Acta fytotechn zootechn, 27, 2024(3): 203–208
http://www.acta.fapz.uniag.sk

Original Paper

Determination of Pit-1 and T Polymorphism in Ukrainian Brown Dairy and Sumy Intrabreed Type of the Ukrainian Black-and-White Dairy Cattle

Alona Malikova[1], Yuliia Pavlenko[1], Yuriy Skliarenko*[1, 2], Tatyana Chernyavska[1], Liudmyla Bula[1], Vytautas Gustaitis[1]

[1]*Sumy National Agrarian University, Ukraine*
[2]*Institute of Agriculture in the North East of the National Academy of Agrarian Sciences of Ukraine, Ukraine*

Article Details: Received: 2024-06-07 | Accepted: 2024-07-02 | Available online: 2024-09-30

https://doi.org/10.15414/afz.2024.27.03.203-208

The research of polymorphism of the PIT-1 and beta-lactoglobulin genes has been provided on the population of Ukrainian brown dairy and Sumy intrabreed type Ukrainian black-and-white dairy cows. The study of genotypes has been carried out using polymerase chain reaction in real time. The existence of interbreed differentiation in the frequencies of genotypes and alleles of the studied genes has been proved. The influence of the studied genes genotype on individual indicators of milk productivity of cows has been established.

Keywords: polymorphism, PIT-1, Beta-Lactoglobulin, genotype frequency, breeds

1 Introduction

Today, in the practical breeding of cattle, the achievements of genetics are widely used, which allows to significantly speed up the selection process (Shelyov et al., 2021).

Such genetic markers include the genes: β-lactoglobulin (BLG) and the pituitary-specific positive transcription factor PIT-1 (Mitioglo et al., 2021; Mattos et al., 2004).

An important technological property of the BLG protein is its reaction with casein, resulting changes in the thermal stability of milk and delaying the process of rennet clotting. The most common allelic variants of the BLG gene are A and B and variants of genotypes AA, BB and AB. Allele A is associated with milk yield, serum protein content, and total protein content in milk (Mitioglo et al., 2021). Allele B is associated with the level of casein proteins and casein coagulate and with a high mass fraction of fat in milk. Knowledge of beta-lactoglobulin gene polymorphism will enable breeders to improve milk productivity of cattle breeds and herds (Mitioglo et al., 2021; Kamiński et al., 2000).

Scientists consider the influence of the beta-lactoglobulin gene on the main indicators of milk productivity in cows. Holstein cows with the AA genotype distinguished by higher milk yields and the amount of milk fat and protein (Sitkowska et al., 2009; Heidari et al., 2012). Animals with homozygous BB genotype have higher fat and protein content in milk (Mohammadi et al., 2013).

It was established that the pituitary-specific positive transcription factor PIT-1 is a regulatory gene (Aytekin et al., 2013). It regulates the development of pituitary zones that control the synthesis of somatotropic hormones, prolactin and thyroid-stimulating hormone. This determines the classification of the PIT-1 gene as a marker of genetic variability of traits related to milk productivity. Scientists think that mutations of this gene can lead to significant changes in the milk productivity traits of cows (Mattos et al., 2004).

The researchers claim that the polymorphism of this gene can serve as a marker of milk productivity, although this issue has not been thoroughly studied. It has been established that the A allele is associated in animals with higher milk yields and quality traits of milk (Zwierzchowski et al., 2002; İbrahim et al., 2022).

***Corresponding Author:** Yuriy Skliarenko, Sumy National Agrarian University, Herasima Kondratieva St, 160, Sumy, Sumy Oblast, Ukrajina, 40000, Ukraine, sklyrenko9753@ukr.net

According to the results of dispersion analysis, it was established that the share of influence of the PIT-1 gene on the amount of milk yield and the amount of milk fat and protein during the first lactation is within 4.1–4.6%. This tendency also observed with the indicators of the second lactation. At the same time, the author notes the superiority animal's milk yield in animals having the AB genotype over animals with the BB genotype (by 499 kg) (Gubarenko 2020).

In animals of different breeds, the frequency of genotypes for the PIT-1 gene has its differences (İbrahim et al., 2022).

The objective of our study was to research BLG and PIT-1 gene polymorphisms in local dairy cattle.

2 Material and Methods

According to the recommendations of the FAO (FAO, 2011), for a reliable assessment of the frequency of alleles, samples should be taken from at least 25 animals of the studied breed. We performed genotyping of cows of the Sumy intrabreed type of the Ukrainian black-and-white dairy breed (SITUBWD) (n = 30) and the Ukrainian brown dairy breed (UBD) (n = 30). The animals belong to the Research Farm located in the Sumy district of the Sumy oblast.

