IDENTIFICATION OF SOME GENETIC MARKERS AS PRODUCTIVE AND REPRODUCTIVE TRAITS IN UKRAINIAN DAIRY CATTLE BREEDING

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Supporting Information

ABSTRACT: Selection based on DNA markers is a breeding technique grounded in the genomic selection value of animals. The aim of the research is to study the genotypic profile of cows of different breeds in Ukrainian breeding in relation to the genes LEP, CSN3, TG5, BLG, and Pit-1 and to identify the probability of using them as markers for reproductive traits in cows. During the study, the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method was used to analyze the genes and determine their polymorphic characteristics. The obtained data indicated that the same gene variants have varying effects in the studied breeds due to their diverse influences on the genomic background. Specifically, the homozygous state of leptin genes (LEP^{CC}) and pituitary-specific transcription factor (PIT-1^{BB}) was observed to impact the reproductive characteristics of cows in the evaluated breeds. Meanwhile, for the genes casein (CSN3^{AB}), thyroglobulin (TG5^{CT}), and beta-lactoglobulin (BLG^{AB}), the heterozygous state of alleles was found to influence the key reproductive traits of dairy cattle of Holstein origin. The polymorphism of the genes CSN3, βLG, TG, PIT-1, and LEP indicated the presence of genetic potential for the reproductive function of cows and can be utilized as molecular markers in selective breeding, providing significant progress in improving not only the traits of dairy cattle productivity but also reproductive function. Therefore, in the implementation of selective breeding work, it is advisable to consider genotyping for the genes CSN3, βLG, TG, PIT-1, and LEP as an additional criterion for the selection of animals to enhance both their milk and reproductive characteristics.

Keywords: CSN3, Dairy cattle, Genetic potential, Marker genes, Polymorphism, Reproductive function.

INTRODUCTION

Genomic selection has proven its effectiveness in improving the genetic structure for key selection traits in dairy cattle breeding systems. In milk production, the reproductive capacity of *Bos taurus primigenius* plays a key role in the efficiency of production, determining the profitability and cost-effectiveness of breeding dairy cows (Mello et al., 2020). Genomic selection programs have demonstrated their utility in enhancing the genetic gain of characteristics related to the productivity of cattle; however, progress in reproductive traits has been considerably slower, primarily due to low heritability (Keogh et al., 2021). Moreover, traits associated with reproductive capability typically manifest in the third trimester of an animal's productive life. Thus, traditional breeding methods require more time to assess the reproductive potential of individual animals (De Melo et al., 2017). However, even with these restrictions, the level of genetic variability in dairy cattle is significant enough to enable breeding programs to enhance reproductive efficiency (Berry et al., 2014). In the era of genomic selection, methodologies for studying cows are numerically extensive, combining phenotypes with high-yield genomic SNP marker data. This allows the implementation of selection strategies for new functional traits. Another important advantage of genomic selection is the substantial reduction in generation intervals (Mahmoud et al., 2017).

Berry et al. (2014) highlighted that in both dairy and beef cattle, there is a multitude of reproductive phenotypes, but most reproductive traits in dairy and beef cows exhibit low heritability (ranging from 0.02 to 0.04). Reproductive phenotypes in male animals, such as sperm quality, as a rule are more clearly heritable than reproductive phenotypes in females. However, the low heritability of reproductive traits, especially in females, does not imply that genetic selection cannot alter phenotypic indicators. This is evidenced by the recent decline in reproductive performance in dairy cows, partially linked to aggressive selection for increased milk production. Furthermore, antagonistic genetic correlations between reproductive traits and milk or meat productivity are not universally applicable, meaning that simultaneous genetic selection for both increased productivity (milk and meat) and reproductive capability is indeed possible (Berry et al., 2014). Meanwhile, Raven et al. (2014) argue that genome-wide association studies (GWAS) in most breeds of cattle result in large genomic intervals of significant associations, complicating the identification of causal mutations. This occurs due to extensive linkage disequilibrium within breeds of cattle. Since there is less linkage disequilibrium between breeds, multi-breed GWAS may enhance the accuracy of mapping causal variants (Raven et al., 2014).

Intra-breed analysis indeed offers the advantage of capturing a higher proportion of variation, but multi-breed GWAS leads to a more precise mapping of QTLs that genuinely differ between breeds (Olson et al., 2012). Therefore, the aim of the research was to leverage SNP associations of the genes LEP, CSN3, TG5, BLG, and Pit-1 with reproductive traits in cows of different breeds in Ukrainian selection. The application of a multi-breed strategy in dairy cattle could be beneficial for refining the location of QTLs and determining the likelihood of using data from these genes as markers for reproduction in *Bos taurus primigenius*.

The application of identified Quantitative Trait Loci (QTL) for the genetic evaluation of animal performance traits, as opposed to traditional breeding programs based solely on phenotype and pedigree information, holds significant potential for enhancing selection accuracy and accelerating genetic improvement in animal productivity (Jiang et al., 2010; Kumar, 2017). The development of Polymerase Chain Reaction (PCR) technologies and the integration of molecular-genetic methods in animal husbandry have facilitated rapid analysis of the correlation between gene allelic variants and performance traits. DNA marker technologies enable the identification of genomic regions (Quantitative Trait Loci or QTL) associated with complex genetically determined traits. The implementation of such molecular-genetic procedures in evaluation significantly enhances selection precision, thereby expediting the genetic enhancement of desirable animal qualities (Kusza et al., 2015; Gritsienko et al., 2022).

Over the past decades, numerous studies have been conducted to identify QTL for dairy performance traits in cattle. However, the investigation of the association between Single Nucleotide Polymorphisms (SNPs) of genes and reproductive traits has revealed various discrepancies among researchers (Oikonomou et al., 2009; Trakovická et al., 2013; Cañizares-Martínez et al., 2021). It is known that leptin genes and leptin receptor genes are considered markers for productivity traits in dairy or beef cattle. Therefore, Trakovická et al. (2013) aimed to investigate associations of polymorphisms in LEP and LEPR genes in cattle breeds, such as Slovak and Pinzgau, with production and reproductive traits. The research results indicated that leptin, in particular, is a candidate gene that primarily influences milk production traits and can be implemented in breeding strategies to enhance the productivity of cattle breeds.

