genotype

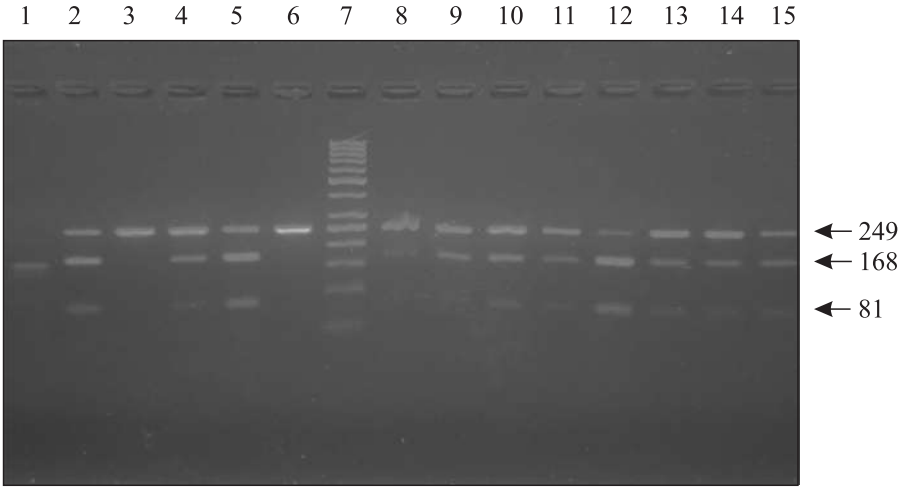

Fig. 4. The electrophoregram of restriction products of the promoter area of gene *TNFα* (-824A>G). 1–15 – numbers of samples; 1 – genotype AA; 2, 4, 5, 9–15 – genotype AG; 3, 6, 8 – genotype GG; 7 – GeneRuler 50 bp DNA Ladder

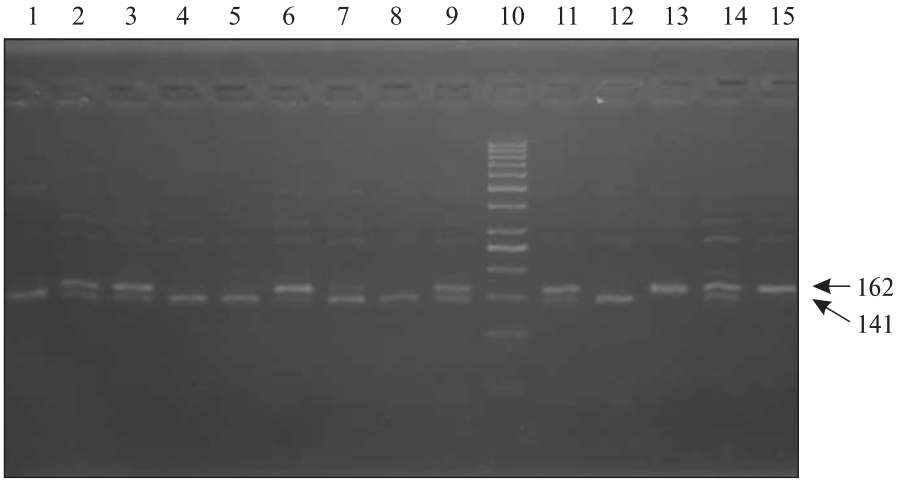

Fig. 5. The electrophoregram of restriction products of the second exon of gene *MBL1* (2651G>A). 1–15 – numbers of samples; 1, 4, 5, 7, 8, 12 – genotype AA (*StyI*+/*StyI*+); 2, 3, 9, 11, 14 – genotype AG (*StyI*+/*StyI*-); 6, 13, 15 – genotype GG (*StyI*-/*StyI*-); 10 – GeneRuler 50 bp DNA Ladder

Table 3. The general genetic and populational parameters of Charolais beef cattle (n = 100) for 6 polymorphic loci

Locus	C_a	n_e	H_o	H_e	F_{is}
TLR1 (1596G>A)	0.505	1.980	0.900	0.495	–0.818
SLC11A1 (7400C>G)	0.668	1.497	0.420	0.332	–0.265
SLC11A1 (7808A>T)	0.970	1.031	0.030	0.030	0
IFNGR2 (1008A>G)	0.620	1.613	0.390	0.380	–0.026
TNFα (–824A>G)	0.539	1.855	0.500	0.461	–0.085
MBL1 (2651G>A)	0.630	1.587	0.230	0.370	0.378

Note: C_a – homozygosity coefficient; n_e – effective number of alleles; H_o – observed heterozygosity; H_e – expected heterozygosity; F_{is} – Wright's fixation index.

