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

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Abstract

Genotyping of 47 stud bulls of Brown breeds was carried out by the gene CSN3. The results of scientific studies indicate that both Brown purebred and cross-breed cattle bred in Ukraine are characterized by a significant difference in the frequency of genotypes by this trait. The genetic equilibrium established by us corresponds to the global trends of breed populations. The creation of the desired micro-populations with the desired homozygous BB genotype by kappa-casein makes it possible to improve the quality of milk as a raw material for milk processing enterprises specialized in cheese making. Therefore, the increased frequency of the BB genotype by kappa-casein may contribute to the preservation and distribution of Brown cattle in Ukrainian farms.

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

INTRODUCTION

Much attention is paid to the conservation of biological diversity at the global level. The vast majority of cattle breeds are represented by local populations that differ significantly not only in morphology, but also in the corresponding co-adaptive genetic complexes formed under the influence of natural and artificial selection against the background of specific growing conditions. During the genesis and further improvement of the breed, the selection of cattle that are selectively valuable from a breeding point of view, it is essential to have information on their productivity potential, the level of genetic polymorphism, genotype by genes of quantitative traits, the availability of unique alleles that are characteristic of representatives of this particular breed and have adaptive significance, and therefore create the basis for the need to preserve its gene pool [7].

Domestic Brown cattle of Ukraine are represented by the Lebedyn, Brown Carpathian breeds, and Ukrainian Brown dairy breed created on their basis. The Lebedyn breed was approved in 1950. It was created as a result of mating local, mainly Gray Ukrainian, cattle with the Swiss breed, the inter se breeding of hybrids, which lasted for more than 40 years. The local Swiss breed, whose stock has dramatically decreased over the past 20 years, is characterized by unique economic and useful traits, including adaptability to local, economic and feed conditions, endurance and resistance to diseases, long-term use, breeding plasticity, universal productivity. To be sure, it is almost impossible for it to compete in terms of milk yield with such world leaders as the Holstein breed. In this case, the quality of the resulting products should come to the fore. And to do this, in our opinion, it is necessary to know the genetic characteristics of cattle. The revival of the Lebedyn cattle breed can be carried out at the expense of both natural and artificial reproduction methods. For this purpose, the banks of genetic resources and breeding centers of Ukraine have a sufficient supply of semen of stud bulls. In particular, Sumy Breeding Center LLC stores the genetic material of 12 bulls of the Lebedyn breed and crossbreeds with the Swiss breed. A small number of stud bulls of the Lebedyn breed, whose semen is stored in breeding centers, make it impossible to completely avoid inbreeding, which may lead to undesirable breeding consequences. Therefore, scientists of Sumy National Agrarian University have proposed a fundamentally new scheme for reproducing the genealogical structure of herds of local breeds, namely, working with the application of the population method of reciprocal reproduction [11, 20].

The Brown Carpathian cattle of the combined productivity are a local breed, whose area of distribution is the south-west of Ukraine. Cattle of this breed are characterized by extremely valuable economic features. They are adapted to mountain breeding conditions, have high viability and resistance, are characterized by a strong body composition, high reproductive capacity, long-term productive use, and high fat and protein content in milk. Genetic resources of the Brown Carpathian breed of the Transcarpathian region are represented by breeding stock, which is located in 69 settlements of 13 districts and has 15,800 heads. In addition, the Bank of Animal Genetic Resources of M. V. Zubets Institute of Animal Breeding and Genetics of the National Academy of Agrarian Science of Ukraine and Transcarpathian Breeding Farm JSC stores more than 259 thousand doses of semen. Today, the issue of preserving the Brown Carpathian cattle is being given more and more attention [18, 19, 20, 21].

Increasing requirements for the quality of dairy products have led to the use of genetic markers in breeding and the search for their relationship with the milk productivity of animals. In this situation, there is a need to change the methods of assessing the breeding characteristics of animals and develop new approaches based on the achievements of genetics and biotechnology [7, 8]. Thanks to the achievements of modern molecular genetics, it is possible to identify genes that control economically useful traits in cows. This makes it possible, in addition to traditional animal selection, to conduct selection directly at the DNA level. Among the many genes that affect the milk quantity and quality, we can distinguish a separate group that make the greatest contribution to the formation of milk productivity traits. These genetic markers include the following genes: kappa-casein (CSN3), beta-lactoglobulin (BLG), prolactin (PRL), and others [7].