At the same time, Oikonomou et al. (2009) presented opposing data. According to the authors, the DGAT1 alleles and growth hormone receptors, responsible for significant increases in milk production, have a negative impact on reproductive function. Additionally, the leptin allele, associated with a significant increase in milk production, was linked to a slight increase in the frequency of endometritis in cows.

Cañizares-Martínez et al. (2021) assessed allele and genotype frequencies of markers in leptin (LEP), pituitary transcription factor (PIT-1), and luteinizing hormone receptor (LHR) genes, examining their impact on reproductive traits and milk productivity in Holstein cattle. The research results showed that polymorphisms in LEP, PIT-1, and LHR may serve as candidates for marker-assisted selection, with alleles A and G being positively associated. Polymorphisms in LEP and LHR positively influenced interval traits and age at first calving, respectively. These findings indicate that these polymorphisms are candidates for selection using reproductive trait markers. Additionally, a significant trend association with the SNP PIT-1 gene was confirmed by the scientists. This polymorphism is strongly linked to aspects of growth and development, particularly with allele A. The AA genotype tends to increase the number of services per conception (NCS), calving to calving interval (CC), and calving to conception interval (CCI) compared to other genotypes.

Al-Shari et al. (2022) investigated the DNA polymorphism of the FGFBP1, leptin, κ-casein, and αs1-casein genes and their association with reproductive function in dromedary camels. The authors concluded that multiple linear regression analysis (MLR) revealed that SNPs in FGFBP1, κ-casein, and αs1-casein significantly influence the age at first calving (AFC), the number of days to first estrus (CCI), the calving interval (CCI), and the number of services per conception (NSC). The coefficients of determination (R^2) also indicated that the variability in phenotypic measurements of the investigated traits may be correlated with the identified SNPs in genes associated with reproduction. Except for the leptin gene, which exhibited a monomorphic pattern and did not confirm its influence on reproductive qualities. Ateya et al. (2023) also pointed out that the identified SNPs in the genes of β-lactoglobulin, κ-casein, and DGAT1 could serve as candidates for marker-assisted selection (MAS) for milk composition, productive, and reproductive traits in Holstein dairy cattle.

Therefore, the use of molecular genetic methods in conjunction with traditional animal breeding approaches is a crucial element in achieving a balanced selection process and optimizing animal breeding programs. Our goal was to study markers that increase milk productivity, its quality indicators, animal health, productive longevity and reproductive function, which will facilitate and accelerate breeding progress, indicating its potential.

MATERIALS AND METHODS

Ethical regulation

The rules for handling animals in the experiment fully complied with European animal legislation protection and comfort on farms (Directive No. 95/58 EU "On the protection of farm animals" of the EU Council on 20.07.1998 as amended by EU Regulation No. 806/203 of 14.04.2003, No. 91/630 EC "Minimum standards for cows protection " dated November 19, 1991 as amended by the EU Regulation). Experimental protocol for blood sampling in cows, approved by the local bioethics commission of the Nikolaev National Agrarian University, Ukraine, on Good Clinical Practice (GCP) for the protection and humane treatment of experimental animals This study is reported in accordance with ARRIVE

guidelines (https://arriveguidelines.org). All methods were performed in accordance with the relevant guidelines and regulations. The study was conducted as part of the partial completion of scientific work for the degree of Candidate of Science (PhD) in genetics (agricultural science) at the Department of Biotechnology and Bioengineering of the Nikolaev National Agrarian University, Nikolaev, Ukraine. Before proceeding with sample collection, consent was obtained from the management of the enterprise.

Materials

To carry out the research, research groups were formed from breeding cows of dairy cattle of the direction of productivity. These included Ukrainian Red Dairy (URD, n = 32 head), Ukrainian Black-and-White Dairy (UBWD, n = 32 head), and Ukrainian Red-and-White Dairy (URWD, n = 28 head) breeds from the leading enterprise in the southern region of Ukraine, LLC "Kolos-2011" in Ochakiv district, Mykolaiv Oblast.

Methods

The polymorphism of the CSN3, βLG, TG, PIT-1, and LEP genes was determined using the PCR-RFLP method (Alexander et al., 1988; Pedrosa et al., 2021). According to the recommendations of the manufacturer's methodology of T. Maniatis and N. A. Zinovieva, genomic DNA was extracted from peripheral blood using the standard commercial kit "DNA-Sorb V" produced by "Amplisense" (Russia), (Pedrosa et al., 2021). The concentration of DNA was checked in a 2% agarose gel using electrophoresis. For the polymerase chain reaction (PCR) in this study, a reaction mixture with a volume of 10 µL was used: dH2O – 4.3 µL, 5x PCR buffer (15 mMg-1.0 mL) – 2.0 µL, dNTP mix 10x (2 mM each) – 0.8 µL, two primers (70 ng each) – 0.8 µL, Taq polymerase (1 U/µL) – 0.1 µL, DNA 50-100 ng – 2.0 µL.

Restriction products were separated by electrophoresis in a 2% agarose gel, followed by staining with ethidium bromide solution. Visualization of electropherograms was achieved using a digital camera on a transilluminator under UV light, with subsequent photography. Differentiation of the amplicons was conducted based on the molecular weight marker GeneRulerTM 50 bp DNA Ladder, SM0378 (Fermentas, Lithuania) to determine their sizes (Oztabak et al., 2008). For each gene, the temperature regime and the number of PCR amplification cycles were determined. To analyze the polymorphism of structural loci CSN3, βLG, TG, PIT-1, and LEP, restriction enzymes specific to each locus were used. Typing was performed immediately after the PCR analysis (Grobet et al., 1998).

Statistical analysis

Statistical data processing was carried out using the standard package "Microsoft Excel 2013."