Genetic studies conducted on DNA samples taken from hair bulbs of cows.

To study the single nucleotide polymorphism of the beta-lactoglobulin (BLG) gene of cattle (chromosome 11, GenBank: X14710.1, exon 4, rs458095482 (Gcc/Ccc, 270A >P) and rs109625649 (gCc/gTc, 270A >V) we used the PCR-RFLP method with specific primers and HaeIII restriction endonuclease. DNA was isolated from hair follicles using the commercial kit "DNK-Sens"). Amplification of BLG gene fragment conducted in thermocycler "Tertsick" (DNA-techologies) using primers: F-5'-TGTGCTGGACACCGACTACAAAAAG-3' I R-5'-GCTCCCGGTATATGACCACCCTCT-3" (Ibatulin et al., 2017).

The PCR mixture (10 ml) contained: 5 µl master mix (10× buffer for DNA polymerase (1 µl), DNA polymerase (Fermentas, Lithuania, 0.25 units), 2.5 mM DNATP (1 µl), deionized H_2O (3 µl)), 1 µµ mixture of primers (5 µl) and DNA (5 µl). Temperature regime: initial denaturation – 2.5 min at 94 °C, next 38 cycles – 94 °C 20 s, 64 °C 30 s, 72 °C 1 min, final elongation at 72 °C 7 min. The size of the amplicon is 247 bps. The studied fragment has one monomorphic restriction site for HaeIII (GG ↓ CC) and one polymorphic one. Expected restriction patterns for genotypes: AA (HaeIII-) – 148/99 bps; BB (HaeIII+) –74/74/99 bps; AB – 148/99/74 bps.

Amplification products were treated with HaeIII endonuclease according to the manufacturer's instructions (Fermentas, Lithuania). The number and length of the restriction products were determined by electrophoresis in a 3% agarose gel (with the addition of 0.5 µg ml^{-1} ethidium bromide) in Tris-borate buffer (TBE: 0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA pH 8.0) with using a molecular weight marker (100 bps Ladder, Simgen). Electrophoresis results visualized on a transilluminator in the UV spectrum (312 nm).

The polymorphism of the locus of the pituitary-specific transcription factor PIT 1 (chromosome 1, GenBank: Y15995.1, exon 6, synonymous transition 1,256 G >A, ctG >ctA) studied by the PCR-PDRF method using HinfI endonuclease (G ↓ ANTC restriction site).

Primers:

- forward: 5'-CAATGAGAAAGTTGGTGC-3';
- reverse: 5'-TCTGCATTCGAGAT GCTC-3') (Moody et al., 1995).

The conditions for PCR amplification of the PIT 1 gene are as follows: initial denaturation – 2.5 min at 94 °C, the next 35 cycles – 94 °C 20 s, 52 °C 30 s, 72 °C 1 min, final elongation at 72 °C 7 min. The size of the amplicon is 1301 bp.

The studied fragment has two monomorphic and one polymorphic (1,256 G >A) restriction sites for HinfI (G ↓ ANTC). Restriction fragments with a length of 617, 424 and 260 bp. correspond to allele A (NinfI), fragments 617, 379, 260 and 45 bp. indicate the B allele (NinfI+).

To calculate the frequency of alleles of the studied genes, the formula was used:

$$P = \frac{2N_1 + N_2}{n}$$

where: N_1 and N_2 – the corresponding number of homozygous genotypes and heterozygous genotypes; n – total number of alleles

The formula was used to calculate the Pearson criterion:

$$\chi^2 = \sum \frac{(A-T)^2}{T}$$

where: A – actual number of genotypes; T – theoretical number of genotypes

The formula was used to calculate the actual heterozygosity:

$$H_O = \frac{N2}{n}$$

The formula was used to calculate the expected heterozygosity:

$$H_E = 1 - \sum_{i=1}^{n} p_i^2$$

where: p_1, p_2... p_n – the corresponding frequency of a certain allele

The formula was used to calculate the fixation index:

$$F_{is} = \frac{H_E - H_O}{H_E}$$

3 Results and Discussion

We conducted a study to determine the genotype of animals based on the PIT-1 and BLG genes. Analysis of PIT-1 gene polymorphism shown in Figure 1.

The purpose of the conducted genetic research on the PIT-1 gene was to study the genetic characteristics of dairy cows that had a common origin from the Lebedyn cattle breed (Table 1).

