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EVALUATION OF STUD BULLS BY BETA-CASEIN GENOTYPE IN THE CONTEXT OF CONSERVATION OF LOCAL CATTLE BREEDS IN UKRAINE

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Abstract

Genotyping of 114 stud bulls of local and specialized dairy breeds based on the beta-casein gene has been carried out. According to the results of the research, it is found that the highest frequency of the A2A2 desired homozygous genotype is characteristic of local cattle of Lebedyn breed and OBV (56%), as well as stud bulls of the Ukrainian White Headed breed (50). A significant difference in the frequency of genotypes for this trait has been established between individual breeds. The creation of micro-populations of cattle based on beta-casein with the A2A2 desired homozygous genotype makes it possible to obtain milk that has a number of properties, which are not characteristic of ordinary milk. Thus, the increased frequency of the A2A2 genotype for beta-casein may contribute to the conservation and spread of local breeds on Ukrainian farms.

Key words*: allele, beta-casein, breed, genotype, stud bull.*

INTRODUCTION

In accordance with international requirements, in 2000 Ukraine developed the first National Program for the Conservation and Rational Use of the Breed Gene Pool. An important aspect of this program is the genetic evaluation of stud bulls of local breeds (Kruhliak, 2017).

Scientists claim that in Ukraine in the period up to 2014, 16 domestic breeds and breed groups disappeared only from the mammalian class, and this is, first of all, the disappearance of valuable genes that characterized adaptability to the conditions of the environment in which cattle existed, resistance to adverse factors, and guaranteed product quality. These genes will not be able to be restored in a number of generations (Reznykova, 2022).

It is believed that breeds, if desired, can be revived and expanded by both natural and artificial reproduction methods. For this purpose, the Bank of Genetic Resources of M.V. Zubets Institute of Animal Breeding and Genetics of the National Academy of Agrarian Sciences has created a sufficient supply of semen of stud bulls in a deep frozen state. It should be added that the semen reserves of stud bulls of individual local breeds are also stored in other semen banks of breeding enterprises in Ukraine that enables to use such reserves for the revival of endangered populations. Theoretically, it is not a significant problem to revive the number of Brown cattle breeds, even in the absence of breeding farms and breeding stock of the active part of the population. Cows of this breed can be found in the population, or absorption crossing may be used if the animal is not purebred (Vyshnevskyi, 2017).

At the present stage, the main purpose of the bank is to accumulate and ensure long-term storage of gene pool material of all types of farm animals, as well as to implement a set of organizational and technological measures for

the preservation and rational use of their gene pool in Ukraine (Baschenko et al., 2017).

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 (Ladyka et al., 2019).

To implement the Gene Pool Preservation Program, the bank of genetic resources of animals stores and uses genetic material from 27 stud bulls of three local breeds - Ukrainian White Headed, Lebedyn and Brown Carpathian. Semen of the Red Steppe breed from two stud bulls is stored in the gene pool bank. Scientists state that to characterize the biological diversity of genetic material stored in the Institute's bank, it is expedient to analyze stud bulls by genealogical origin. This study should be combined with their molecular genetic characteristics. It is believed that an increase in the genetic potential of cattle is mainly determined by the availability of information on genes that control signs of productivity and enable to ensure the targeted selection of animals (Ladyka et al., 2019).

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 (Gorkhali et al., 2021; Ivankovic et al., 2021; Park & Haenlen, 2021).

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 (Amatya et al., 2021; Gorkhali et al., 2020; Mumtaz et al., 2021).

The frequency of the A2/A2 genotype in Holstein cattle is 48%, A1/A2 heterozygotes are amounted to 25%, and A1/A1 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% (Parashar & Saini, 2015). 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.

MATERIALS AND METHODS

The research was conducted at the premises of the Laboratory of O.O. Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine. The semen of stud bulls of the Ukrainian White Headed (n = 8), Ukrainian Gray (n=11), Red Steppe (n = 2) breeds stored in the Bank of Genetic Resources of M.V. Zubets Institute of Animal Breeding and Genetics of the National Academy of Agrarian Sciences, Lebedyn (n = 12) and Ukrainian Black-and-White dairy (n = 30), Simmental (n = 13) breeds of the semen bank of Sumy Breeding Center, Holstein (n = 15) breed of the semen bank of the Ukrainian Genetic Company was examined and studied. In addition, blood samples from the raised stud bulls of Lebedyn and OBV crossbreeds were examined (n=23).

Blood samples were taken under the sterile conditions into 2.7 mL Monovette contains EDTA potassium salt as an anticoagulant ("Sarstedt", Germany) with the following freezing of samples and their storage at -20ºC. DNA for genotyping was extracted from the samples using Monarch® Genomic DNA Purification Kit New England BioLab kits (USA) according to manufacturer's protocol. TaqMan®Custom was used to perform allelic discrimination. The TaqMan® SNP Genotyping Assays use TaqMan® 5′-nuclease chemistry for amplifying and detecting specific polymer-phisms in purified genomic DNA samples. All assays are developed using Life Technologies robust bioinformatics assay design process relying on a pipeline using heuristic rules deduced from both manufacturing and assay performance data. The TagMan@Genotyping system and a set of primers and probes were used to perform allelic discrimination.