RESULTS AND DISCUSSION

Over the past few decades, a widespread decline in reproductive capacity has been observed in dairy cows, partially attributed to unfavorable genetic correlations with key traits selected for milk productivity. Inadequate reproductive performance and an increase in involuntary culling escalate herd maintenance costs and veterinary sanitary measures, resulting in significant additional expenditures. Therefore, genomic selection for female fertility requires careful examination. One challenge in genomic selection for reproductive traits is the complexity and high polygenicity of reproduction in large ruminants. Genes identified as candidates for such traits may also exert pleiotropic effects on other economically important characteristics (Zhang et al., 2023). Hence, the results of this study could positively contribute to the genetic improvement of the reproductive function of dairy cattle and enhance the understanding of genes with pleiotropic effects for intricately conditioned reproductive traits.

Leptin is a protein actively involved in the growth and metabolism of animals, playing an important role in the regulation of feed consumption, energy metabolism, growth, and reproduction in large ruminants. The leptin gene consists of three exons separated by two introns and is localized on chromosome 4 in cattle (Gritsienko et al., 2022). Analyzing reproductive performance indicators in three groups of cows (Table 1) of different breeds during the I-III lactations and beyond, a certain tendency of correlation between respective traits and polymorphism in the LEP gene was identified. In Ukrainian Red dairy cattle, a positive and significant trend is observed in favor of cows with the CT genotype for the service period duration (88.43 ± 33.18 days) and, consequently, the calving index (4.42 ± 1.66 days). However, for the duration of the intercalving period, the difference favored the homozygous CC genotype. While this difference was statistically insignificant, cows with the CC genotype showed a reduction in the calving interval to 369 days.

In Ukrainian Red Pied dairy cows with the CC genotype, during the first lactation, they exceeded cows with CT and TT genotypes in the service period duration (137 ± 106 days) and calving index (6.85 ± 5.30 days). However, in the second lactation, homozygous animals with the CC genotype exhibited a poorer manifestation of this trait compared to other genotypes. Meanwhile, cows homozygous for the TT genotype demonstrated better reproductive performance. Analyzing indicators of the third lactation revealed a similar trend, with homozygous TT cows having an advantage in reproductive parameters. The interval from calving to first estrus was 44.0 days, the udder recovery period was 54 days, and the intercalving period reached 319 days. Accordingly, the calving index value was 2.2. Characteristics of the higher lactation showed similar trends, but in heterozygous cows with the CT in accordance calving interval was 362 ± 27.5 and 438 ±

68.5 days, respectively. It's worth noting that animals with the C allele in their genotype (CC/CT) had an advantage over homozygous cows with the TT genotype in reproductive traits. Ukrainian Black-and-White dairy cows with a homozygous CC genotype were the best in terms of the LEP gene polymorphism and reproductive performance.

In Ukrainian Black-and-White Dairy cows with the LEP[CT] genotype, during the first lactation, they outperformed homozygous cows with CC and TT genotypes in the service period duration (102 ± 49.9 days), calving index (5.10 ± 2.46), and calving interval (381 ± 50.6 days). As they aged (second and third lactations), homozygous cows with the *CC* genotype had an advantage in reproductive traits: service period duration (79 ± 19 and 166 ± 60 days); dry period (40 ± 22 and 75 ± 15 days), A certain trend was also noted in the duration of the dry period. Animals of all three breeds with the CT genotype had a shorter udder recovery period than homozygous cows with CC/TT genotypes: Ukrainian Red Dairy cows with the CT genotype by 11 days and TT by 6 days; Ukrainian Red Pied Dairy cows with the CT genotype by 25 days and TT by 9 days; Ukrainian Black-and-White Dairy cows by 19 and 18 days, respectively. It is noteworthy that throughout the entire research period, no statistically significant differences were observed in the polymorphism of the LEP gene concerning reproductive quality traits.

Similar data were obtained by Rambachan et al. (2017) It is now well established that leptin may be a strong candidate gene for cost-effective production as it controls traits such as subcutaneous fat thickness, feed intake and reproductive function. It is now well established that leptin may be a strong candidate gene for cost-effective production, as it controls traits such as subcutaneous fat thickness, feed intake, and reproductive function. it is reported that the LEP gene is located on the 4th chromosome in Bosprimigeniustaurus, is synthesized by adipose tissue and is involved in the regulation of feed intake, energy balance, and is responsible for fertility and immune functions of cattle. According to the authors, this polymorphism can be additionally evaluated for selection with the help of markers, and the developed PCR methodology will accelerate the screening of a large number of animals, and improve the selection of animals for reproductive qualities (Javanmard et al., 2008).

Similar data were obtained by Rambachan et al. (2017) which indicated that the SNP LEP/BsaAI significantly affected the gestation period and dry period of the evaluated population of Hariana cows. Therefore, the authors concluded that leptin is a candidate gene that affects reproductive traits and can be used in breeding, a strategy to improve the reproductive function of Harian cattle (Rambachan et al., 2017). One of the main milk proteins is casein, and of particular interest among the four casein milk proteins is β-casein, which contains 209 amino acid residues in the protein chain, and its gene (CSN3) belongs to a cluster of four casein genes located on chromosome 6, and has a decisive role in the quality of milk and its syrupability (Zhang et al., 2023).

During the analysis of signs of the reproductive capacity of cows of Ukrainian selection according to the kappa-casein locus (Table 2), it was established that representatives of the Ukrainian red dairy breed with the homozygous AA genotype differed in better reproductive capacity in terms of lactations. With the exception of the first lactation, where heterozygous AB individuals had a significant advantage, which had the shortest service period (96±33.5 days) and interval between calvings (381±36.7 days), which in turn improved the insemination index (4.84±1.68). During the II-III and higher lactations, the opposite tendency was noticed – the carriers of the homozygous genotype AA were better in all breeding traits, as their clear advantage was observed. Carriers of another homozygous BB genotype were not found in this group of cows during the study.

