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STUDY OF BETA-CASEIN GENE POLYMORPHISM IN DAIRY CATTLE POPULATIONS OF UKRAINE

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Abstract: *Using real-time polymerase chain reaction methods, we have researched the CSN2 polymorphism gene in 2 populations of cows in the North-East of Ukraine. The observed distributions of genotype frequencies A1A1, A1A2, A2A2 and A1 or A2 alleles varies considerably in different cattle populations of cows: population of brown cattle – 1.7%, 38.5, 59.8% (0.209 and 0.791); black and spotted population – 18.3%, 44.7%, 37.0% (0.406 and 0.594). The ratio of genotypes were found significant differences when comparing genotypes A1A1 ($p<0.001$) and A2A2 ($p<0.001$). The results of DNA testing of the beta-casein locus for A1 and A2-allelic variants in stud bulls of the studied populations have shown that the highest frequency of the A2A2 desired homozygous genotype is characteristic of the breeders of brown cattle (46%). The obtained results indicate the prospects of breeding work to create herds with genotype A2A2 animals - specifically with the brown cattle.*

Keywords: *genetic polymorphism, beta-casein A2, genotype frequency, cattle breeds.*

INTRODUCTION

In all developed countries of the world, genotyping of cattle breeds has become routine practice. The search for gene variants that determine the formation of the optimal phenotype of a cow for industrial use is focused primarily on pairs of genes, for which significant associations milk proteins: beta-casein (CSN2), kappa-casein (CSN3), beta-lactoglobulin (BLG) [14]. These include polymorphism of beta-casein, the second most abundant protein in cow's milk representing 27% of total protein, which is being studied most extensively given its medical significance. It is encoded by the CSN2 gene mapped on chromosome 6q31 (Gene ID: 281099) and consists of the 209-amino-acid single polypeptide chain and molecular mass of about 24 kDa [4]. Beta-casein is expressed as 13 genetic variants [3], which is the result of single nucleotide polymorphisms (SNP) in the CSN2 gene [13]. Much attention in dairy cattle breeding is paid to the quality characteristics of milk. In recent years, scientists have found that cow milk usually contains two main types of beta-casein, such as A1 and A2 [7, 9].

Researchers have found a possible relationship between milk consumption and certain diseases, such as Type 1 diabetes, cardiovascular disease, Sudden Infant Death Syndrome, schizophrenia and autism, gastrointestinal diseases, prostate cancer, and other diseases [2, 7].

In recent years, the discussion about A2 milk has been intensifying. There is only one difference between the A1 and A2 variants. At position 67, the amino acid proline in variant A2 is replaced by histidine in variant A1. During digestion, beta-casein A1 releases the peptide beta-casomorphin 7, not produced in milk A2. It is the beta-casomorphin 7

peptide (BCM). It was mentioned that BCM is a risk factor for some diseases and has been linked to digestive and other problems [1, 17].

Environmental studies conducted in nineteen countries (United Kingdom, Finland, Ireland, Sweden, Denmark, France, Germany, Iceland, Norway, Austria, Switzerland, United States, Japan, Israel, Australia, New Zealand, Hungary, Venezuela, and Canada) found a strong association between β-casein A1 consumption and the incidence of type 1 diabetes. Epidemiological studies reported in a patent application show a link between β-casein A1 consumption and worsening neurological disorders such as autism and schizophrenia [1].

Studies by scientists that have studied the relationship between β-casein genotypes and milk productivity traits are very contradictory. Citing the authors note that a positive effect of Variant A2 compared to Variant A1 on the amount of milk in Holstein cows has been established. Proved that the A2A2 genotype positively affects the amount of milk protein in first lactation cows and the fat and protein content in second lactation animals compared to the A1A1 genotype. According to, which the authors cite, animals with the A2A2 genotype are characterized by more protein content. A higher fat content, on the contrary, was characteristic of animals with an A1A1 genotype. So, variant A2 is positively associated with higher milk yield and protein content in milk, while variant A1 has a positive effect on fat content. According to the authors also note that animals of Holstein breed of Dutch selection with variant A2 had higher productivity and protein content in milk than cows with variant A1. At the same time, the authors argue that found that Holstein steers with the A2A2 genotype have a high breeding value in milk yield and protein content compared to A1A1 genotype animals, but the low breeding value in fat content. At the same time, scientists have shown that as a result of research. found that Czech Simmental bulls with A2A2 genotype had a negative breeding evaluation, while animals with A1A1 genotype, on the contrary, had positive milk yield evaluation. With regard to suitability for cheese making, the researchers cite the results of, according to which milk from animals of genotype A2A2 has longer clotting time and lower gel stability of Holstein cows of the Danish breed significantly compared to milk of animals of genotype A1A1. Variant B had a substantially shorter clotting time and higher gel stability than variant A1 [1].