Milk protein genes, especially kappa-casein (CSN3), are important for milk quality, as they significantly affect the cheese production process, which has been proven by a large number of studies [1, 12, 15]. Polymorphism of the kappa-casein gene is quite well studied today, scientists distinguish the following most common variants – A and B. Less common gene variants are: C, D, E, F, H, J, I, X, Az, A1. The most common genetic variants are A and B [17]. The kappa-casein B allele is associated with the production of milk with a more favorable chemical composition for cheese production. Therefore, for the production of cheese, it is better to use milk from animals with the BB genotype compared to milk from animals with a different genotype [4]. This leads to the use of the kappa-casein bovine genotype indicator to ensure successful breeding for cheese production. It is proved that different breeds of dairy cattle differ significantly in the frequency of genotypes and alleles by kappa-casein [2, 3, 8, 13, 14, 22].

One of the important productive features of the Brown cattle is that among all other breeds, the milk of these cattle contains the largest kappa-casein fraction of milk protein, which may determine its preservation [3, 7, 18, 22].

The objective of our research is to study the frequency of kappa-casein genotypes and alleles of purebred and local stud bulls of the Brown cattle in Ukraine.

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 study was conducted on DNA samples obtained from the long-term storage semen of bulls: of Lebedyn breed (5 heads), Lebedyn crossbreeds with Swiss (7 heads) and original Brown German (19 heads); Brown Carpathian (4 heads), Brown Carpathian crossbreeds with Swiss (11 heads). The study was also conducted on blood samples taken from live stud bulls: Lebedyn crossbreeds with the original Brown German (19 heads); crossbreeds of Ukrainian Brown dairy and original German Brown (5 heads).

Blood samples were taken under sterile conditions into 2.7 mL monovettes containing EDTA potassium salt as an anticoagulant (Sarstedt, Germany), with the subsequent freezing and storage of samples at -20ºC.

For molecular genetic studies, one semen dose was used from 47 stud bulls (of the corresponding breed). 4 µL of a sample from the semen dose of each of the stud bulls of these breeds was taken to isolate genomic DNA.

Monarch® Genomic DNA Purification Kit New England BioLab kits (USA) according to the manufacturer's protocol was used to obtain DNA from samples for genotyping.

The TaqMan® SNP Genotyping Assays use TaqMan® 5′-nuclease chemistry for amplifying and detecting specific polymorphisms 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. These assays use TaqMan® minor groove-binding (MGB) probes for superior allelic discrimination, improved signal-to-noise ratios, and design flexibility. TaqMan real-time PCR Two primers were designed to amplify the 101 bp product involving SNP rs43703011 (genomic DNA: X14711 (http://www.ncbi.nih.gov); forward primer, 5′- AAG CAG TAG AGA GCA CTG TAG CTA -3′; reverse primer, 5′- TGA TCT CAG GTG GGC TCT CAA TAA -3′). Two fluorogenic TaqMan probes were designed with different fluorescent dye reporters to allow single-tube genotyping. The first probe was targeted at the wild type allele A (5′-VIC-CTTCTGGAGAAGCTTCTA-3′) and the second one at the mutated allele B (5′-FAM- CTTCTGGAGAATCTTCTA -FAM-3′) of the CSN3 gene. The 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 obtained 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 a 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. The analyses of amplification products were performed using SDS software, version 4.2. The allele frequency was calculated taking into account the number of homozygotes and heterozygotes found in the corresponding allele using the following formula:

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

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:

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

where:

A – actual number of genotypes;

T – theoretical number of genotypes.

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

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

The expected heterozygosity was determined using the following formula:

$$H_E = 1 - \sum_{l=1}^{n} p_i^2 \text{.......................................} (4)$$

where:

$p_1, p_2, \ldots p_n$ – frequency of alleles.