Individuals of the Ukrainian red-spotted dairy breed, which had the BB genotype, differed in better reproductive capacity at the age of the first and higher lactations, their index of the duration of the service period was 46±0 and 64±0 days, respectively. This affected the values of the insemination index (2.30±0 and 3.20±0), respectively, and contributed to an excellent interval between calvings (329±0 and 342±0 days), respectively. During the second II-III lactations, carriers of the heterozygous AB genotype had an advantage in terms of reproductive ability. Thus, the interval from calving to fertile insemination was 94±20.5 and 95±50.2 days, respectively, the dry period was 54±10.7 and 77±36.0 days, respectively, and the interval between calvings was at the level of 389±34.7 and 385±45.8 days, respectively. This was also reflected in the average values of the insemination index (4.72±1.02 and 4.79±2.51), respectively. In general, it should be noted that carriers of the B allele (AB/BB) have a better reproductive capacity among UCherRM cows, compared to females of the same age with the homozygous AA genotype. Research has not revealed carriers of the homozygous BB genotype among cows of the Ukrainian black-spotted dairy breed. At the same time, cattle of the Ukrainian black-spotted dairy breed, which had the AB genotype, with the exception of the first lactation, had better indicators of reproductive capacity. The duration of the service period in them during the II and III lactations was lower, compared to homozygous CSN3[AA] cows, by 27 days and 115 days, respectively, and was 111±22.5 and 120±60.7 days, and in the latter case by the second level of probability. The indicator of the insemination index was 5.58±1.13 and 6.00±3.03, respectively, which was a difference of 1.3 and 4.7

According to the data of higher lactation, animals with genotype AB again had significantly better indicators of reproduction than animals with genotype AA. Since no carriers of the homozygous BB genotype were found among the representatives of the UCM, it should be noted that the B allele has a greater influence on the indicators of reproductive capacity for the CSN3 gene, since cows with the AB genotype had better reproductive function values than their homozygous counterparts for the A allele. Similar studies were conducted by Ardicli et al. (2019).

Table 1 - Relationship of LEP Gene SNPs gene with reproductive traits of cows

Genotype g.0000 C>T ($\bar{X}\pm S_x$) / Trait/Indicator	CC			CT			TT		
	URD	URSD	UBWD	URD	URSD	UBWD	URD	URSD	UBWD
1st lactation	n =4	n =10	n =2	n =7	n =4	n =7	n =2	n =1	n =6
Duration of service period, days	124±45.3	137±106	108±10	124±47.9	261±104.6	102±49.9	115±33	193±0	161±76.9
Insemination index, times	6.23±2.26	6.85±5.3	5.40±0.5	6.21±2.39	13.09±5.23	5.10±2.46	5.75±1.65	9.65±0	8.08±3.84
Inter-calving period duration, days	408±48.1	414 ±104.4	369±9.5	406±48.1	441±57.5	381±50.6	399±34.5	485±0	433±69.8
2nd lactation	n =4	n =10	n =2	n =7	n =4	n =7	n =2	n =1	n =6
Duration of service period, days	254±157.9	204±114.7	79±19	134±57.1	125±55.8	89±20.5	178±28.5	86±0	179±53.2
Dry period duration, days	62±6.1	45±8.2	40±22	74±28	57±7.9	51±20.1	46±6.5	77±0	53±10.3
Insemination Index, times	12.71±7.89	10.24±5.73	3.95±0.95	6.74±2.86	6.28±2.79	4.48±1.03	8.93±1.43	4.3±0	8.99±2.66
Inter-calving Period Duration, days	445±54.6	418±51.7	362±27.5	398±55.6	378±18.7	377±28.2	466±30	362±0	475±56.7
3rd lactation	n =4	n =10	n =2	n =7	n =4	n =7	n =2	n =1	n =6
Duration of service period, days	100±42.2	113±21.8	166±60	111±40	88±15.8	188±130.7	111±53	44±0	181±67.6
Dry period duration, days	77±52.1	70±30	75±15	66±18.9	45±4	51±8.5	73±7	54±0	69±10.4
Insemination index, times	5.03±2.11	5.70±1.28	8.30±3	5.55±2	4.42±0.79	9.43±6.53	5.55±2.65	2.20±0	9.05±3.38
Inter-calving period duration, days	345±10.2	394±26.6	438±68.5	454±70.2	342±10.5	498±105.4	396±50.5	319±0	415±76.7
Higher Lactation	n =4	n =10	n =2	n =7	n =4	n =7	n =2	n =1	n =6
Duration of service period, days	96±59.3	127±49.4	138±88	88±33.2	121±58	219±111.7	129±18	183±0	192±55
Dry period duration, days	45±9.6	69±21.9	73±17	57±15.5	54±9.23	51±5.7	61±3.0	219±0	61±12.4
Insemination index, times	4.83±2.96	6.36±2.47	6.90±4.4	4.42±1.66	6.05±2.9	10.99±5.58	6.45±0.9	9.15±0	9.61±2.75
Inter-calving period duration, days	336±113.5	394±43.5	507±20	430±73.8	353±3.4	498±165.9	415±12	–	431±83.3

Significant: *=P<0.05; **=P<0.01; ***=P<0.001 (compared to the animals of the first control group); a = P<0.05; b= P<0.01. (compared to the animals of the third experiment group with analogues of the second experimental group). Mean values with different superscripts in the column differ significantly. Leptin (LEP); Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD)