The mutational allele G, which has a restriction site for *BsiHKAI*, was found with a frequency of 0.255. The animals with the homozygous genotype GG amounted to 6 % of the total number in the group, and the share of heterozygotes AG was 0.39 (**Table 2**).

SacI-restriction of *TNFα,* also yielded polymorphism (in mutation –824A>G, see **Fig. 4**).

Allele G dominated (0.64); the share of homozygous genotypes GG was 0.39. Homozygotes AA occurred with a frequency of 0.11 (11 % of the total number of animals).

As for *StyI*-polymorphism of the second exon of gene *MBL1* (2651G>A), we found animals with all the possible variants (AA, AG, and GG), see **Fig. 5**.

Frequency of allele A was 0.245; the amount of heterozygous (AG) and homozygous (AA) animals was 23 and 13 %, respectively.

It should be noted that for the polymorphism of gene *SLC11A1* two SNPs (SNP5 and SNP6) were studied.

The general genetic and populational parameters based on the 6 polymorphic loci studied are presented in **Table 3**.

DISCUSSION

For locus *TLR1* we observed a near equivalence in the frequencies of alleles A and G (0.45 vs 0.55), however, the frequencies of genotypes differed considerably with an excess of heterozygous animals (81.8 %). There were no animals homozygous by allele A in the animal population. This locus showed the highest value of the effective number of alleles (1.98) among the 5 genes studied, which actually is the maximal level of polymorphism for two-allele systems. Negative values of the fixation index were also noted for other cattle breeds reared in Ukraine (Kulibaba et al, 2021), but

they did not reach such extreme values. The number of animals, homozygous for allele G, as compared to homozygotes AA was high 90 against 0. By this index, meat Charolais cattle was similar to the Ukrainian Gray cattle of dual-purpose cows, which did not have animals with genotype AA either, although AG and GG percentages were substantially different (Kulibaba et al, 2021).

For *TLR4*, our findings were completely opposite to those of *TLR1*. There was no variation for the investigated mutations (8732G>A, 8834G>C, and 2021C>T), i.e. the locus was monomorphic. The results obtained are in full agreement with other data obtained for dairy and dual purpose cattle. The locus was monomorphic also in cattle breeds of dairy (Ukrainian Black-and-White dairy breed, Ukrainian Red-and-White dairy breed) and combined (Ukrainian Gray cattle) directions of productivity (Kulibaba et al, 2021; Okuni et al, 2021).

The monomorphism of the locus excludes it of being a candidate for marker-assisted selection.

For locus *SLC11A1* there was complete domination of the frequency of allele A over T (0.985 vs 0.015) for SNP6 7808A>T. Allele T actually even did not exceed the commonly accepted threshold value for the polymorphism of the locus (5 %) (King et al, 2006). Moreover, allele T occurred only in the heterozygotes (3 % of the total number of the sampling). This means that there is a final fixation of allele A, becoming completely monomorphic in terms of this mutation. As a result, the value of the effective number of alleles was the lowest among those of the other genes studied.

For the second SNP of locus *SLC11A1* studied (SNP5 7400C>G), the general tendency was similar to that of SNP6 7808A>T – no animal was homozygous for allele G, but this polymorphism had relatively high values of heterozygosity, both observed (0.42) and expected (0.332). Due to the presence of a considerable number of heterozygous animals, allele G was present in rather a large number (21 %).

This fact is also highlighted by Liu et al. In their study, they found that the linkage disequilibrium analysis of single nucleotide polymorphisms (SNP5 and SNP6) in the *SLC11A1* gene suggests a close linkage between these SNPs (Liu et al, 2017). The high frequencies of alleles C (SNP5) and A (SNP6) observed for Charolais cattle in this case, were also observed for some other breeds, both dairy and dual-purpose cattle reared in Ukraine (Kulibaba et al, 2021). However, the maximum tendency towards allele A fixation by SNP6 7808A>T was found only in the Charolais breed.

