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Original Article

Association of polymorphic variants of prolactin (*PRL*) and beta-lactoglobulin (*BLG*) genes with resistance/susceptibility to mastitis in holstein cows

Associação de variantes polimórficas dos genes da prolactina (PRL) e betalactoblobulina (BLG) com resistência/suscetibilidade à mastite em vacas holandesas

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Abstract

The work aims to analyze the associations of polymorphic variants of the *PRL* and *BLG* genes with resistance and susceptibility to mastitis in Holstein cows. The experimental study consisted of the selection of biomaterial samples from 250 heads of Holstein cows aged 3 years divided into two groups (healthy and with a confirmed diagnosis of mastitis). The determination of animal genotypes was carried out using polymerase chain reaction and restriction fragment length polymorphism. The study of the nature of the association of polymorphic variants of the *PRL* and *BLG* gene with resistance/increased risk of mastitis established a significant deviation from the theoretically expected distribution of *bBLG*-HaeIII genotypes in the group of animals suffering from mastitis (the value of χ^2 was 0.24). The *bBLG*-HaeIII^{BB} genotype can act as a marker of an increased risk of developing mastitis in Holstein cows; its frequency in the group of sick animals exceeds the frequency in the control group by more than 2 times (44.0 compared to 17.0%, respectively). The *bBLG*-HaeIII^{AB} genotype is significantly associated with mastitis resistance in Holstein cows; its frequency is 2 times lower than in the control group (28.0 compared to 54.0%).

Keywords: cattle, polymorphism, holstein breed, resistance, mastitis.

Resumo

O trabalho tem como objetivo analisar as associações de variantes polimórficas dos genes PRL e BLG com resistência e suscetibilidade à mastite em vacas holandesas. O estudo experimental consistiu na seleção de amostras de biomateriais de 250 cabeças de vacas holandesas com 3 anos de idade divididas em dois grupos (saudáveis e com diagnóstico confirmado de mastite). A determinação dos genótipos dos animais foi realizada utilizando reação em cadeia da polimerase e polimorfismo de comprimento de fragmentos de restrição. O estudo da natureza da associação de variantes polimórficas do gene PRL e BLG com resistência/risco aumentado de mastite estabeleceu um desvio significativo da distribuição teoricamente esperada dos genótipos bBLG-HaeIII no grupo de animais que sofrem de mastite (o valor de χ^2 foi 0,24). O genótipo bBLG-HaeIIIBB pode atuar como marcador de risco aumentado de desenvolvimento de mastite em vacas holandesas; sua frequência no grupo de animais doentes excede a frequência no grupo controle em mais de duas vezes (44,0% contra 17,0%, respectivamente). O genótipo bBLG-HaeIIIAB está significativamente associado à resistência à mastite em vacas holandesas; sua frequência é duas vezes menor que no grupo controle (28,0% contra 54,0%).

Palavras-chave: bovinos, polimorfismo, raça holandesa, resistência, mastite.

1. Introduction

An important role in increasing the productivity of the dairy industry is played not only by modern technologies of cattle breeding and selection (Zhang et al., 2022; Behren et al., 2023; Bui et al., 2023) but also by the use of modern methods of infectious disease control (Runtuwene et al., 2022; Maurić Maljković et al., 2023). Mastitis is considered the most common disease, dangerous both for public health and production development (Mussynov et al., 2019). Mastitis is an inflammatory disease of the udder caused by infection with bacteria, which

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results in a decrease in the quality and quantity of milk obtained from animals (Ulyanov et al., 2021; Kober et al., 2022; Cattaneo et al., 2023).

Consumption of mastitis-affected milk can be harmful to humans since drug-resistant pathogens (Alessandri et al., 2023; Mussynov et al., 2014) can be transmitted through contaminated unpasteurized milk (Suchshikh et al., 2023). Therefore, it is also a serious problem and a danger to public health (Pascu et al., 2022; Mussayeva et al., 2021). Losses occur in the dairy industry due to reduced milk yields, poor milk quality and discarded milk, the cost of treating sick animals (Zaitsev et al., 2024; Madenova et al., 2019), as well as a decrease in the fertility of cows (Detilleux et al., 2015; Gomes and Henriques 2016; Côté-Gravel and Malouin 2019). On average, the total loss due to mastitis is estimated at $147 per cow per year, especially due to milk losses and animal culling, which is approximately 11 to 18% of gross profit per cow per year (Hogeveen et al., 2019; Popov et al., 2017).

Based on the focus of our study and to understand the relevance of the chosen research topic, it should be noted that livestock farming in Kazakhstan occupies about 43% of the total gross agricultural output and is the main source of employment, nutrition, and income of the rural population. Its development is one of the main strategic economic objectives of Kazakhstan (Bokayev et al., 2023; Sarsekova et al., 2023). The dairy industry accounts for about 20% of the volume produced in Kazakhstan. The most common bacterial infectious cattle disease in Kazakhstan is mastitis, which has a direct impact on the development of the dairy industry.

2. Literature Review

Along with marker-associated breeding measures aimed at increasing the profitability of the industry by increasing the genetic potential of productivity of farm animals, the use of genetic markers of resistance to bacterial infections can affect the reduction of treatment costs and losses from mortality, abortions, and culling of sick animals (Baymenov et al., 2023; Mendybayeva et al., 2023).

An important aspect of cattle resistance is the immune system of animals (Khan et al., 2023; Powell et al., 2023). Animals with a strong immune system can effectively fight infectious diseases and recover quickly from stressful situations. In addition, genetic factors can influence individual predisposition to certain diseases. An integrated approach combining genetics, the immune system, hygiene, prevention, and herd management will help to increase the resistance of cattle to mastitis.

The prolactin gene (*PRL*) encodes the protein prolactin, which is a hormone that plays a key role in the regulation and maintenance of lactation in mammals. Prolactin, like growth hormone, belongs to the same family of protein hormones that are involved in the initiation and maintenance of lactation in mammals (Malintha et al., 2023). Prolactin is also synthesized in various tissues, including endothelial cells, neurons, breast cells, etc. (Beishova et al., 2023). Prolactin secretion is regulated by pituitary cells (paracrine regulation), as well as by several intracellular factors secreted by lactotrophic cells (autocrine regulation).

Studies related to the effect of *PRL* on mastitis resistance in cattle are a subject of scientific interest. For example, some polymorphisms in the PRL gene may be associated with changes in immune function and response to infection, including mastitis. This may mean that certain variants of the *PRL* gene may be associated with increased or decreased resistance to mastitis in cows.