Table 2 - Relationship of SNPs of the CSN3 gene with reproductive traits of cows

Genotype g.0000 C>T ($X \pm S_x$) Trait/Indicator	AA			AB			BB		
	URD	URSD	UBWD	URD	URSD	UBWD	URD	URSD	UBWD
1st lactation	n =10	n =8	n =9	n =6	n =6	n =6	n =0	n =1	n =0
Duration of service period, days	136±42.5	226±147.6	104 ±32.8	96±33.5	133±37.8	160±89.7	-	46±0	-
Insemination index, times	6.82±2.13	11.31±7.38	5.21±1.64	4.84±1.68	6.68±1.89	8.03±4.48	-	2.30±0	-
Inter-calving period duration, days	419±43.6	453±117.2	377 ±34.5	381±36.7	415±41.5	435±82.17	-	329±0	-
2nd lactation	n =10	n =8	n =9	n =6	n =6	n =6	n =0	n =1	n =0
Duration of service period, days	142±49.6	198±113.5	138±71.2	217±123.2	94±20.5	111±22.5	-	167±0	-
Dry period duration, days	66±21.6	51±7.4	48±8.9	65±11.8	54±10.7	54±10.3	-	51±0	-
Insemination index, times	7.11±2.48	9.90±5.68	6.91±3.56	10.86±6.16	4.72±1.02	5.58±1.13	-	8.35±0	-
Inter-calving period duration, days	418±56.8	409±49.8	435±70.5	427±76.2	389±34.7	387±25.0	-	443±0	-
3rd lactation	n =10	n =8	n =9	n =6	n =6	n =6	n =0	n =1	n =0
Duration of service period, days	117±41.9	111±23.5	235±85.4	134±61.2	95±50.2	120±60.7 [b] **	-	109±0	-
Dry period duration, days	73±28.3	51±12.5	62±11.5	134±61.2	77±36	61±12.7	-	46±0	-
Insemination index, times	5.86±2.09	5.57±1.4	11.77±1.27	6.71±3.06	4.79±2.51	6.00±1.03 [b] ***	-	5.45±0	-
Inter-calving period duration, days	406±57.8	385±42.7	473±116.2	450±50.5	385±45.8	443±69.9	-	385±0	-
Higher Lactation	n =10	n =8	n =9	n =6	n =6	n =6	n =0	n =1	n =0
Duration of service period, days	112±56.1	145±57.7	220±87.6	149±80.8	119±53.7	157±81.7	-	64±0	-
Dry period duration, days	54±10.1	55±7.9	58±11.1	56±15.4	107±44.8	60±11.3	-	109±0	-
Insemination index, times	5.63±2.81	7.26±2.88	11.04±4.38	7.49±4.04	5.95±2.68	7.88±4.08	-	3.20±0	-
Inter-calving period duration, days	400±81.3	411±49.2	471±117.6	402±88	367±23	481±149.5	-	342±0	-

Significant: *=P<0.05; **=P<0.01 (compared to the animals of the first control group); a = P<0.05; b= P<0.01. (compared to the animals of the third experiment group with analogues of the second experimental group). Mean values with different superscripts in the column differ significantly. CSN3 - kappa-casein , Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD)

The results of their experiments showed that CSN3 affects the length of the independence period and the interval between calvings. The authors also indicate that the SNP of the CSN3 gene was significantly associated with the duration of pregnancy, in addition, the effect of CSN3 on the age of first calving was observed. At the same time, ambiguous results of research by Tsiaras et al. (2005). Thus, the authors did not find any associations between polymorphisms in the CSN3 locus and reproductive capacity. However, there was a tendency to increase the age of cows at the first and second calving with the AB genotype.

The TG5 gene is one of the longest mammalian genes. In cattle it is located in the centromeric region of the 14th chromosome and consists of 37 exons. The TG5 gene has two allelic variants, TG5^T and TG5^C, and three genotypes: TG5^{CC}, TG5^{CT}, and TG5^{TT}. Glycoprotein of thyroglobulin is a precursor of iodothyronine hormones of the thyroid gland, which regulate many physiological and biochemical processes in almost all tissues of the body by regulating gene expression, and affect the performance indicators of cows (Safina, 2018).

Analyzing the relationship between the polymorphism of the TG5 gene and the indicators of the reproductive capacity of cows of different breeds according to the data of I-III and higher lactation (Table 3).

It was established that in all three experimental breed groups there are no individuals with the homozygous TT genotype, which is probably related to the individual characteristics of the animals, and a small frequency of occurrence of this genotype. But at the same time, comparing the effect of the TG5 gene on the reproductive characteristics of cows in the red dairy breed, it should be noted that there is a clear influence of the T allele on the characteristics of reproductive capacity - carriers of the heterozygous CT genotype had better indicators of reproductive function, with the exception of the first lactation, where the advantage was on sides of homozygous CC individuals. At the same time, the advantage of heterozygous organisms in the 3rd lactation according to the main signs of reproduction was probable (P<0.01).

Characterizing the influence of the TG5 gene polymorphism on the reproduction characteristics of Ukrainian red-spotted dairy cattle in the section I-III and higher lactations, no dependence was established between the above-mentioned parameters. Since the signs of reproduction had an oscillatory nature of their manifestation, both in terms of lactation and in terms of the investigated genotypes (CC or CT).

That is, the effect of the homo- or heterozygous state of the TG5 gene on the manifestation of indicators of the signs of the reproductive function of Ukrainian red-spotted dairy cows has not been established. The assessment of reproduction indicators and the effect on them of the TG5 gene polymorphism in representatives of Ukrainian black-spotted dairy cattle established the opposite trend - a clear effect of heterozygosity of individuals for the TG5 gene on reproductive characteristics was observed. So, for the I-III lactations by the length of the service period (101, 101 and 50 days, respectively), the dry period (52 and 38 days, respectively), the interval between calvings (377, 331 and 384 days, respectively), as well as the index insemination (5.05; 5.05 and 2.50, respectively) had a clear advantage of heterozygous individuals with the ST genotype, although only one animal fell into this group.

In the scientific literature, there is very little data confirming or refuting the influence of SNPs of the TG5 gene on the characteristics of animal reproduction. According to Deb et al. (2021), relatively similar research results were obtained. Thus, the analysis of the growth dynamics of first-born cows with different genotypes according to TG5 showed that individuals with the TT genotype had an advantage in live weight, and as a result, were fertilized at an earlier age. However, in terms of reproductive qualities, first-born cows with the TG5^{TC} genotype were inferior to cows with other genotypes. After the first calving, heterozygous TG5^{TC} individuals had a shorter service period and a high Doha index. In this follows a similar tendency to our researches.