It is generally known that the progress of breed in modern breeding conditions is provided by stud bulls with high breeding value. This has become possible by storing deep-frozen semen and applying a large-scale breeding system in practice [5, 6].

The frequency of the A2A2 genotype in Holstein cattle is 48%, A1A2 heterozygotes are amounted to 25%, and A1A1 homozygotes are amounted to 27%. This frequency in Ayrshire stud bulls is 22%, 47%, and 31%, respectively. At the same time, A. Parashar shows that the frequency of the A1 allele in the Guernsey breed is in the range of 4-2%, Swiss – 34-30%, Jersey – 50-37%, Holstein – 56-47%, Ayrshire – 60-51%, Red Danish – 77%. Thus, DNA monitoring of the ratio of beta-casein alleles in the genotype of stud bulls will enable to predict the possibility of creating dairy herds with the programmed milk quality, since an increase in beta-casein homozygosity of A2A2 in the next generation is possible, especially when using A2A2 homozygous stud bulls. This, in turn, will increase the competitiveness of local breeds, and accordingly give additional options for their preservation [10, 12].

The role of beta-casein A1 as an undesirable variant led to an attempt to select dairy cows based on beta-casein polymorphism. Breeding programs have attempted to use bulls with the A2A2 genotype. In New Zealand, this led to the selection of cows in the herd producing milk only with the A2 variant.Since 2003 A2 milk had been solved in New Zealand and Australia as a premium brand that proposes a natural selection of proteins. The company started marketing A2 milk in Asia, Russia, and the USA (A2 Corporation 2006) [17].

A separate task for geneticists is to determine the distribution of different beta-casein variants in local cattle breeds. For example, Iranian researchers analyzed the frequency of the A1 allele of the CSN2 gene in the following breeds: Holstein - 50 %, Simmental 51.57%, Sistani 54.5 %, 49.4%, and 46.6% in Taleshi and Mazandarani cattle populations, respectively [15, 18].

In Ukraine, given the long tradition of cattle breeding, there is an excellent variety of different breeds, among which the unique Ukrainian Lebedyn breed, genetic studies of which began only recently [11, 16].

The purpose of this study was to perform genotyping of different Ukrainian breeds for the CSN2 gene (rs43703011), calculation, evaluation, and analysis of genotype and allele frequencies.

MATERIALS AND METHODS

Genotyping of 990 cattle of the following populations was carried out: brown cattle of various bloodlines according to the Swiss breed (n = 243), black-spotted cattle of different conventional bloodlines according to the Holstein breed (n = 747). Blood samples were taken under sterile conditions into 2.7 mL Monovette containing EDTA potassium salt as an anticoagulant ("Sarstedt," Germany) with the following samples' freezing and storage at -20ºC. DNA for genotyping was extracted from the samples using Monarch® Genomic DNA Purification Kit New England BioLab kits (USA) according to the manufacturer's protocol.

The TaqMan@Genotyping real-time PCR system was used to perform allelic discrimination.

Two primers were designed to amplify the 101 bp product involving SNP rs43703011 (genomic DNA: X14711 (http://www.ncbi.nih.gov); forward primer, 5′-CCCAGACACAGTCTCTAGTCTATCC-3′; reverse primer, 5′- GGTTTGAGTAAGAG-GAGGGATGTTT -3′). Two fluorogenic TaqMan probes were designed with different fluorescent dye reporters to allow single-tube genotyping. The first probe was targeted to the wild type allele A (5′- VIC-CCCATCCATAACAGCC-3′) and the second one to the mutated allele B (5′- FAM- CCATCCCTAACAGCC -FAM-3′) of the CSN2 gene. The powerful NFQ quencher was linked to the 3′ end of both probes. Primers and probes were designed using Primer Express software, version 3.0 (Applied Biosystems, CA, USA) and were obtained from Applied Biosystems. The accuracy of the used sequence source was verified by comparison with sequences from the GenBank database using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Real-time PCR was performed in 20 µl reactions with 10 µl of TaqMan universal PCR master mix containing AmpliTaq Gold DNA Polymerase (Applied Biosystems, CA, USA), 200 nM concentration of forward and reverse primer, 100 nM of each probe and 2 µl (50–100 ng) of sample DNA. The PCR reaction was realized using the FAST 7500 Real Time PCR System (Applied Biosystems). The time and temperature profile of the PCR reaction consisted of the following steps: 2 min at 50°C for UNG activation, 10 min at 95°C for starting AmpliTaq Gold activity, 40 cycles of 95°C for 15 s and 60°C for 1 min. As a negative control, we used a sample without template. An allelic discrimination experiment consisted of three steps: a pre-read run, an amplification run and a post-read run. Each sample was visually verified by analyzing the generated PCR curves. Analyses of amplification products were performed using SDS software, version 4.2.