Breeding features of the genotypes frequency of the studied gene has been established. In UBD animals, a higher frequency was characteristic of the BB genotype than AA. The difference in the proportions of this genotype between animals of the studied breeds was statistically significant (P <0.01). In SITUBWD animals, a higher frequency is characteristic of the AB genotype. The difference in the proportions of this genotype between animals of the studied breeds was also statistically significant (P <0.01). The homozygous AA genotype had a low frequency in UBD animals and was absent among SITUBWD cows.

The degree of correspondence of the actual distribution of genotypes to the expected value, calculated using the χ^2 criterion, indicates that it corresponds to the theoretical distribution in UBD animals (P <0.01). In SITUBWD animals, on the contrary, the actual distribution of genotypes did not correspond to the expected values with a high degree of reliability (P <0.01).

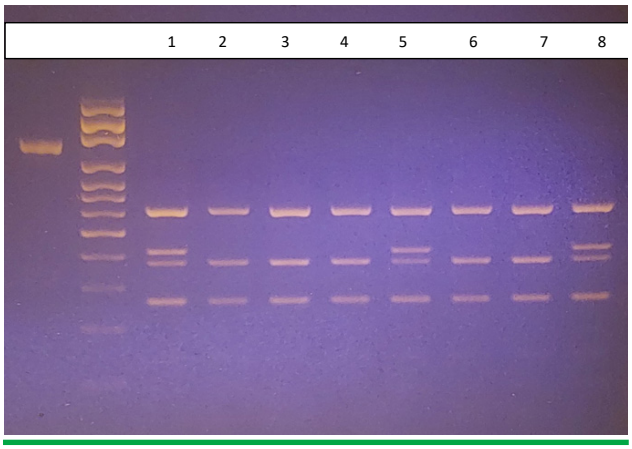

Fig. 1 Electrophorogram of PIT-1 gene amplification products
1, 5 and 8 lines – AB; 2, 3, 4, 6, and 7 lines – BB

The frequency of allele B was higher than the frequency of allele A in animals of both studied breeds. It has been established that allele frequencies in animals of both studied breeds are statistically significant (reliability criterion tA and tB >3).

The studied populations differed in the ratio of actual to expected heterozygosity. In UBD animals, the actual heterozygosity was inferior to the expected one, and in SITUBWD cows, the actual heterozygosity, on the contrary, prevailed over the theoretical one. This is also confirmed by the value of the fixation index (Table 2).

The BLG gene polymorphism analysis shown in Figure 2.

In both UBD and SITUBWD animals, a highest frequency was characteristic of the AB genotype. The difference in the proportions of this genotype between animals

Table 1 The genotype and allele frequencies and the expected heterozygosity of PIT-1 polymorphism

Distribution[1]	Genotype						Allele (pcs.)		χ^2	Statistical significance
	AA		AB		BB		A	B		
	n	frequency	*n*	frequency	*n*	frequency				
UBD										
A	2	0.07	11	0.37**	17	0.56**	0.25 ±0.056	0.75 ±0.056	0.015	P >0.05
T	–	0.06	–	0.38	–	0.56				
SITUBWD										
A	0	0.00	24	0.80a	6	0.20a	0.40 ±0.063	0.60 ±0.063	13.3	P <0.01
T	–	0.16	–	0.48	–	0.36				

1 A – actual number of genotypes; T – theoretical number of genotypes; **P – level of significance according, to Fisher's test: P <0.01; a – the difference is likely to be relative to the indicator indicated by the superscript

Table 2 The value of the main variability indicators for the PIT-1 gene

Breed	Ho	He	Fis
UBD	0.367	0.375	0.022
SITUBWD	0.800	0.480	-0.667

Ho – actual heterozygosity, He – expected heterozygosity, Fis – fixation index

of the studied breeds was not statistically significant. Share of homozygous AA genotype was higher in SITUBWD animals, and BB genotype – in UBD cows.

The degree of conformity of the actual distribution of genotypes with the expected value, calculated using the χ^2 criterion, indicates that it corresponds to the theoretical distribution in UBD animals, while in SITUBWD animals, on the contrary, the actual distribution of genotypes did not correspond to the expected values with a high degree of reliability (P <0.05). According to the frequency of alleles, animals of both researched breeds were characterized by the superiority of allele B. According to the results of the calculations, it was proved that the frequencies of alleles were statistically significant (criterion of reliability tA and tB >3) in both researched breeds (Table 3).