The fixation index was calculated using the following formula:

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

RESULTS AND DISCUSSIONS

The results of DNA testing of the kappa-casein locus for the presence of A and B-allelic variants in stud bulls of the studied breeds and cross cattle have revealed that cattle of the Brown Carpathian breed are characterized by the frequency of the desired (BB) genotype at 50%, which is higher than all other breeds studied (Table 1).

Stud bulls of the Ukrainian Brown dairy breed were somewhat inferior to them in this respect. Crossbreeds of the Lebedyn breed with Swiss, original German and Brown Carpathian with the Swiss breed had a significantly lower frequency of this genotype. The desired genotype was not found at all in stud bulls of the Lebedyn breed.

Brown Carpathian and Swiss breeds differed in a higher proportion of the heterozygous genotype. Cattle of other breeds were inferior to them in terms of the share of this genotype.

Stud bulls of the Lebedyn breed were characterized by the availability of the AA genotype with a proportion of 50%, and none of them was found in cattle of the Brown Carpathian breed at all.

The use of the χ2 criterion has enabled to determine the degree of compliance of the actual distribution of genotypes with the expected values. Calculation using the Hardy-Weinberg formula shows no difference between the actual and expected genotype frequencies for most breeds.

Table 1. Frequency of alleles and genotypes by kappa-casein gene locus

Breed/crossbreed	Distribution	Frequency of genotypes			Frequency of alleles		χ2
		AA	AB	BB	A	B	
Lebedyn purebred	A	0.50	0.50	0	0.75	0.25	0.444
	E	0.56	0.38	0.06			
Crossbreeds of Lebedyn breed with Swiss breed	A	0.42	0.29	0.29	0.57	0.43	1.215
	E	0.33	0.49	0.18			
Purebred Brown Carpathian	A	0	0.50	0.50	0.25	0.75	0.444
	E	0.06	0.38	0.56			
Crossbreeds of Brown Carpathian with Swiss breed	A	0	0.73	0.27	0.36	0.64	3.591
	E	0.13	0.47	0.40			
Crossbreeds of Lebedyn breed with original German Brown breed	A	0.26	0.57	0.17	0.55	0.45	0.555
	E	0.31	0.49	0.20			
Crossbreeds of Ukrainian Brown dairy breed with original German Brown breed	A	0.20	0.40	0.40	0.40	0.60	0.139
	E	0.16	0.48	0.36			

Source: Own research.

This may indicate a lack of selection based on this trait and the preservation of genetic balance. It means cattle breeding is carried out on the basis of traditional methods for assessing milk productivity, without taking into account genetic factors that affect the qualitative composition of milk protein. The exception is made by local cattle of the Brown Carpathian breed with the Swiss one.

There is a popular opinion that a violation of random crossing should cause a deviation in genotype frequencies from the expected equilibrium according to the Hardy-Weinberg law. In cattle of the Lebedyn, Brown

Carpathian breeds, crossbreeds of Brown Carpathian and Swiss breeds, Lebedyn with the original Brown and Simmental breeds, the actual heterozygosity exceeded the expected one. A negative value of the Wright fixation index indicates a slight excess of heterozygotes in these samples.

In our opinion, measures for the preservation of the Lebedyn breed developed by specialists of Sumy National Agrarian University, namely, the work by the method of population reciprocal reproduction using stud bulls of the original Brown German breed, enables to increase the frequency of the kappa-casein B allele in gene pool herds.

This, in turn, will make it possible to obtain cattle with the desired quality indicators of dairy raw materials and stud bulls of the Lebedyn breed with the BB genotype by kappa-casein for custom mating. Therefore, we can safely assume that the conducted research and cooperation of scientists and producers can provide an opportunity to create dairy herds of Brown cattle to obtain milk for cheese making that will significantly increase the profitability of its production and processing, as well as the demand for dairy products. In turn, this may contribute to further measures to preserve the gene pool of Brown cattle in Ukraine.

Work with the Brown Carpathian breed should include individual work of scientists and breeders with livestock owners in order to obtain cattle with the desired kappa-casein genotype from custom pairs. This, in turn, will enable to obtain milk for cheese making from cows that will increase the competitiveness of this breed. However, it should be noted that due to the low share of cows of this breed among farmers, we can only talk about the manufacture of craft products (cheese). One of the options for popularizing the breed can be green tourism in the Carpathians.