Scientists have established a close coupling of the *PRL* gene with genes of class I and class II of the main histocompatibility complex. The maximal coupling occurs with *BoLA-DRB3* (Lewin et al., 1992; van Eijk et al., 1995). The *BoLA-DRB3* gene is responsible for the primary immune response, as alleles associated with resistance and sensitivity to diseases such as persistent lymphocytosis, bovine leukemia, and dermatophilosis are known. The relationship of this gene with signs of milk productivity, somatic cell content, and resistance to mastitis has also been studied.

The incidence of mastitis in animals is highest during the calving period, which is associated with increased production of *PRL*. In the research by M.G. Salgado-Lora et al., and also in early studies by L. Lara-Zarate et al.,, it is suggested that *PRL* plays a role in the development of mastitis. They found that *PRL* promoted an inflammatory reaction in the epithelial cells of the mammary gland of cattle through the activity of NF-kB (Salgado-Lora et al., 2020; Barajas-Mendiola et al., 2022).

The *BLG (β-LG)* gene refers to the gene encoding the beta-lactoglobulin protein, which is one of the main proteins in cow's milk and is present in significant amounts. Beta-lactoglobulin plays an important role in the immune system of animals and may be associated with resistance to mastitis (Chaneton et al., 2011; Singh et al., 2023; Tomanić et al., 2023). This protein is a source of biologically active peptides. Peptides, in turn, have a wide range of physiological activity, such as immunomodulatory properties, antimicrobial, antihypertensive properties, etc. The fragments obtained as a result of hydrolysis with alkalase, pepsin, or trypsin have bacteriostatic activity against *E.coli, Bacillus*, and *S. aureus* (Wong and Chai 2023).

Studies on the effect of the *BLG* gene on milk production and the risk of mastitis in animals were conducted to understand the genetic factors that may affect these parameters. These studies indicate a potential link between genetic variants of the *BLG* gene and the risk of mastitis in cows, especially in the Holstein breed (Zemanova et al., 2022). We should note the importance of this breed of cows for Kazakhstan. Studies collectively show that Holstein cows are raised in Kazakhstan because of their high milk productivity (Shamshidin et al., 2023; Uskenov et al., 2023), ability to adapt to local climatic conditions (Mylostyvyi et al., 2023), as well as the benefits that cows receive from the addition of mineral and vitamin premixes and food additives (Bayazitova et al., 2023; Karynbayev et al., 2023). These factors make them a valuable asset for the dairy industry in Kazakhstan.

In this regard, the study aims to analyze the links between polymorphic variants of the *PRL* and *BLG* genes and the level of resistance or susceptibility to mastitis in Holstein cows.

3. Materials and Methods

To achieve this goal, we conducted an experimental study in the farms of Kostanay region (Kazakhstan) in the period from 2022 to 2023.

The study was conducted in strict accordance with the principles set out in the Basel Declaration (2010), as well as standards and recommendations for animal experiments (ICLAS, 2024). All stages of the experiment were carried out considering ethical norms and rules for the welfare and protection of animals. The results of the study were obtained in compliance with all ethical principles and norms, and the principles of caring for the welfare of animals and respect for their rights were considered.

The material for the study included samples of biomaterial from 250 heads of Holstein cows aged 3 years with leveled conditions of keeping, feeding, and breeding. Of these, 150 heads had a confirmed diagnosis of mastitis (m), and an experimental group of 100 heads consisted of healthy (h) animals of the Holstein breed.

3.1. DNA typing of animals

Sampling and preparation of samples for analysis (carried out by employees of the farm providing samples).

DNA extraction from hair follicles was carried out using a commercial DNA Extran-2 kit (Syntol LLC, Moscow, Russia). The qualitative analysis of the isolated DNA was carried out using gel electrophoresis, and the DNA was characterized by good quality since there were no nonspecific fragments on the electrophoregram. The quantitative analysis was carried out by measuring the DNA concentration on an Agilent Cary 60 spectrophotometer. The average DNA concentration was 80 ng/ul. The A260/A280 ratio was 1.9.

The determination of the genotypes of the studied animals by *BLG*-HaeIII polymorphism was carried out using a technique optimized by selecting and standardizing polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) conditions to a level suitable for organizing mass routine screening. The PCR-RFLP method for DNA typing of animals by DpnI polymorphism was developed within the framework of this project.

For DNA typing of Holstein breed animals by the *PRL* gene, the polymorphism rs211032652 was selected, accompanied by the substitution of the amino acid alanine for serine in the 13th position of the protein. DpnI restrictase was selected for the PCR-RFLP method. This restrictase can effectively recognize and cleave the GmATC sequence (where A is methylated) and cannot cleave the GATC sequence (where A is not methylated), since according to the latest available data, this mutation is associated with increased milk productivity in Romanian spotted and Romanian brown cattle (Ilie et al., 2023).

The amplification of the *PRL* gene fragment containing rs211032652 was performed using the following primers:
F: 5′GCTCCAGAAGTCGTTGTTTTC3′
R: 5′CGAGCTTATGAGCTTGATTCTT3′ (Cowan et al., 1989)

The composition of the reaction mixture was optimized: H_2O up to 25 µl, 10x buffer to 2.5 µl, $MgCl_2$ (25 mM) to 2 µl, dNTP (25 mM) to 0.2 µl, direct primer F to 1 µl, reverse primer R to 1 µl, Taq polymerase (5 activity units (a.u.)/µl) to 0.25 µl.

The optimal PCR regime was selected by experimental methods: 94°C for 5 min, (94°C for 30 sec, 56°C for 30 sec, 72°C for 30 sec) x 35 cycles. The density of the agarose gel should be increased to 3% for optimal visualization of restriction fragments.

The length of the amplified fragment of the *PRL* gene is 156 base pairs (bp).

3.2. Determination of cattle genotypes based on the HaeIII polymorphism of the BLG gene

The technique of PCR formulation for DNA typing of animals by the *BLG* gene was optimized. The optimization of animal genotyping techniques was carried out by selecting and standardizing PCR-RFLP conditions to a level suitable for organizing mass routine screening.

To amplify the polymorphic region of the *BLG* gene, we used oligonucleotide primers *BLG*1 and *BLG*2 with the following sequence (Medrano and Aguilar-Cordova 1990):
*BLG*1: 5' TGTGCTGGACACCGACTACAAAAAG 3'
*BLG*2: 5' GCTCCCGGTATATGACCACCCTCT 3'

For DNA typing of Holstein animals using the *BLG* gene, the *BLG*-HaeIII polymorphism was selected, located in the coding region of the gene (exon IV) and leading to changes in the amino acid sequence of the protein. The composition of the reaction mixture was optimized for *BLG*-HaeIII polymorphism: H_2O: up to 25 µl, 10x buffer: 2.5 µl, $MgCl_2$ (25 mM): 2 µl, dNTP (25 mM): 0.2 µl, direct primer (*BLG*-HaeIII-F): 1 µl, reverse primer (*BLG*-HaeIII-R): 1 µl, Taq polymerase (5 a.u./µl): 0.25 µl.