Cows UBD and SITUBWD were characterized by the advantage of actual heterozygosity over the expected one, which also confirms the value of the fixation index (Table 4).

Among UBD animals, the majority had the complex PIT 1/BLG genotype – AB/AB (30%) and AB/BB (30%). Among SITUBWD cows, the majority had the AB/AB genotype (48%). SITUBWD animals lacked the complex AA/AA genotype found in UBD cows (Table 5, 6).

On the contrary, the complex AB/AA genotype, which was present in SITUBWD animals, was not found in UBD animals. Complex genotypes: AA/AB, AA/BB were not found in animals of both breeds.

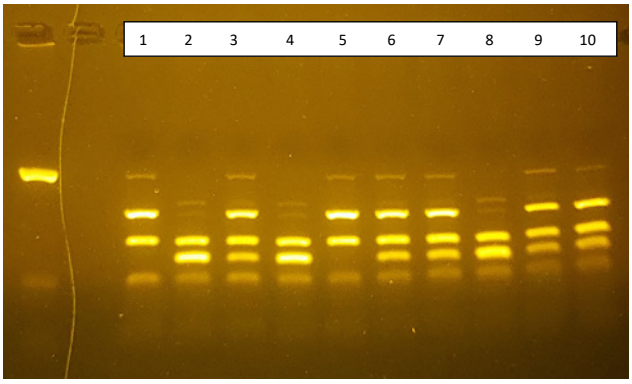

Fig. 2 Electrophorogram of BLG gene amplification products
1, 5 lines – AA; 3, 6, 7, 9, and 10 – AB; 2, 4, and 8 lines – BB

Table 4 The value of the main indicators of variability by gene BLG

Breed	Ho	He	Fis
UBD	0.600	0.480	-0.250
SITUBWD	0.667	0.498	-0.339

Ho – actual heterozygosity, He – expected heterozygosity, Fis – fixation index

In contrast to the previously obtained results, it was established that animals of the Swiss breed are characterized by the absence of animals with the AA genotype by the PIT-1 gene and a high frequency of the AB genotype (0.8) (İbrahim et al., 2022). Our results indicate that in the animals of UBD, obtained with the participation of Swiss cattle, the presence of AA genotypes and a low frequency of AB genotype (0.37) is characteristic. However, other researchers (Aytekin et al., 2013) testified the presence of the AA genotype in animals of this breed. Regarding SITUBWD animals, in the selection of which Holstein cattle were used, our results correspond to a certain extent with those obtained by other researchers. In terms of allele frequencies, our studies also correspond to previous

Table 3 The genotype and allele frequencies and the expected heterozygosity of BLG polymorphism

Distribution[1]	Genotype						Allele (pcs.)		χ2	Statistical significance
	AA		AB		BB		A	B		
	n	frequency	n	frequency	n	frequency				
UBD										
A	3	0.10	18	0.60	9	0.30	0.40 ±0.063	0.60 ±0.063	1.870	P >0.05
T	–	0.16	–	0.48	–	0.36				
SITUBWD										
A	4	0.13	20	0.67	6	0.20	0.47 ±0.064	0.53 ±0.064	3.45	P <0.05
T	–	0.22	–	0.50	–	0.28				

1 A – actual number of genotypes; T – theoretical number of genotypes; NS; not significant (P > 0.05)

Table 5 Frequency distribution of the studied combinations of PIT-1 and BLG genotypes in UBD cows

Genotypes BLG	Frequency of composite genotypes (%)		
	genotypes PIT-1		
	AA	AB	BB
AA	7	–	3
AB	–	30	30
BB	–	7	23

Table 6 Frequency distribution of the studied combinations of PIT-1 and BLG genotypes in SITUBWD cows

Genotypes BLG	Frequency of composite genotypes (%)		
	genotypes PIT-1		
	AA	AB	BB
AA	–	14	3
AB	–	48	14
BB	–	18	3

studies (Vargas et al., 2004; Thuy et al., 2018; İbrahim et al., 2022).

According to the BLG gene, the SITUBWD animals did not fully correspond to the previously obtained results of other researchers. According to the results of their research, the share of the AB genotype, although it was the highest (0.47), was inferior to our results (0.67). In terms of allele frequencies, our results were consistent with those of other researchers (Doosti et al., 2011). In UBD animals, the frequencies of alleles of this gene obtained by our studies (A – 0.4; B – 0.6) coincided with the results of other researchers (A – 0.375; B – 0.625) (Unsal 2015).