SNP rs43703011 in the beta-casein gene (CSN2) was determined according to our methods using the next primers: upstream 5'-CCCAGACACAGTCTCTAGTCTATCC-3',

downstream 5'-GGTTTGAGTAAGAGGAGGGATGTTT-3' and the next probe - 5'-VIC-CCCATCCATAACAGCC-MGB-3` and 5'-FAM-CCATCCCTAACAGCC-MGB-3` (Thermo Scientific, USA). Amplification realized using Fast Real-time PCR System (Applied Biosystems, США) in total volume 10 мкл with 2X TaqMan Universal Master Mix (Applied Biosystems, USA), primers, probes, and DNA. Amplification of 84 bp fragment of CSN2 consisted of two steps: denaturation (95°C) during 3 s and annealing and elongation (60°C) during 30 s. Data were analyzed using 7500 Fast Real-Time PCR Software.

The data analysis was performed in the R statistical environment (www.R-project.org, V.4.0) and STATISTICA 10.

The allele frequency was calculated taking into account the number of homozygotes and heterozygotes found in the corresponding allele using the following formula:

$$P(A) = \frac{2N_1+N_2}{2n} \quad (1)$$

where:

N_1 and N_2 - number of homozygotes and heterozygotes for the studied allele, respectively;

n - sample number.

In order to assess the statistical reliability of the discrepancy between the distribution of the obtained results the Pearson criterion was used:

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

where:

A - actual number of genotypes;

T - theoretical number of genotypes.

The actual (available) heterozygosity was determined by direct calculation using the following formula:

$$H_0 = \frac{N_2}{n} \quad (3)$$

The expected heterozygosity was determined using the following formula:

$$H_E = 1 - \sum_{I=1}^{n} p_i^2 \quad (4)$$

where:

p_1, p_2,….p_n - frequency of alleles.

The fixation index was calculated using the following formula:

$$F_{is} = \frac{H_E-H_0}{H_E} \quad (5)$$

RESULTS AND DISCUSSIONS

The results of DNA testing of the beta-casein locus for A1 and A2-allelic variants in stud bulls of the studied breeds and cross cattle have shown that the highest frequency of the A2A2 desired homozygous genotype is characteristic of the Lebedyn and OBV crossbreeds. They were somewhat inferior to stud bulls of the Ukrainian White Headed (Table 1).

It should be noted that this genotype was found in all local breeds studied, with the exception of two stud bulls of the Red Steppe breed characterized by the A1A1 and A1A2 genotypes (not shown in Table). In commercial breeds, Simmental cattle had a higher frequency of the desired genotype. Stud bulls of the Holstein breed had the lowest frequency of the A2A2 genotype.

Values of the frequency of the A2A2 genotype in stud bulls of the Ukrainian Gray and Lebedyn breeds which are slightly lower than the expected ones, in our opinion, require a more detailed study. Stud bulls of the Ukrainian Black-and-White dairy breed had a mediocre value of the frequency of the desired genotype.

Stud bulls of the Brown Carpathian and Holstein breeds are characterized by a greater frequency of the A1A2 homozygous genotype. The least frequency is found in stud bulls of the Ukrainian White Headed and Simmental breeds.

Stud bulls of the Ukrainian Gray breed have the highest frequency of the A1A1 genotype. These genotypes are not found in stud bulls of the Brown Carpathian breed and Lebedyn crossbreeds with OBV.

The local Lebedyn crossbreeds with OBV were dominated by frequency of the desired A2 allele. Cattle of Ukrainian White Headed, Simmental of the Austrian breeding and Brown Carpathian breeds have values close to the above. An interesting fact is the predominance of the frequency of this allele over the A1 allele in stud bulls of Holstein and Ukrainian Black-and-White dairy breeds. The predominance of the A1 allele in cattle of the Ukrainian Gray and Lebedyn breeds requires a more detailed study.

Table 1. Frequency of alleles and genotypes by the beta-casein gene locus in stud bulls

Breed/crossbreed	Distribution	Frequency of genotypes			Frequency of alleles		$\chi 2$
		A2A2	A1A2	A2A2	A1	A2	
Ukrainian White Headed	A	12.5	37.5	50.0	31.25	68.75	0.1295
	E	9.8	43.0	47.2			
Ukrainian Gray	A	36.4	45.5	18.1	59.10	40.90	0.0393
	E	34.9	48.3	16.8			
Lebedyn purebred	A	25.0	58.3	16.7	54.20	45.80	0.3667
	E	29.3	49.7	21.0			
Brown Carpathian	A	0.0	70.0	30.0	35.00	65.00	2.8994
	E	12.3	45.5	42.2			
Crossbreeds of Lebedyn breed with original German Brown breed	A	0.0	43.5	56.5	21.7	78.3	1.7746
	E	4.7	34.0	61.3			
Ukrainian Black-and-White dairy	A	26.7	43.3	30.0	48.3	51.7	0.5256
	E	23.4	49.9	26.7			
Holstein	A	6.7	73.3	20.0	43.0	57.0	3.6488
	E	18.8	49.1	32.1			
Simmental	A	15.4	38.5	46.1	34.6	65.4	0.2938
	E	12.0	45.2	42.8			

Source: Own research.