Using experimental methods, the optimal temperature and annealing time of primers for genotyping according to the *BLG*-HaeIII polymorphism were selected, allowing for producing a pure amplification in an amount sufficient for electrophoretic detection as follows: 94°C: 5 min, (94°C: 30 sec, 60°C: 30 sec, 72°C: 30 sec) x 35 cycles. The number of PCR cycles was increased to 35, which made it possible to take 5 µl of amplification for restriction in a volume of 10 µl of the reaction mixture and thereby reduce the consumption of reagents for restriction by 2 times. It was found that for optimal visualization of *BLG*-HaeIII restriction fragments, the density of the agarose gel should be increased to 3%.

The length of the amplified fragment of the *BLG* gene is 247 bp. The length of the fragments after restriction is 148, 99, and 74 bp. Fragment size variants with 148 and 99 bp correspond to the *BLG*-HaeIII^{AA} genotype; 148, 99, and 74 bp correspond to the *BLG*-HaeIII^{AB} genotype; 99 and 74 bp correspond to the *BLG*-HaeIII^{BB} genotype (Figure 1).

The genotypes of the studied populations were determined using PCR-RFLP by the following polymorphisms: *BLG*-HaeIII^{AA}, *BLG*-HaeIII^{AB}, and *BLG*-HaeIII^{BB} by the *BLG* gene. Among animals of the Holstein breed with an established diagnosis of mastitis by *BLG*-HaeIII polymorphism, the *BLG*-HaeIII^{BB} genotype has the highest frequency of occurrence, followed by the *BLG*-HaeIII^{AB} genotype; the most rare is the *BLG*-HaeIII^{AA} genotype. Among healthy animals of the Holstein breed, by *BLG*-HaeIII polymorphism, the *BLG*-HaeIII^{AB} genotype has the highest frequency of occurrence, followed by the *BLG*-HaeIII^{AA} genotype; the most rare is the *BLG*-HaeIII^{BB} genotype.

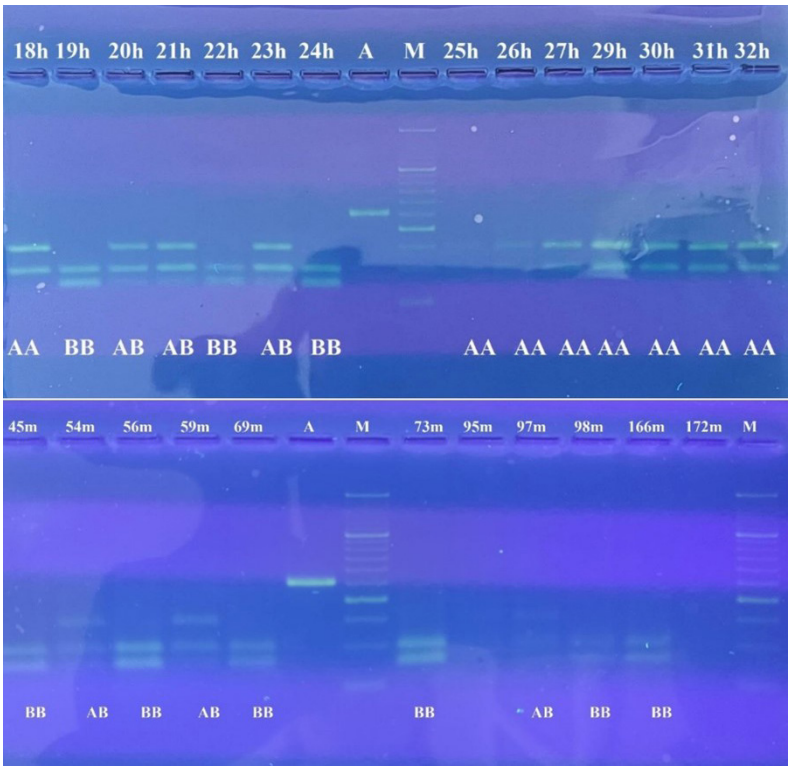

Figure 1. Electrophoregram of DNA polymorphism typing, *BLG*-HaeIII in 3% agarose gel. M is the O'range Ruler ™ 50 bp DNA Ladder molecular mass marker, Fermentas, Lithuania; A is the amplification of 247 bp fragment of the *BLG*-HaeIII gene; h are the samples from healthy animals; m are the bands from animals with mastitis; bands 18h, 25h, 26h, 27h, 29h, 30h, 31h, and 32h are the restriction fragments with 148 and 99 bp corresponding to the *BLG*-HaeIII^{AA} genotype; bands 19h, 22h, 24h, 45m, 56m, 69m, 73m, 98m, and 166m are the restriction fragments with 99 and 74 bp corresponding to the *BLG*-HaeIII^{BB} genotype; bands 20h, 21h, 23h, 54m, 59m, and 97m are the restriction fragments with 148, 99, and 74 bp corresponding to the *BLG*-HaeIII^{AB} genotype. The position of specific stripes on the gel is indicated by arrows.

3.3. Statistical processing

The observed genotype frequencies were determined by direct counting.

The frequency of alleles was calculated using Formula 1 (Haddrill, 2021):

$$P_A = \left(2nAA + nAB\right)/2N$$
$$q_B = \left(2nBB + nAB\right)/2N \quad (1)$$

where P_A is the frequency of the A-allele; q_B is the frequency of the B-allele; and *N* is the total number of alleles.

The statistical error of the relative frequencies of alleles was calculated using Formula 2:

$$S_Q = \sqrt{\left(Q\left(1- Q\right)/2n\right)} \quad (2)$$

where Q is the relative frequency of the allele under study, and *n* is the sample size (Clarke and PLOS Biology Staff Editors, 2022).

The comparison of samples according to the frequency distribution of allelic variants of the studied genes was carried out using the χ^2 criterion, Formula 3. The number of degrees of freedom was equal to the number of genotypes minus the number of alleles:

$$\chi^2 = \sum(H_o - H_e)/H_e \quad (3)$$

where H_o is the observed frequencies of alleles, and H_e is the expected frequencies of alleles (Clarke and PLOS Biology Staff Editors 2022).