The analysis of polymorphism at the *IFNGR2* locus revealed a predominance of allele A over G, with frequencies of 0.745 and 0.255, respectively. The AA genotype was the most prevalent, accounting for 55 % of individuals in the population. This locus suggested a balanced population state with a heterozygotes excess of 2.6 %. The effective number of alleles for the *IFNGR2* gene is at an intermediate level, indicating an average level of polymorphism. In comparison to dairy breeds of Ukrainian selection, the Charolais breed exhibits the most significant differences in allele frequencies (A vs G) and the highest number of animals with genotype AA (Ivashchenko et al, 2023). A (limited) prevalence of allele A frequency was also obtained for indigenous and crossbred animals in India (Prakash et al, 2014).

Locus *TNFα* (–824A>G) demonstrated the prevalence of the frequency of allele G over A (almost twofold) due to a larger number of animals with genotype GG (39 %) and 50 % heterozygous animals. An excess of heterozygotes was also shown to be present in a population of Holstein dairy cattle (Kulibaba et al, 2024). Furthermore, in the population of black-breed dairy cattle in Poland, similar values of A and G allele frequencies were observed, with frequencies of 0.47 and 0.53 respectively, indicating a slight predominance of the G allele frequency (Bojarojć-Nosowicz et al, 2011). Research conducted by Konnai et al. also demonstrated a predominance of the G allele frequency over the A allele in populations of Japanese Black and Holstein-Friesian cattle breeds (Konnai et al, 2006). Conversely, a prevalence of the A allele frequency over the G allele frequency (0.54 vs 0.46) was observed in the population of Holstein cattle of Canadian selection (Bimenova et al, 2018). Hence, the observed pattern of *TNFα* gene allele frequency distribution may suggest differences in breeding practices among different cattle breeds.

For locus *MBL1,* we found a prevalence of the frequency of allele G (*StyI*-) as compared to A (*StyI*+) due to a considerable number of homozygous animals GG (64 %). A similar tendency was also observed in a population of Chinese Holstein and some indigenous cattle breeds (Liu et al, 2011). However, for the Ukrainian dairy and dual-purpose cattle breeds studied earlier (Ivashchenko, 2023; Kulibaba et al, 2024), a prevalence of allele A was observed, which potentially may be a result of selection. It should be noted for locus *MBL1* was the only one studied so far, where we determined a high level of proper homozygous animals (the inbreeding level was 37.8 %).

In general, it was demonstrated that among the six polymorphic loci studied, the population of Charolais beef cattle was in genetic Hardy-Weinberg equilibrium for *SLC11A1* (7808A>T), *IFNGR2*, and *TNFα*. However, *TLR1*, *SLC11A1* (7400C>G), and *MBL1* were not in equilibrium. The deviation of the latter loci from the state of genetic equilibrium may indicate drifting of genes, or selection pressure in the said population. We intend to do further similar studies in a larger population of Charolais beef cattle and also perform transition studies (effects on disease resistance/susceptibility, qualitative traits) in the investigated populations of cattle studied so far.

CONCLUSION

Some specificities in the genetic structure of Charolais beef cattle population (cows, n = 100) were analyzed by DNA-markers. In the population studied, genes *TLR1*, *IFNGR2*, *SLC11A1*, *TNFα,* and *MBL1* were polymorphic, while *TLR4* by three mutant variants (8732G>A, 8834G>C, and 2021C>T) was found to be monomorphic. *BclI*-polymorphism of the first exon of the *TRL1* gene (1596G>A) was used to determine an excess of heterozygous animals (81.8 %); no animals was homozygous for allele A. Polymorphism of locus *SLC11A1* for SNP6 (7808A>T) showed a complete domination of the frequency of allele A over T (0.985 vs 0.015), and animals, homozygous for allele T, were completely absent. As for SNP5 7400C>G, there were no animals homozygous for allele G; the ratio of alleles C and G was 0.79 and 0.21, respectively. Polymorphism in locus *IFNGR2* (1008A>G), showed the prevalence of the frequency of allele A over G (0.745 vs 0.255); genotype AA was most common. As for gene *TNFα* (–824A>G), we determined a two-fold prevalence of the frequency of allele G over A due to a higher number of animals with genotype GG (39). Some 50 of the animals were heterozygous for this gene. Locus *MBL1,* showed a prevalence of the frequency of allele G (*StyI*-) as compared to A (*StyI*+) due to a considerable number of homozygous animals GG (64 %); The percentage of homozygous animals was 37.8 %. In general, out of six observed polymorphic loci, only by *SLC11A1* (7808A>T), *IFNGR2,* and *TNFα* were genetic Hardy-Weinberg equilibrium. The loci *TLR1*, *SLC11A1* (7400C>G), and *MBL1* were not in equilibrium. The genes *TLR1*, *IFNGR2*, *SLC11A1* likely offer little perspective for further association studies due to a lack of sufficient genotypes found in the Charolais population. The other genes *TNFα* and *MBL1* are more promising in this respect.