There is a generally accepted view that a violation of random crossing should cause a deviation in genotype frequencies from the expected equilibrium according to the Hardy-Weinberg principle. In cattle of the Lebedyn and Brown Carpathian breeds, crossbreeds of the Lebedyn and Original Brown and Holstein breeds, the actual heterozygosity exceeds the expected one. A negative value of the Wright Fixation Index indicates a slight excess of heterozygotes in these samples (Table 2). In our opinion, measures to preserve the Lebedyn breed developed by specialists of Sumy National Agrarian University (Ladyka et al., 2021; Ladyka et al., 2019), namely, the work by the population method of reciprocal reproduction using stud bulls of the Original Brown German breed, enables to increase the frequency of the A2 beta-casein allele in gene pool herds (according to our research, the frequency of the desired allele in stud bulls increased from 45.8% to 78.3%).

This, in turn, has made it possible to obtain cattle with the desired quality indicators of dairy raw materials and a number of stud bulls of the Lebedyn breed with the A2A2 beta-casein genotype for custom mating.

Table 2. Values of the main indicators of variability by the kappa-casein gene

Breed	H_o	H_e	F_{is}
Ukrainian White Headed	0.375	0.430	0.128
Ukrainian Gray	0.455	0.483	0.060
Lebedyn	0.583	0.497	-0.175
Brown Carpathian	0.700	0.455	-0.538
Crossbreeds of Lebedyn breed with original German Brown	0.435	0.340	-0.278
Ukrainian Black-and-White dairy	0.433	0.499	0.132
Holstein	0.733	0.491	-0.493
Simmental	0.385	0.453	0.150

Ho - actual heterozygosity, He - expected heterozygosity, Fis - fixation index
Source: Own research.

Therefore, we can say with confidence that due to the research and cooperation of scientists and producers, it is possible to create dairy herds of Brown cattle in order to obtain milk with the A2A2 genotype for beta-casein that will significantly increase the profitability of its production. In turn, this may contribute to further measures to preserve the gene pool of Brown cattle in Ukraine. It is also promising to use stud bulls of other (local) breeds in order to further preserve them and create herds of cattle with the A2A2 genotype.

Differentiation of the frequency of genotypes by beta-casein in cows of autochthonous breeds is established based on the results of studies that have been previously conducted (Table 3).

Cattle of the Lebedyn breed 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 breed. Cattle of the Ukrainian Gray breed being the parent one in the creation of the Lebedyn breed have a low frequency of the A2A2 genotype - 0.046. 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 semen is stored in a deep frozen state is equal to 18.2 %. Cows of the Ukrainian White Headed breed have a share of heterozygous genotype at the level of 52%, while 50% of stud bulls have the desired homozygous genotype that enables to work on creating herds of cattle with the A2A2 genotype.

The similar work with the Brown Carpathian cattle will be longer, which is due to the absence of cows with the A2A2 and A1A2 genotypes and a low proportion of stud bulls with the A2A2 genotype.

Table 3. Frequency of beta-casein genotypes in cows of local cattle breeds

Breed	Share of genotypes	Author
Lebedyn	A1A1-0; A1A2-0.7; A2A2-0.3	Ladyka V., Pavlenko Y., Sklyarenko Y., 2021
Ukrainian Gray	A1A1-0.110; A1A2-0.844; A2A2-0.046	Mokhnacheva N. B., 2021
Ukrainian White Headed	A1A1-0.48; A1A2-0.52; A2A2-0	Mokhnacheva N. B., 2021
Brown Carpathian	A1A1-1.00; A1A2-0; A2A2-0	Mokhnacheva N. B., 2021

Source: Links to other studies

CONCLUSIONS

This work results in finding frequencies of alleles and genotypes by the beta-casein locus in stud bulls of autochthonous breeds of Ukraine. It is established that according to this feature, stud bulls of local Ukrainian breeds differ significantly from each other. The results obtained indicate that there is no targeted breeding work on creating herds with the A2/A2 genotype cattle. An exception is the breeding of the Brown cattle, as evidenced by the obtaining of stud bulls from custom mating, in which the frequency of the desired genotype is higher than 50%. Stud bulls of the Ukrainian White Headed breed are also characterized by a similar frequency. Therefore, it can be argued that there are prospects for creating such herds in the population of Lebedyn and Ukrainian White Headed cattle. Such work with dairy herds of the Ukrainian Gray, Brown Carpathian and Red Steppe breeds requires further in-depth study.

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