If the expected values of the number in at least one of the classes turn out to be less than five, then the calculation of χ^2 was carried out with the Yates correction, Formula 4:

$$\chi^2 = \sum((H_o - H_e) - 0.5)^2/H_e \quad (4)$$

The correspondence of the actual and expected genotype distribution was checked using the chi-square method, Formula 5. The number of degrees of freedom was equal to the number of genotypes minus the number of alleles.

$$\chi^2 = \sum(H_o - H_e)^2/H_e \quad (5)$$

where H_o is the observed frequencies of genotypes; H_e is the expected frequencies of genotypes: $AA=p^2$; AB=2pq; $BB=q^2$ (Clarke and PLOS Biology Staff Editors, 2022).

The acceptable value of χ^2 for one degree of freedom and a 95% significance level was 3.84 (Clarke and PLOS Biology Staff Editors, 2022).

4. Results

The genetic structure of samples of animals with mastitis and the control group for *bPRL*-DpnI polymorphism is shown in Table 1.

The frequency ratio of the *bPRL*-DpnI^{C} and *bPRL*-DpnI^{A} alleles in the group of animals suffering from mastitis was 0.967±0.001 and 0.033±0.001, respectively. Among healthy animals, the frequency ratio of the *bPRL*-DpnI^{C} and *bPRL*-DpnI^{A} alleles was 0.980±0.002 and 0.020±0.002, respectively.

The genetic structure of samples of animals suffering from mastitis and the control group for *bBLG*-HaeIII polymorphism is shown in Table 2.

The genetic structure of the studied livestock by the b*BLG* polymorphic gene is characterized by an almost identical distribution of alleles A and B in groups of both sick and healthy animals. The ratio of *BLG*-HaeIII^{A} and *BLG*-HaeIII^{B} alleles in animals suffering from mastitis is 0.420±0.003 to 0.580±0.003. In the control group, this ratio is 0.560±0.005 to 0.440±0.005.

The association of polymorphic variants of the *PRL* gene with susceptibility/resistance to mastitis was studied by evaluating the correspondence of the nature of the observed genotype frequencies to the theoretically expected equilibrium by calculating the χ^2 criterion, as well as by comparative analysis of the distribution of different genotypes in the group of animals suffering from mastitis and the control group of healthy cows.

The results of the assessment of the compliance of the observed frequencies of genotypes with the theoretically expected equilibrium and the values of the χ^2 criterion are shown in Table 3.

Table 1. Distribution of relative frequencies of alleles of the studied b *PRL* gene.

Polymorphism	Allele	Mastitis (n=150)	Healthy (n=100)
bPRL-DpnI	*bPRL*-DpnI^{C}	0.967±0.001	0.980±0.002
	bPRL-DpnI^{A}	0.033±0.001	0.020±0.002

Table 2. Distribution of relative frequencies of alleles of the studied *bBLG* gene.

Polymorphism	Allele	Mastitis (n=150)	Healthy (n=100)
bBLG-HaeIII	*BLG*-HaeIII^{A}	0.420±0.003	0.560±0.005
	BLG-HaeIII^{B}	0.580±0.003	0.440±0.005

Table 3. Distribution of genotype frequencies for the polymorphic *bPRL* gene in groups of healthy and sick animals.

Genotype	Mastitis (n=150)*			Healthy (n=100)*		
	n_o	n_e	χ^2	n_o	n_e	χ^2
bPRL-DpnI^{CC}	138	137		92	91	
bPRL-Dpn^{CA}	11	13	0.24	7	9	0.58
bPRL-Dpn^{AA}	1	0		1	0	

Notes: 1. The value of χ^2 for a significance level of 0.05 is 3.84; 2. *The values of χ^2 were calculated with the Yates correction.

According to the data shown in Table 3, the observed frequencies of the CC, CA, and AA genotypes in the group of animals suffering from mastitis were 138, 11, and 1, and in the control group, 92, 7, and 1, respectively. The expected frequencies according to the Hardy-Weinberg law should be 137, 13, and 0 in the mastitis group and 91, 9, and 0 in the control group. The values of χ^2 were 0.24 in the group of cows with mastitis, and in healthy cows χ^2=0.58, which indicates that the observed frequencies of genotypes correspond to the theoretically expected equilibrium frequencies in the group of sick animals.

The results of the analysis of the degree of excess of the observed frequencies of homozygous genotypes in a group of sick animals are shown in Table 4.

In the groups of animals diagnosed with mastitis, the relative frequencies of the CC, AC, and AA genotypes were 92.0, 7.3, and 0.7%, respectively. The proportions of these genotypes in the control group were 92.0, 7.0, and 1.0%, respectively.

The results of the assessment of the correspondence of the observed frequencies of HaeIII-polymorphic variant genotypes of the *bBLG* gene to the theoretically expected equilibrium and the values of the χ^2 criterion are shown in Table 5.

According to the data in Table 5, the observed frequencies of the *bBLG*-HaeIII^{AA}, *bBLG*-HaeIII^{AB}, and *bBLG*-HaeIII^{BB} genotypes in the group of animals suffering from mastitis were 42, 42, and 66, and in the control group 29, 54, and 17, respectively. The expected frequencies according to the Hardy-Weinberg law should be 26, 73, and 51 in the mastitis group and 32, 49, and 19 in the control group. The deviation of the genotype distribution from the equilibrium in the group of animals suffering from mastitis was significant and χ^2 amounted to 27.13. In healthy cows, the value of χ^2=0.92, which indicates the significance of the deviation of the observed genotype frequencies from the theoretically expected equilibrium ones in the group of sick animals.

The results of the analysis of the degree of excess of the observed frequencies of homozygous genotypes in a group of sick animals are shown in Table 6.

Table 4. The proportion of genotypes of polymorphic *bPRL* genes in groups of healthy and sick animals (% of the surveyed livestock).

Gene	Genotype	Mastitis, %		Healthy, %	
		observed	expected	observed	expected
PRL	*bPRL*-DpnI^{CC}	92.0	91.9	92.0	83.5
	bPRL-DpnI^{AC}	7.3	8.1	7.0	8.2
	bPRL-DpnI^{AA}	0.7	0.0	1.0	8.3

Table 5. Distribution of genotype frequencies for the polymorphic *bBLG* gene in groups of healthy and sick animals.

Genotype	Mastitis (n=150)			Healthy (n=100)		
	n_o	n_e	χ^2	n_o	n_e	χ^2
bBLG-HaeIII^{AA}	42	26	27.13	29	32	0.92
bBLG-HaeIII^{AB}	42	73		54	49	
bBLG-HaeIII^{BB}	66	51		17	19	

Notes: 1. The value of χ^2 for a significance level of 0.05 is 3.84.