Beta-lactoglobulin (BLG) is the main protein of cow's milk and affects milk yield, fat and protein content, as well as syrup properties. Until now, several variants of BLG have been identified, but the most common are A and B. The genes for the whey protein are located on two different chromosomes: the BLG gene encoding β-LG on chromosome 11, and the BLG gene encoding α-LA on chromosome 5 (Pedrosa et al., 2021).

Our studies of the indicators of the reproductive capacity of cows of three experimental breeds of Ukrainian selection according to the BLG locus (Table 4) established that in the individuals of the Ukrainian red dairy breed, which have the BB genotype, the indicators of the duration of the service period for the first lactation were significantly better, compared to the carrier females of the same age genotypes AA and AB on 51 days and 73 days, and, accordingly, the indicator of the insemination index was lower by 2.55 and 3.67, and the difference between the indicator of the intercalving period was smaller by 58 and 75 days, respectively. The results of research show a probable effect only during the first lactation. During the second lactation, the difference in the duration of the service period of the above-mentioned genotypes was 108 days and 37 days, but it was unlikely. In general, a better manifestation of signs of reproduction is observed in carriers of the B allele, compared to homozygous AA organisms. Which may indicate a certain influence of beta-lactoglobulin β-LG on indicators of reproductive capacity of cows.

Among Ukrainian red-spotted dairy cows, a similar tendency was found when comparing reproductive traits with the BLG gene polymorphism. Thus, cows that are carriers of the B allele genotype and especially heterozygous organisms - AB, had better indicators of reproductive capacity compared to their homozygous AA counterparts. Thus, their interval before fertile insemination, the dry period and the period between calvings were significantly shortened during the I-III lactations. With the exception of higher lactation, where cows carrying the AA genotype had better reproductive capacity. Accordingly, heterozygous females had a significantly better insemination index, compared to other experimental groups.

Table 3 - Relationship of SNPs of the *TG5* gene with reproductive traits of cows

Genotype g.0000 C>T ($X \pm S_x$) / Trait/Indicator	CC			CT			TT		
	URD	URSD	UBWD	URD	URSD	UBWD	URD	URSD	UBWD
1st lactation	n =13	n =13	n =14	n =3	n =2	n =1	n =0	n =0	n =0
Duration of service period, days	121±38	178±117.53	128±61.6	123±60.9	167±25.5	101±0	-	-	-
Insemination index, times	6.05±1.9	8.93±5.88	6.43±3.08	6.18±3.04	8.38±1.28	5.05±0	-	-	-
Inter-calving period duration, days	404±38.6	426.23±90.2	402±59.5	408±64.4	454±30.5	377±0	-	-	-
2nd lactation	n =13	n =13	n =14	n =3	n =2	n =1	n =0	n =0	n =0
Duration of service period, days	181±82.5	166±95.6	128±50.7	123±60.4	114±28	101±0	-	-	-
Dry period duration, days	67±19.4	50±6.8	50±10.1	58±16.2	68±8.5	52±0	-	-	-
Insemination index, times	9.06±1.12	8.31±4.78	6.43±2.53	6.17±1.02 [a] *	5.70±1.4	5.05±0	-		-
Inter-calving period duration, days	428±60	401±42.1	419±50.9	394±70	419±57	331±0	-	-	-
3rd lactation	n =13	n =13	n =14	n =3	n =2	n =1	n =0	n =0	n =0
Duration of service period, days	134±27.5	100±26.1	193±92.5	64±11.0 [a] *	125±81.5	50±0	-	-	-
Dry period duration, days	63±14.4	62±23.3	63±11.4	99±15.3 [a] *	50±3.5	38±0	-	-	-
Insemination index, times	6.73±1.38	4,97±1.4	9.66±4.63	3.20±0.55 [a] **	6.28±4.08	2.50±0	-	-	-
Inter-calving period duration, days	430±27.3	392±40.8	458±100.8	391±17.5 [a] *	356±37.5	384±0	-	-	-
Higher lactation	n =13	n =13	n =14	n =3	n =2	n =1	n =0	n =0	n =0
Duration of service period, days	130±67.7	120±52.8	197±93	109±70.2	192±9	202±0	-	-	-
Dry period duration, days	57±11.2	63±16.3	59±11.6	38±8	160±59	55±0	-	-	-
Insemination index, times	6.52±3.39	6.0±2.64	9.90±4.65	5.48±3.51	9.60±0.45	10.1±0	-	-	-
Inter-calving period duration, days	395±92.9	386±39.7	454±115.4	427±51.6	–	680±0	-	-	-

Significant: *=P<0.05; **=P<0.01 (compared to the animals of the first control group); a = P<0.05; b= P<0.01 (compared to the animals of the third experiment group with analogues of the second experimental group). Mean values with different superscripts in the column differ significantly (p<0.05). TG5 thyroglobulin gene (TG-5); Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD)

Table 4 - Connection of *BLG* gene SNPs with reproductive characteristics of cows