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

Breed	H_o	H_e	F_{is}
Lebedyn purebred	0.500	0.375	-0.333
Crossbreeds of Lebedyn breed with Swiss breed	0.286	0.490	0.417
Purebred Brown Carpathian	0.500	0.375	-0.333
Crossbreeds of Brown Carpathian with Swiss breed	0.727	0.463	-0.571
Crossbreeds of Lebedyn breed with original German Brown breed	0.579	0.494	-0.171
Crossbreeds of Ukrainian Brown dairy breed with original German Brown breed	0.400	0.480	0.167

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

Based on the results of our studies, it was found that the highest frequency of the desired allele is characteristic of stud bulls of the Brown Carpathian cattle (0.750). In cattle of the Lebedyn breed, on the contrary, the frequency of A allele was 0.750, respectively, and the B allele – 0.25. The results obtained by us do not coincide with the previously obtained results of other scientists. According to the results of our studies, the frequency of the A allele is three times higher than the frequency of the B allele in stud bulls of the Lebedyn breed. While according to the results of other scientists, this difference is 1.16-1.9. Crossbreed stud bulls of the Lebedyn and Swiss breeds are characterized by a higher frequency of the A allele compared to the published results of other scientists. A allele is 1.32 times higher, while according to the literature data, the frequencies of the A and B alleles are the same. According to the results of our research, the Brown Carpathian breed has a very high frequency of the B allele – 0.75, which does not coincide with the previous studies. Cattle crossbred with the Swiss breed have a lower frequency of the desired allele. In our opinion, the increase in the frequency of the desired B allele in cattle of the Lebedyn breed subject to the use of stud bulls of the Swiss breed can be explained by the fact that cattle of the Swiss breed have a higher frequency of the B allele than A, which is proved by the previous studies (Table 3).

Table 3. Main Frequency of kappa-casein alleles (according to researchers)

Breed	Frequency of alleles		Source
	A	B	
Lebedyn*	0.600	0.380	Ovcharenko, V., Ladyka, V., 1999 [16]
Crossbreeds of Lebedyn* and Swiss	0.500	0.500	Ovcharenko, V., Ladyka, V., 1999 [16]
Lebedyn*	0.533	0.454	Eremenko V., Oblivantsov V., 2004 [5]
Ukrainian Brown dairy	0.500	0.500	Eremenko V., Oblivantsov V., 2004 [5]
Lebedyn	0.660	0.340	Ladyka V., Sklyarenko Yu., Pavlenko Yu., 2018 [10]
Lebedyn	0.463	0.537	Huzeiev, Yu.V., 2012 [7]
Brown Carpathian	0.564	0.436	Huzeiev, Yu.V., 2012 [7]
Brown Carpathian	0.619	0.381	Huzeiev, Yu., Sydorenko, O., Vishnevskyi, L., 2017 [9]
Swiss*	0.312	0.653	Gladyr ,O. O., 2001 [6]
Swiss of German* breeding	0.474	0.518	Eremenko V., Oblivantsov V., 2004 [5]

*Other alleles are available.
Source: Links to other studies.

CONCLUSIONS

This work has resulted in detecting the frequencies of alleles and genotypes in the kappa-casein locus. It is established that Brown breeds that are bred in Ukraine differ significantly in this trait among stud bulls. The genetic equilibrium revealed by our research reflects global trends in breed populations and indicates the absence of targeted breeding towards increasing BB homozygotes. Accelerated formation of the desired genetic combinations in micro-populations (herds), if required, by the processing industry, primarily cheese producers, is possible in populations of Brown cattle. Such micro-populations with the desired homozygous BB genotype by kappa-casein create prerequisites for improving the quality of milk as a raw material for milk processing enterprises specialized cheese making. The stabilization of the dairy cattle industry and its development in Ukraine will be significantly facilitated by an increase in the price of raw materials (milk), which is possible when selling batches of milk exclusively with BB genotypes by kappa-casein for cheese making. Due to the increase in the frequency of the BB genotype in Brown cattle, it is possible to preserve and spread them in Ukrainian farms.

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