Table 6. The proportion of genotypes of polymorphic *bBLG* genes in groups of healthy and sick animals (% of the surveyed livestock).

Gene	Genotype	Mastitis, %		Healthy, %	
		observed	expected	observed	expected
bBLG	*bBLG*-HaeIII^{AA}	28.0	17.4	29.0	31.3
	bBLG-HaeIII^{AB}	28.0	49.0	54.0	49.5
	bBLG-HaeIII^{BB}	44.0	33.6	17.0	19.2

5. Discussion

In the group of mastitis cows, by the *bBLG* polymorphism, one can note a significant redistribution of the relative frequencies of genotypes towards an increase in the frequency of the BB genotype. Thus, the percentage ratio of *bBLG*-HaeIII^{AA}, *bBLG*-HaeIII^{AB}, and *bBLG*-HaeIII^{BB} genotypes in the group of sick animals was 28.0, 28.0, and 44.0%, respectively, and in the control group, 29.0, 54.0, and 17.0%, respectively.

The distribution of *bBLG*-HaeIII genotypes is characterized by a significant increase in the frequency of *bBLG*-HaeIII^{AA} and *bBLG*-HaeIII^{BB} genotypes relative to the expected ones, 28.0 compared to 17.4% and 44.00 compared to 33.6% for *bBLG*-HaeIII^{AA} and *bBLG*-HaeIII^{BB} genotypes, respectively. The opposite, but statistically insignificant redistribution is observed in the control group. However, the observed frequency of occurrence of the *bBLG*-HaeIII^{AA} genotype in the group of mastitis cows is 28.0%, and in the control group, the number is almost the same (29.0%). However, heterozygous genotypes in the group of sick animals occur almost 2 times less often than in the control group (28.0 compared to 54.0%), and animals with the *bBLG*-HaeIII^{BB} genotype among mastitis cows occur more than 2 times more often than in the control group (44.0 compared to 17.0%, respectively).

This observation suggests that the *bBLG*-HaeIII^{AB} genotype is significantly associated with mastitis resistance in Holstein cows, while animals carrying the *bBLG*-HaeIII^{BB} genotype can be included in the increased risk group.

The data we obtained partly confirms the information received by other authors. Thus, according to R. Luhar et al. (2006), there is an association between the *BLG* gene polymorphisms and somatic cell count (mastitis indicator). The results of the study showed that certain variants of the *BLG* gene were associated with a reduced somatic cell count, which is a good indicator of udder health.

According to U. Singh et al. (2015), a link was established between genetic polymorphisms of the BLG gene and subclinical mastitis in Frieswal (HF×Sahiwal) cows in India. The study found a statistically significant association between certain gene variants and the risk of developing subclinical mastitis.

A similar relationship was established in piebald (black-white) cows in the studies by I.M. Kriventsov et al. (1975). They showed a connection between polymorphisms of the *BLG* gene and a predisposition to mastitis. The results showed that some gene variants were associated with an increased risk of developing mastitis.

These studies indicate a potential link between genetic variants of the *BLG* gene and the risk of mastitis in cows, especially dairy breeds.

6. Conclusions

The developed method of PCR-RFLP DNA typing of cattle by the *bPRL*-DpnI rs211032652 polymorphism of the *PRL* gene, accompanied by the replacement of the amino acid alanine with serine in the 13th position of the protein, made it possible to identify the presence/absence of this single-nucleotide polymorphism (SNP) in Holstein cattle. The observed frequencies of CC, CA, and AA genotypes in the group of animals suffering from mastitis equaled 138, 11, and 1, and in the control group, 92, 7, and 1, respectively.

The optimized PCR technique for conducting DNA typing of animals by the *BLG* gene made it possible to identify 42, 42, and 66 cases of carrying the AA, AB, and BB genotypes in the group of animals suffering from mastitis and 29, 54, and 17 cases, respectively, in the control group.

The genetic structure of the samples of animals suffering from mastitis and the control group for *bPRL*-DpnI polymorphism is consistent concerning the frequency distribution of the rare/frequent alleles; they practically do not differ from each other. The most common allele is *bPRL*-DpnI^{C} with 0.967±0.001 and 0.980±0.002 in the group of animals with mastitis and the control group, respectively. The most rare is the bPRL-DpnI^{A} allele (0.033±0.001 and 0.020±0.002 in the groups of sick and healthy animals, respectively).

The genetic structure of the samples of animals with mastitis and the control group for *bBLG*-HaeIII polymorphism is not consistent concerning the rare/frequent allele distribution. In the group of animals with mastitis, the *BLG*-HaeIII^{B} allele is characterized by a predominant frequency (0.580±0.003), while in the control group of healthy animals, the frequency of the BLG-HaeIII^{B} allele is reduced to 0.440±0.005.

A study of the nature of the association of polymorphic variants of the *PRL* and *BLG* gene with resistance/increased risk of mastitis established a significant deviation from the theoretically expected distribution of *bBLG*-HaeIII genotypes in a group of animals with mastitis (the χ^2 value was 0.24).

The *bBLG*-HaeIII^{BB} genotype can act as a marker of an increased risk of developing mastitis in Holstein cows. Its frequency in the group of sick animals exceeds the frequency in the control group by more than 2 times (44.0 compared with 17.0%, respectively). The *bBLG*-HaeIII^{AB} genotype is significantly associated with mastitis resistance in Holstein cows; its frequency is 2 times lower than in the control group (28.0 compared to 54.0%).

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References

ALESSANDRI, G., SANGALLI, E., FACCHI, M., FONTANA, F., MANCABELLI, L., DONOFRIO, G. and VENTURA, M., 2023. Metataxonomic analysis of milk microbiota in the bovine subclinical mastitis. *FEMS Microbiology Ecology*, vol. 99, no. 12, pp. fiad136. http://doi.org/10.1093/femsec/fiad136. PMid:37880979.

BARAJAS-MENDIOLA, M.A., SALGADO-LORA, M.G., LÓPEZ-MEZA, J.E. and OCHOA-ZARZOSA, A., 2022. Prolactin regulates H3K9ac and H3K9me2 epigenetic marks and miRNAs expression in bovine mammary epithelial cells challenged with Staphylococcus aureus. *Frontiers in Microbiology*, vol. 13, pp. 990478. http://doi.org/10.3389/fmicb.2022.990478. PMid:36212825.