Genotype g.0000 C>T ($X\pm S_x$) / Trait/Indicator	AA			AB			BB		
	URD	URSD	UBWD	URD	URSD	UBWD	URD	URSD	UBWD
1st lactation	n =7	n =3	n =1	n =5	n =1	n =6	n =4	n =11	n =8
Duration of service period, days	127±38.1	258±212.5	118±0.0	149±32.9	94±0.0	120±68.8	76±22.3 [a] **	173±93.4	132±53.3
Insemination index, times	6.36±1.91	12.93±10.63	5.9±0.0	7.48±1.64	4.7±0.0	6.03±0.0	3.81±1.11 [a] *	8.65±4.67	6.62±2.82
Inter-calving period duration, days	414±39.9	344±15.0	360±0.0	431±33.4	371±0.0	395±58.6	356±21.8	451±94.2	409±58.2
2nd lactation	n =7	n =3	n =1	n =5	n =1	n =6	n =4	n =11	n =8
Duration of service period, days	219±104.2	119±48.0	60±0.0	148±54.1	81±0.0	104±26.1	111±51.8	176±106.6	149±63
Dry period duration, days	65±12.7	52±1.0	18±0.0	54±13.0	26±0.0	48±10.5	81±38.4	54±8.5	56±5.2
Insemination index, times	10.98±5.21	5.95±2.40	3.0±0.0	7.43±2.70	4.05±0.0	5.22±1.30	5.56±2.59	8.81±5.33	7.45±3.15
Inter-calving period duration, days	434±66.4	385±0.0	335±0.0	426±57.9	364±0.0	385±22.0	395±53.0	398±43.6	444±62.9
3rd lactation	n =7	n =3	n =1	n =5	n =1	n =6	n =4	n =11	n =8
Duration of service period, days	115±41.8	138±0.0	226±0.0	108±39.6	86±28.9	161±127.2	156±51.7	110±1.5	191±69.4
Dry period duration, days	86±27.5	77±0.0	90±0.0	53±19.9	64±24.7	62±13.9	61±15.8	46±0.50	57±8.3
Insemination index, times	5.76±2.09	6.90±0.0	11.30±0.0	5.40±1.98	4.14±1.41	8.05±6.36	7.83±2.59	5.53±0.07	9.55±3.47
Inter-calving period duration, days	414±59.4	418±0.0	507±0.0	415±64.4	379±50.5	472±139.0	450±37.3	385±0.0	447±78
Higher lactation	n =7	n =3	n =1	n =5	n =1	n =6	n =4	n =11	n =8
Duration of service period, days	121±75.3	88±24.0	226±0.0	132±55.1	181±0.0	165±133.4	128±71.0	126±57.3	215±55.4
Dry period duration, days	59±11.9	78±31.00	90±0.0	54±12.1	62±0.0	60±13.8	49±11.8	76±34.6	54±4.1
Insemination index, times	6.06±3.76	4.4±1.20	11.3±0.0	6.63±2.76	9.05±0.0	8.26±6.67	6.40±3.55	6.30±2.86	10.77±2.77
Inter-calving period duration, days	344±107.4	342±0.0	507±0.0	455±76.1	380±0.0	438±155.8	432±46.3	392±44.8	493±110.2

Significant: *=P<0.05; **=P<0.01; ***=P<0.001 (compared to the animals of the first control group); a = P<0.05; b= P<0.01. (compared to the animals of the third experiment group with analogues of the second experimental group) Mean values with different superscripts in the column differ significantly (p<0.05). *BLG* - betalactoglobulin; Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD)

Table 5 - Relationship of SNPs of the *Pit-1* gene with reproductive traits of cows

Genotype g.0000 C>T ($X \pm S_x$) / Trait/Indicator	AA			AB			BB		
	URD	URSD	UBWD	URD	URSD	UBWD	URD	URSD	UBWD
1st lactation	n =10	n =4	n =1	n =0	n =8	n =9	n =6	n =3	n =5
Duration of service period, days	112±42.2	206±177.5	101±0	-	186±91.1	151±80.4	136±39	114±62	86±22.6
Insemination index, times	5.64±2.11	10.30±8.88	5.05±0	-	9.32±4.56	7.58±4.02	6.80±1.95	5.70±3.10	4.34±1.13
Inter-calving period duration, days	396±52.3	481±175.1	379±0	-	417±57.6	426±76.1	420±37.8	394±64.2	357±17.3
2nd lactation	n =10	n =4	n =1	n =0	n =8	n =9	n =6	n =3	n =5
Duration of service period, days	180±102.5	247±158	286±0	-	120±46.7	129±38	153±48.3	130±47.6	81±17.8
Dry period duration, days	67±21.2	43±8.6	67±0	-	55±0	52±6.7	64±12.8	58±2	44±13.3
Insemination index, times	9.02±5.12	12.38±7.9	14.30±0	-	6.01±2.33	6.45±1.90	7.68±2.42	6.52±2.38	4.09±0.89
Inter-calving period duration, days	423±66.1	410±55.5	570±0	-	385.7±23.3	421±36.6	420±60.7	434±53.1	356±23
3rd lactation	n =10	n =4	n =1	n =0	n =8	n =9	n =6	n =3	n =5
Duration of service period, days	118±54.5	102±28.9	285±0	-	88±22.3	202±100.6	132±40.5	140±54	115±55.1
Dry period duration, days	78±23.8	52.0±12.5	67±0	-	67±33.9	60±10.9	56±18.8	60±8.9	63±14.5
Insemination index, times	5.92±2.72	4.93±1.98	14.25±0	-	4.40±1.12	10.13±5.03	6.63±2.03	7.00±2.7	5.79±2.76
Inter-calving period duration, days	410±44.2	378±40.0	569±0	-	356±26.6	448±118.7	444±82.2	438±29.8	454±42.4
Higher lactation	n =10	n =4	n =1	n =0	n =8	n =9	n =6	n =3	n =5
Duration of service period, days	114±54.1	150±65.5	285±0	-	117±50.1	222±97	147±84.3	133±49.8	142±74.3
Dry period duration, days	53±12.5	52±4.8	67±0	-	93±44.3	56±9.9	57±12.8	74±18	61±13.9
Insemination index, times	5.71±2.7	7.50±3.28	14,25±0	-	5.89±2.5	11.11±4.85	7.35±12.83	6.68±2.49	7.12±3.72
Inter-calving period duration, days	410±45.3	404±58.5	569±0	-	367±22.8	467±125.9	386±153.5	389±34.5	462±131.3

Significant: *=P<0.05; **=P<0.01; ***=P<0.001 (compared to the animals of the first control group); a = P<0.05; b= P<0.01 (compared to the animals of the third experiment group with analogues of the second experimental group). Mean values with different superscripts in the column differ significantly. Pituitary-specific transcription factor (Pit-1); Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD).