4 Conclusions

It was established that the dairy breeds of cattle, which are bred in the north-east of Ukraine, differ significantly from each other in terms of PIT-1 and BLG genotypes, as well as the complex PIT-1/BLG genotype. It was established that the general population genetic balance among the studied breeds coincides with the data of other researchers and indicates the absence of targeted selection for these genotypes.

Among UBD cows, the majority had complex PIT 1/BLG genotypes – AB/AB and AB/BB, and among SITUBWD cows – AB/AB. Complex genotypes AA/AB and AA/BB were not found in animals of both breeds.

References

Aytekin, I., & Boztepe, S. (2013). Associations of Pit-1 gene polymorphism with milk yield and composition traits in brown swiss cattle. *Journal of Animal and Plant Sciences*, 23, 1281–1289.

Doosti, A. et al. (2011). Kappa-casein gene polymorphism in Holstein and Iranian native cattle by polymerase chain reaction restriction fragment length olymorphism (PCR-RFLP). *African Journal of Biotechnology*, 10(25), 4957–4960.

FAO. 2011. *Molecular genetic characterization of animal genetic resources*. FAO Animal Production and Health Guidelines. No. 9. Rome.

Gubarenko, N. (2020). Energy evaluation of cows using genetic markers. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 3(3), 3–7. https://doi.org/10.32718/ujvas3-3.01

Heidari, M. et al. (2012). Effect of Polymorphic Variants of GH, Pit1, and βLG Genes on Milk Production of Holstein Cows. *R. Journal of Genetics*, 48(4), 417–421.

Ibatulin, I. et al. (2017). *Methodology and organization of scientific research in animal husbandry*. Agrarian science.

İbrahim, A., & Mervan, B. (2022). Pit-1 Gene Polymorphisms in Anatolian Black, Holstein Friesian, Brown Swiss and Simmental Cattle Reared in Turkey. *Journal of Agricultural Sciences*, 28(1),139–144.

Mattos, K. et al. (2004). Association of bGH and Pit-1 gene variants with milk production traits in dairy Gyr bulls. Pesq. agropec. bras. *Brasilia*, 39, 147–150. https://doi.org/10.1590/S0100-204X2004000200007

Mitioglo, I. et al. (2021). Genetic structure of cows of the Ukrainian red-spotted dairy breed according to the complex of genotypes GH, CSN3 and BLG. *Herald of Agrarian Science*, 4(817), 51–58.

Mohammadi, Y. et al. (2013). Allelic polymorphism of K-casein, β-Lactoglobulinand leptin genes and their association with milk production traits in Iranian Holstein cattle. *Journal of Cell and Molecular Research*, 5(2), 75–80. https://doi.org/10.22067/jcmr.v5i2.21086

Moody, D. et al. (1995). Restriction fragment length polymorphism in amplification products of the bovine Pit-1 gene and assignment of Pit-1 to bovine chromosome 1. *Animal genetics*, 97, 45–47.

Shelyov, A. et al. (2021). Formation of the genetic structure of cattle populations by single locus DNA fragments depending on their productivity direction and origin. *Agricultural Science and Practice*, 8(3), 35–49. https://doi.org/10.15407/agrisp8.03.035

Sitkowska, B. et al. (2009). Effect of the polymorphic composite forms of betalactoglobulin on the milk yield and chemical composition in maximum lactation. *Journal of Central European Agriculture*, 10(3), 251–254.

Kamiński, S., & Zabolewicz, T. (2000). Associations between bovine beta-lactoglobulin polymorphism within coding and regulatory sequences and milk performance traits. *J. Appl. Genet.,* 41(2), 91–99.

Thuy, N. et al. (2018). Polymorphism of PIT-1 and Prolactin Genes and Their Effects on Milk Yield in Holstein Frisian Dairy Cows Bred in Vietnam. *Russian Journal of Genetics*, 54, 346–352. https://doi.org/10.1134/S1022795418030146

Unsal, D. (20t15). β-lactoglobulin genetic variants in Brown-Swiss dairy cattle and their association with milk yield and quality traits. *The Journal of Animal and Plant Sciences*, 25(2), 595–598.

Vargas, L. et al. (2004). Pit-1 gene polymorphism in dairy cows from Central Chile. *Archivos de Zootecnia,* 53, 217–220.

Zwierzchowski, L. et al. (2002). Effects of polymorphism of growth hormone (GH), Pit-1 and leptine (LEP) genes, cow`s age, lactation stage, and somatic cell count on milk yield and composition of Polish Black and White cows. *Animal science*, 20(4), 213–227.