BASEL DECLARATION, 2010 [viewed 13 February 2024]. *A call for more trust, transparency, and communication on animal research* [online]. Animal Research Tomorrow. Available from: https://animalresearchtomorrow.org/en/basel-declaration

BAYAZITOVA, K., RAMAZANOV, A., BAYAZITOV, T., IL, D. and IL, Y., 2023. Effect of plant-based whole milk substitute on calves' growth rate. *Online Journal of Biological Sciences*, vol. 23, no. 2, pp. 210-218. http://doi.org/10.3844/ojbsci.2023.210.218.

BAYMENOV, B.M., BULASHEV, A.K., CHUZHEBAYEVA, G.D., ALIYEVA, G.K., BEISHOVA, I.S., KOKANOV, S.K. and RAKETSKY, V.A., 2023. Phenotypic and genotypic resistance to antibiotics in Staphylococcus aureus strains isolated from cattle milk in Northern Kazakhstan. *Veterinary World*, vol. 16, no. 9, pp. 1815-1820. http://doi.org/10.14202/vetworld.2023.1815-1820. PMid:37859965.

BEHREN, L.E., KÖNIG, S. and MAY, K., 2023. Genomic selection for dairy cattle behaviour considering novel traits in a changing technical production environment. *Genes*, vol. 14, no. 10, pp. 1933. http://doi.org/10.3390/genes14101933. PMid:37895282.

BEISHOVA, I.S., BELAYA, A.V., YULDASHBAYEV, Y.A., CHUZHEBAYEVA, G.D., ULYANOV, V.A., ULYANOVA, T.V., KOVALCHUK, A.M., KUZHEBAYEVA, U.Z. and NAMET, A.M., 2023. Genetic polymorphism of prolactin and nitric oxide synthase in Holstein cattle. *Veterinary World*, vol. 16, no. 1, pp. 161-167. http://doi.org/10.14202/vetworld.2023.161-167. PMid:36855359.

BOKAYEV, Z., KAISHATAYEVA, A., DZHULAMANOV, T., AISIN, M. and MAUKENOVA, A., 2023. Development of marketing tools to raise funds for green projects: experience of the Republic of Kazakhstan. *Journal of Environmental Management and Tourism*, vol. 14, no. 3, pp. 689-697. http://doi.org/10.14505/jemt.v14.3(67).08.

BUI, A.P.N., TAM, T.L.H., PHUONG, P.T. and LINH, N.T., 2023. Overdominance in livestock breeding: examples and current status. *Advancements in Life Sciences*, vol. 10, no. 4, pp. 525-529.

CATTANEO, L., MINUTI, A., DAHL, G.E. and TREVISI, E., 2023. Graduate student literature review: the challenge of drying-off high-yielding dairy cows. *Journal of Dairy Science*, vol. 106, no. 9, pp. 6416-6426. http://doi.org/10.3168/jds.2022-23113. PMid:37500440.

CHANETON, L., PÉREZ SÁEZ, J.M. and BUSSMANN, L.E., 2011. Antimicrobial activity of bovine β-lactoglobulin against mastitis-causing bacteria. *Journal of Dairy Science*, vol. 94, no. 1, pp. 138-145. http://doi.org/10.3168/jds.2010-3319. PMid:21183025.

CLARKE, J. and PLOS BIOLOGY STAFF EDITORS, 2022. Mendel's legacy in modern genetics. *PLoS Biology*, vol. 20, no. 7, pp. e3001760. http://doi.org/10.1371/journal.pbio.3001760. PMid:35901028.

CÔTÉ-GRAVEL, J. and MALOUIN, F., 2019. Symposium review: features of Staphylococcus aureus mastitis pathogenesis that guide vaccine development strategies. *Journal of Dairy Science*, vol. 102, no. 5, pp. 4727-4740. http://doi.org/10.3168/jds.2018-15272. PMid:30580940.

COWAN, C.M., DENTINE, M.R., AX, R.L. and SCHULER, L.A., 1989. Restriction fragment length polymorphisms associated with growth hormone and prolactin genes in Holstein bulls: evidence for a novel growth hormone allele. *Animal Genetics*, vol. 20, no. 2, pp. 157-165. http://doi.org/10.1111/j.1365-2052.1989.tb00853.x. PMid:2569284.

DETILLEUX, J., KASTELIC, J.P. and BARKEMA, H.W., 2015. Mediation analysis to estimate direct and indirect milk losses due to clinical mastitis in dairy cattle. *Preventive Veterinary Medicine*, vol. 118, no. 4, pp. 449-456. http://doi.org/10.1016/j.prevetmed.2015.01.009. PMid:25638330.

GOMES, F. and HENRIQUES, M., 2016. Control of bovine mastitis: old and recent therapeutic approaches. *Current Microbiology*, vol. 72, no. 4, pp. 377-382. http://doi.org/10.1007/s00284-015-0958-8. PMid:26687332.

HADDRILL, P.R., 2021. Developments in forensic DNA analysis. *Emerging Topics in Life Sciences*, vol. 5, no. 3, pp. 381-393. http://doi.org/10.1042/ETLS20200304. PMid:33792660.

HOGEVEEN, H., STEENEVELD, W. and WOLF, C.A., 2019. Production diseases reduce the efficiency of dairy production: A review of the results, methods, and approaches regarding the economics of mastitis. *Annual Review of Resource Economics*, vol. 11, no. 1, pp. 289-312. http://doi.org/10.1146/annurev-resource-100518-093954.

ILIE, D.E., MIZERANSCHI, A.E., MIHALI, C.V., NEAMȚ, R.I., CZISZTER, L.T., CARABAȘ, M. and GRĂDINARU, A.C., 2023. Polymorphism of the prolactin (PRL) gene and its effect on milk production traits in Romanian cattle breeds. *Veterinary Sciences*, vol. 10, no. 4, pp. 275. http://doi.org/10.3390/vetsci10040275. PMid:37104430.

INTERNATIONAL COUNCIL FOR LABORATORY ANIMAL SCIENCE – ICLAS, 2024 [viewed 13 February 2024]. *Harmonization International Standards* [online]. Available from: https://iclas.org/harmonization-committee-international-standards/

KARYNBAYEV, A., NASIYEV, B., ZHARYLKASYN, K., ZHUMADILLAYEV, N. and ZHUMADILLAYEV, N., 2023. Development of a methodology for determining the nutritional value of pasture feed considering the fractions of easily digestible carbohydrates in the desert zone of Southern Kazakhstan. *Online Journal of Biological Sciences*, vol. 23, no. 4, pp. 458-469. http://doi.org/10.3844/ojbsci.2023.458.469.