Statistical analysis was performed in the R (www.R-project.org, V.4.0).

RESULTS AND DISCUSSION

The frequency of allelic variants in 2 different Ukrainian cattle populations gave the following information: the ratio of A1A1, A1A2, A2A2 genotypes in the brown cattle was 1.7 %, 38.5%, 59.8%; black-spotted cattle – 18.3%, 44.7%, 37.0%. Accordingly, the highest frequency of A2A2 genotype is characterized by population of brown cattle. A higher frequency of heterozygotes distinguished animals of population of brown cattle population. The A1A1 genotype was more frequent in animals of black-spotted cattle population. (Table 1).

Table 1. **Frequency of CSN2 (rs43703011) genotypes and alleles in different populations of Ukrainian dairy cattle**

Distribution	Genotypes						Allele, un		χ^2
	A1A1		A1A2		A2A2		A1	A2	
	n	%	n	%	n	%			
Brown cattle									
Actual	3	1,7***	69	38,5	107	59,8***	0,209	0,791	4,803
Expected	7,9	4,4	59,3	33,1	111,8	62,5			
Black-spotted cattle									
Actual	128	18,3	314	44,7	260	37,0	0,406	0,594	3,702
Expected	115,7	16,5	338,6	48,2	247,7	35,3			

*** $p<0.001$

When comparing the ratio of genotypes in different breeds, there was a reliable difference between studied populations ($p<0.001$).

Analysis of the data obtained clearly shows that brown cattle populations have a significantly higher frequency of A2 allele than black-spotted cattle population.

There was no statistically significant difference between the actual value and the theoretically calculated genotype frequency. The difference between the actual and expected heterozygosity is confirmed by the data shown in Table 2.

Table 2. Values of the main volatility indicators

Populations	H_o	H_e	F_{is}
Brown cattle	0.385	0.331	-0.164
Black-spotted cattle	0.447	0.482	0.073

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

The expected heterozygosity in cows of the black and spotted population outweighed the actual one. However, in cattle of the brown populations, on the contrary, the actual heterozygosity exceeded the expected one. This is also indicated by the negative value of Wright's fixation index. This indicates a slight excess of heterozygotes in these samples.

It is generally known that the progress of breed in modern breeding conditions is provided by stud bulls with high breeding value. This has become possible by storing deep-frozen semen and applying a large-scale breeding system in practice.

The results of DNA testing of the beta-casein locus for A1 and A2-allelic variants in stud bulls of the studied populations have shown that the highest frequency of the A2A2 desired homozygous genotype is characteristic of the breeders of brown cattle. Stud bulls of the black-spotted population had a less value of the frequency of the desired genotype. The brown cattle was dominated by frequency of the desired A2 allele. An interesting fact is the predominance of the frequency of this allele over the A1 allele in stud black-spotted bulls (Table 3).

Table 3. Frequency of alleles and genotypes at the beta-casein gene locus in breeders

Distribution	Genotypes						Allele, un		χ^2
	A1A1		A1A2		A2A2		A1	A2	
	n	%	n	%	n	%			
Brown cattle									
Actual	7	10.9	31	48.5	26	40.6	0.352	0.648	0.249
Expected	7.9	12.4	29.2	45.6	26.9	42.0			
Black-spotted cattle									
Actual	9	20.0	24	53.3	12	26.7	0.467	0.533	0.229
Expected	9.8	21.8	22.4	49.8	12.8	28.4			

Animals of the brown cattle are characterized by a higher frequency of the desired A2A2 genotype, which confirms our opinion about the prospects of creating herds of cattle with the A2A2 genotype of this particular populations. Cattle of the black-spotted population have a low frequency of the A2A2 genotype – 0.37. At the same time, they are characterized by a high frequency of heterozygous genotypes that creates conditions for obtaining cattle of the desired genotype subject to the use of stud bulls with the A2A2 genotype. As our studies have shown, the share of such stud bulls whose is equal to 0.267.

CONCLUSIONS

The existing genetic structure of Ukrainian cattle breeds allows the formation of homozygous populations for these characteristics in subsequent generations.

The breeding stock of the brown cattle (59.8% of A2A2 homozygous and 38.5% of heterozygous) predicts a significant increase in the frequency of occurrence of individuals with the A2A2 genotype by beta-casein in subsequent generations, especially in case of using homozygous A2A2 sires by beta-casein.

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