Adherence to ethical principles. All procedures performed in the studies involving animal participants were in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 1986.

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Поліморфізм генів у популяції шаролезької м'ясної худоби за використання ДНК-маркерів

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Мета. Дослідити поліморфізм генів, асоційованих з резистентністю до різних захворювань (*TLR1*, *TLR4, SLC11A1, TLR1*, *TLR4*, *IFNGR2*, *SLC11A1*, *TNFα,* та *MBL1*) у популяції корів м'ясної породи шароле. **Методи.** Дослідження проведено за використання методів полімеразної ланцюгової реакції та рестрикційного аналізу (PCR-RFLP), а також, для дослідження поліморфізму гену *MBL1*, за використання методу створення штучного сайту рестрикції (ACRS-PCR). **Результати**. У популяції (n = 100) корів породи шароле гени *TLR1*, *IFNGR2*, *SLC11A1*, *TNFα* та *MBL1* є поліморфними, в той час як ген *TLR4* за трьома мутантними варіантами (8732G>A, 8834G>C, and 2021C>T) виявився мономорфним. За *BclI*-поліморфізмом першого екзону гену *TRL1* (1596G>A) встановлений ексцес гетерозиготних особин (81,8 %), повністю відсутні гомозиготні за алелем А особини. За поліморфізмом локусу *SLC11A1 для* SNP6 (7808A>T) встановлено повне домінування частоти алелю А над Т (0,985 vs 0,015), особин, гомозиготних за алелем Т, не виявлено. За SNP5 7400C>G повністю відсутні гомозиготні за алелем G особини, співвідношення алелів

C та G становить 0,79 та 0,21 відповідно. За локусом *IFNGR2* (1008A>G) встановлено переважання частоти алелю A над G (0,745 vs 0,255); найчастіше зустрічалися особини з генотипом AA. За геном *TNFα* (–824A>G) встановлено двократне превалювання частоти алелю G над A за рахунок більшої кількості особин з генотипом GG (39 %). Близько 50 % особин були гетерозиготними за цим геном. За локусом *MBL1* виявлено переважання частоти алелю G (*StyI*-) у порівнянні з A (*StyI*+) за рахунок значної кількості гомозиготних особин GG (64 %). Відсоток гомозиготних тварин становив 37,8 %. **Висновки**. Встановлено загальні характеристики генетичної мінливості популяції великої рогатої худоби породи шароле за локусами *TLR1*, *TLR4*, *IFNGR2*, *SLC11A1*, *TNFα* та *MBL1*. Для SNP6 7808A>T локусу *SLC11A1* виявлено фінальну стадію фіксації алелю A, за результатами якої ген повністю стане мономорфним за цією мутацією. За результатами аналізу співвідношення частот алелів і генотипів за виявленими поліморфними маркерами, показано недоцільність використання особин з різними алельними варіантами генів *TLR1*, *IFNGR2*, *SLC11A1* у подальших дослідженнях з пошуку асоціацій з проявом господарсько-корисних ознак у корів м'ясної породи шароле дослідної популяції, внаслідок недостатньої чисельності особин з різними генотипами. За локусами *TNFα* та *MBL1* виявлено достатню кількість особин з різними генотипами для проведення подальших асоціативних досліджень. За локусами *TLR1*, *SLC11A1* (7400C>G) та *MBL1* у дослідній популяції встановлено відхилення від стану генетичної рівноваги за Харді-Вайнбергом.

Ключові слова: поліморфізм, мінливість, алель, ген, популяція, резистентність.

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