KHAN, M.Z., WANG, J., MA, Y., CHEN, T., MA, M., ULLAH, Q., KHAN, I.M., KHAN, A., CAO, Z. and LIU, S., 2023. Genetic polymorphisms in immune- and inflammation-associated genes and their association with bovine mastitis resistance/susceptibility. *Frontiers in Immunology*, vol. 14, pp. 1082144. http://doi.org/10.3389/fimmu.2023.1082144. PMid:36911690.

KOBER, A.K.M.H., SAHA, S., ISLAM, M.A., RAJOKA, M.S.R., FUKUYAMA, K., ASO, H., VILLENA, J. and KITAZAWA, H., 2022. Immunomodulatory effects of probiotics: a novel preventive approach for the control of bovine mastitis. *Microorganisms*, vol. 10, no. 11, pp. 2255. http://doi.org/10.3390/microorganisms10112255. PMid:36422325.

KRIVENTSOV, I.M., KRIVENTSOVA, V.F. and BORISOVA, G.V., 1975. Izuchenie vzaimosviazi ingibitornoĭ aktivnosti moloka s razlichnymi tipami beta-laktoglobulinov i rezistentnosti korov k mastitam [The interrelationship between the inhibitory activity of milk with different types of beta-lactoglobulins and the resistance of cattle to mastitis]. *Genetika*, vol. 11, no. 12, pp. 37-44. PMid:5340.

LEWIN, H.A., SCHMITT, K., HUBERT, R., VAN EIJK, M.J. and ARNHEIM, N., 1992. Close linkage between bovine prolactin and BoLA-DRB3 genes: genetic mapping in cattle by single sperm typing. *Genomics*, vol. 13, no. 1, pp. 44-48. http://doi.org/10.1016/0888-7543(92)90200-C. PMid:1577492.

LUHAR, R., PATEL, R.K. and SINGH, K.M., 2006. Studies on the possible association of beta-lactoglobulin genotype with mastitis in dairy cows. *Indian Journal of Dairy Science*, vol. 59, no. 3, pp. 155.

MADENOVA, A.K., ATISHOVA, M.N., KOKHMETOVA, A.M., GALYMBEK, K. and YERNAZAROVA, G.I., 2019. Identification of carriers of resistance to common bunt (Tilletia caries) of winter wheat. *Research on Crops*, vol. 20, no. 4, pp. 782-790.

MALINTHA, G.H.T., CELINO-BRADY, F.T., STOYTCHEVA, Z.R. and SEALE, A.P., 2023. Osmosensitive transcription factors in the prolactin cell of a euryhaline teleost. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, vol. 278, pp. 111356. http://doi.org/10.1016/j.cbpa.2022.111356. PMid:36535574.

MAURIĆ MALJKOVIĆ, M., VLAHEK, I., PIPLICA, A., EKERT KABALIN, A., SUŠIĆ, V. and STEVANOVIĆ, V., 2023. Prospects of toll-like receptors in dairy cattle breeding. *Animal Genetics*, vol. 54, no. 4, pp. 425-434. http://doi.org/10.1111/age.13325. PMid:37051618.

MEDRANO, J.F. and AGUILAR-CORDOVA, E., 1990. Polymerase chain reaction amplification of bovine β-lactoglobulin genomic sequences and identification of genetic variants by RFLP analysis. *Animal Biotechnology*, vol. 1, no. 1, pp. 73-77. http://doi.org/10.1080/10495399009525730.

MENDYBAYEVA, A., ABILOVA, Z., BULASHEV, A. and RYCHSHANOVA, R., 2023. Prevalence and resistance to antibacterial agents in Salmonella enterica strains isolated from poultry products in Northern Kazakhstan. *Veterinary World*, vol. 16, no. 3, pp. 657-667. http://doi.org/10.14202/vetworld.2023.657-667. PMid:37041849.

MUSSAYEVA, A., YEGOROVA, N., YERISHOV, M., DOSSANOVA, A., SUCHSHIKH, V., NAMET, A., SIYABEKOV, S., NUSSUPOVA, S., YESPEMBETOV, B. and SYRYM, N., 2021. Molecular-biological properties of the attenuated strain of salmonella abortus-equi E-841, used in the creation of a vaccine against abortion of mares. *American Journal of Animal and Veterinary Sciences*, vol. 16, no. 2, pp. 144-150. http://doi.org/10.3844/ajavsp.2021.144.150.

MUSSYNOV, K.M., KIPSHAKBAEVA, A.A., ARINOV, B.K., UTELBAYEV, Y.A. and BAZARBAYEV, B.B., 2014. Producing capacity of safflower on dark brown soils of the northern Kazakhstan. *Biosciences Biotechnology Research Asia*, vol. 11, no. 3, pp. 1121-1130. http://doi.org/10.13005/bbra/1497.

MUSSYNOV, K.M., SULEIMENOVA, Z.S., BEKENOVA, S.S., UTELBAYEV, Y.A., BAZARBAYEV, B.B., YESSENBEKOVA, G.T. and SAGATBEK, S.D., 2019. Diseases of Flax (Linum usitatissimum) and substantiation of protective measures in the conditions of the dry steppe zone of Northern Kazakhstan. *Annals of Agri Bio Research*, vol. 24, no. 1, pp. 82-87.

MYLOSTYVYI, R., IZHBOLDINA, O., MIDYK, S., GUTYJ, B., MARENKOV, O. and KOZYR, V., 2023. The relationship between warm weather and milk yield in Holstein Cows. World's. *Veterinary Journal (London, England)*, vol. 13, pp. 134-143. http://doi.org/10.54203/scil.2023.wvj14.

PASCU, C., HERMAN, V., IANCU, I. and COSTINAR, L., 2022. Etiology of mastitis and antimicrobial resistance in dairy cattle farms in the Western part of Romania. *Antibiotics (Basel, Switzerland)*, vol. 11, no. 1, pp. 57. http://doi.org/10.3390/antibiotics11010057. PMid:35052934.

POPOV, V., SEREKPAEV, N., ZHARLYGASOV, Z., STYBAEV, G. and ANSABAEVA, A., 2017. Adaptive technology of environmentally - friendly production of legumes in the dry steppe zones. *Journal of Central European Agriculture*, vol. 18, no. 1, pp. 73-94. http://doi.org/10.5513/JCEA01/18.1.1869.

POWELL, J., TALENTI, A., FISCH, A., HEMMINK, J.D., PAXTON, E., TOYE, P., SANTOS, I., FERREIRA, B.R., CONNELLEY, T.K., MORRISON, L.J. and PRENDERGAST, J.G.D., 2023. Profiling the immune epigenome across global cattle breeds. *Genome Biology*, vol. 24, no. 1, pp. 127. http://doi.org/10.1186/s13059-023-02964-3. PMid:37218021.