However, in cows of the Ukrainian black-spotted dairy breed, which have the homozygous AA genotype, the indicator of the duration of the service period and the interval between calvings during the II lactation was lower, compared to other homozygous BB organisms - by 89 days and 109 days, respectively, the indicator of the insemination index - at 4.45. A similar trend was observed in the first lactation, although comparatively, with a small advantage. At the time when during the 3rd and higher lactations, better reproductive capacity was characteristic of cows with the heterozygous AB genotype. What can indicate the influence of the state of heterozygosity of the organism for the BLG gene on indicators of the reproductive capacity of cows, which was observed in cows regardless of their breed affiliation.

The association of milk protein genes with the fertility of Holstein cattle was also studied by Peñagaricano and Khatib (2012), who, after correcting for polymorphisms in LALBA and BLG genes, established a significant relationship with successful fertilization and the number of blastocysts, which may indicate a relationship between whey protein genes and cow fertility. At the same time Demeter et al. (2010) confirm that the selection of cows based on the polymorphism of milk protein variants with improved production properties does not have a negative effect on the reproductive performance of cows, but on the contrary, is a promising direction of breeding programs. Because this will allow establishing the most successful combinations of genes to improve reproductive traits.

The Pit-1 or POU1F1 gene is a key transcription factor located on the first chromosome in cattle, weighs approximately 33 kDa, has 5 introns and 6 exons, and consists of 291 amino acids. Pit-1 plays a role in pituitary development and somatic cell proliferation, as well as the secretion of the hormones growth hormone (GH) and prolactin (PRL) in mammals. Since it is related to pituitary hormones, it affects the ovulation rate in cattle (Fındık et al., 2022).

The analysis of the reproductive function of cows revealed the connection of its features with polymorphism in the gene of the pituitary-specific transcription factor PIT-1 (Table 5): a positive trend was observed in favor of cows of the BB genotype according to the insemination index for the 1st and 2nd lactations among the three experimental breeds, as well by the duration of the service and inter-hotel periods, we established a difference in favor of the BB genotype, although it was statistically unreliable.

Cows of the PIT-1BB genotype of three breeds had a shorter service period for I-III and higher lactation, compared to peers of the AA and AB genotypes. Accordingly, this affected the duration of the interval between calvings. And as a result, the insemination index among cows of the Ukrainian black and spotted dairy breed was the lowest, precisely among individuals of the homozygous PIT-1BB genotype. A similar influence of the B allele on reproductive performance indicators was also established among cows of the Ukrainian red-spotted dairy breed - carriers of the B allele genotypes, as well as AB heterozygotes and BB homozygotes, were distinguished by better reproductive qualities in the section I-III and higher lactations, compared to homozygotes analogues of AA.

Among individuals of the Ukrainian red dairy breed, a clear influence of the PIT-1 gene on reproductive qualities has not been established, although cows with the BB genotype have a lower index of insemination in the first and second lactations than animals with the AA genotype, and, accordingly, the interval to fertile insemination and the intercalving period are shorter. The results of studies by Pytlewski et al. (2018) indicated the relationship between the PIT-1 gene and reproductive traits that proved the existence of dependencies between the PIT-1 gene and the reproductive potential and body weight of cows and calves. But according to the authors, more favorable results were observed in homozygous AA calves. Al-Khuzai and Al-Anbari (2018), advise using the PIT-1 gene as a genetic marker to improve reproductive traits of high-fertility sheep. Since a polymorphism was established between this gene and sheep fertility.

CONCLUSION

The identified trends in inter-breed differentiation of polymorphism in the genes CSN3, BLG, TG5, PIT-1, and LEP based on reproductive performance indicators in cows of different Holstein-origin breeds are somewhat diverse and occasionally statistically inconclusive due to the small number of groups of investigated animals carrying a specific genotype, the frequency of which is low in the populations of the studied farm. However, it has been established that the same genetic variants influencing reproductive function have different effects in specific breeds due to the specificity of their genomic background. The influence of the leptin gene on the reproductive traits of cows was noted in carriers of the C allele, who generally exhibited better reproductive function compared to carriers of the A allele. The impact of the homozygous or heterozygous state of the B allele in the CSN3 gene on reproductive indicators was established, as cows of the investigated breeds that were carriers of the B allele (AB/BB) showed better reproductive performance compared to the homozygous genotype AA. The analysis of the polymorphism of the TG5 gene in relation to the reproductive traits of cows of different breeds revealed the absence of individuals with a homozygous TT genotype among the evaluated representatives, which is likely related to individual characteristics of the animals and the low frequency of occurrence of this genotype. However, a clear influence of heterozygosity for the TG5 gene was observed on the reproductive characteristics of most investigated dairy breeds. Research on the reproductive traits of cows from three studied breeds of Ukrainian selection at the BLG locus indicates the influence of the allelic combination of the BLG locus on the reproductive traits of cows, irrespective of their breed. The analysis of the reproductive function in cows revealed a connection between its traits and the polymorphism in the PIT-1 gene. A positive dynamics in reproductive performance was observed in favor of cows with the PIT-1BB genotype for the calving index in the first and second lactation among the three studied breeds. Also a difference in the duration of the service and intercalving periods was noted in favor of animals with the PIT-1BB genotype, although it was statistically insignificant.

Therefore, the polymorphism of the CSN3, BLG, TG5, PIT-1, and LEP genes can be utilized as molecular markers in selective breeding to ensure expected progress in improving not only the traits related to the milk productivity of cattle but also their reproductive function.

DECLARATIONS

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Authors' contribution
The first three authors should be considered first authors. M. Gill planned, designed, spell checked and led this study. Y. Gritsienko collected data and samples, conducted laboratory tests, analyzed and visualized data. O. Karatieieva described the results obtained, formulated the initial version of the manuscript and critically edited the final version of the manuscript. M. Gil also supervised the data and reviewed the manuscript. All authors read and approved the submitted version of the manuscript.

Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Consent to publish
Not applicable.

Competing interests
The authors did not declare any conflict of interest.

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