RUNTUWENE, L.R., SATHIRAPONGSASUTI, N., SRISAWAT, R., KOMALAMISRA, N., TUDA, J.S.B., MONGAN, A.E., ABOGE, G.O., SHABARDINA, V., MAKALOWSKI, W., NESTI, D.R., ARTAMA, W.T., NGUYEN-THI, L.A., WAN, K.L., NA, B.K., HALL, W., PAIN, A., ESHITA, Y., MAEDA, R., YAMAGISHI, J. and SUZUKI, Y., 2022. Global research alliance in infectious disease: a collaborative effort to combat infectious diseases through dissemination of portable sequencing. *BMC Research Notes*, vol. 15, no. 1, pp. 44. http://doi.org/10.1186/s13104-022-05927-2. PMid:35151353.

SALGADO-LORA, M.G., MEDINA-ESTRADA, I., LÓPEZ-MEZA, J.E. and OCHOA-ZARZOSA, A., 2020. Prolactin and estradiol are epigenetic modulators in bovine mammary epithelial cells during staphylococcus aureus infection. *Pathogens (Basel, Switzerland)*, vol. 9, no. 7, pp. 520. http://doi.org/10.3390/pathogens9070520. PMid:32605209.

SARSEKOVA, D., MAZARZHANOVA, K., DOSMANBETOV, D., KOPABAYEVA, A., OBEZINSKAYA, E., NURLABI, A. and MUKANOV, B., 2023. Assessment of the degree of landscaping in Astana, Kazakhstan and recommendations for its development. *Caspian Journal of Environmental Sciences*, vol. 21, no. 3, pp. 585-594.

SHAMSHIDIN, A., KHARZHAU, A., GABDULLIN, D., BATYRGALIYEV, Y., KULBAYEV, R. and ZHOLDASBEKOV, A., 2023. Development of feeding and reproduction technology in dairy cattle breeding based on the use of digital technologies. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, pp. e276748. http://doi.org/10.1590/1519-6984.276748. PMid:37909559.

SINGH, M.K., KUMAR, A., NIMMANAPALLI, R. and PANDEY, A.K., 2023. Detection of the β-lactoglobulin genotype in zebu cattle (Gangatiri) milk using high-resolution accurate mass spectroscopy. *The Journal of Dairy Research*, vol. 90, no. 3, pp. 287-291. http://doi.org/10.1017/S0022029923000481. PMid:37622324.

SINGH, U., DEB, R., KUMAR, S., SINGH, R., SENGAR, G. and SHARMA, A., 2015. Association of prolactin and beta-lactoglobulin genes with milk production traits and somatic cell count among Indian Frieswal (HF × Sahiwal) cows. *Biomarkers and Genomic Medicine*, vol. 7, no. 1, pp. 38-42. http://doi.org/10.1016/j.bgm.2014.07.001.

SUCHSHIKH, V., KARIMOV, A., YUSSUPOV, M., AITZHANOV, B., ABUTALIP, A., MUSSAYEVA, A., YEGOROVA, N., MAMANOVA, S. and KANATOV, B., 2023. Effectiveness of different means of disinfection against soil foci of anthrax (Bacillus anthracis) burials at a depth of up to 3.5 m: an experimental study. *Caspian Journal of Environmental Sciences*, vol. 21, no. 4, pp. 893-902.

TOMANIĆ, D., SAMARDŽIJA, M. and KOVAČEVIĆ, Z., 2023. Alternatives to antimicrobial treatment in bovine mastitis therapy: a review. *Antibiotics (Basel, Switzerland)*, vol. 12, no. 4, pp. 683. http://doi.org/10.3390/antibiotics12040683. PMid:37107045.

ULYANOV, V.A., KUBEKOVA, B.Z., BEISHOVA, I.S., BELAYA, A.V. and PAPUSHA, N.V., 2021. Preferred and undesirable genotypes of bGH and bIGF-1 genes for the milk yield and quality of black-and-white breed. *Veterinary World*, vol. 14, no. 5, pp. 1202-1209. http://doi.org/10.14202/vetworld.2021.1202-1209. PMid:34220122.

USKENOV, R., ISSABEKOVA, S., BOSTANOVA, S., SHAIKENOVA, K., SHAMSHIDIN, A. and KHARZHAU, A., 2023. The influence of productivity indicators on the culling of dairy cows in the sharply continental climate of Kazakhstan. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, pp. e274719. http://doi.org/10.1590/1519-6984.274719. PMid:37672436.

VAN EIJK, M.J., BEEVER, J.E., DA, Y., STEWART, J.A., NICHOLAIDES, G.E., GREEN, C.A. and LEWIN, H.A., 1995. Genetic mapping of BoLA-A, CYP21, DRB3, DYA, and PRL on BTA23. *Mammalian Genome*, vol. 6, no. 2, pp. 151-152. http://doi.org/10.1007/BF00303266. PMid:7767004.

WONG, F.C. and CHAI, T.T., 2023. Bioactive peptides and protein hydrolysates as lipoxygenase inhibitors. *Biology (Basel)*, vol. 12, no. 7, pp. 917. http://doi.org/10.3390/biology12070917. PMid:37508348.

ZAITSEV, V., KOROTKIY, V., BOGOLYUBOVA, N., ZAITSEVA, L. and RYZHOV, V., 2024. Prevention of heat stress in lactating cows. *American Journal of Animal and Veterinary Sciences*, vol. 19, no. 1, pp. 7-12. http://doi.org/10.3844/ajavsp.2024.7.12.

ZEMANOVA, M., LANGOVA, L., NOVOTNÁ, I., DVORAKOVA, P., VRTKOVA, I. and HAVLICEK, Z., 2022. Immune mechanisms, resistance genes, and their roles in the prevention of mastitis in dairy cows. *Archives Animal Breeding*, vol. 65, no. 4, pp. 371-384. http://doi.org/10.5194/aab-65-371-2022. PMid:36415759.

ZHANG, H., WANG, K., AN, T., ZHU, L., CHANG, Y., LOU, W., LIU, L., GUO, G., LIU, A., SU, G., BRITO, L.F. and WANG, Y., 2022. Genetic parameters for dairy calf and replacement heifer wellness traits and their association with cow longevity and health indicators in Holstein cattle. *Journal of Dairy Science*, vol. 105, no. 8, pp. 6749-6759. http://doi.org/10.3168/jds.2021-21450. PMid:35840408.