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STUDY OF THE FREQUENCY OF COMPOSITE BETA- AND KAPPA-CASEIN GENOTYPES OF CATTLE POPULATIONS AS A FACTOR IMPROVING THE MILK QUALITY

Volodymyr LADYKA[1], Yuriy SKLIARENKO[2], Yuliia PAVLENKO[1], Viktoriia VECHORKA[1], Alona MALIKOVA[1]

[1]Sumy National Agrarian University, 160, H. Kondratiiev St., Sumy, Ukraine, E-mail: v.i.ladyka@ukr.net, jasjulia@ukr.net, vvvechorka@gmail.com, lelikunique2@gmail.com
[2]Institute of Agriculture in the North East of the National Academy of Agrarian Sciences of Ukraine, Ukraine, Sumy region, Sumy district, Sad village, Zelena Street 1 E-mail: Sklyrenko9753@ukr.net

Corresponding author: Sklyrenko9753@ukr.net

Abstract

The study of polymorphism of beta-casein genes CSN2 and kappa-casein CSN3 in cows of Brown cattle, Simmental breed, Black and White cattle has been carried out with the use of polymerase chain reaction in real time. It is shown that the frequency distribution of CSN2 / CSN3 composite genotypes differs significantly in different breeds: Brown cattle – 22.0 A1A2/AB, 22.7% A2A2/AB and 22.0% A2A2/BB; Simmental – 26.8% A1A2/AB; A2A2/AF, 22.0%, A2A2/AB 19.5%; Black and White cattle – 26.9% A1A2/AA; A1A1/AB, 23.1%, A2A2/AA 23.1%. The obtained results indicate the prospects of breeding work concerning the formation of herds (micropopulations) by the A2A2/BB composite genotype, which is only possible among Brown breeds. This makes it possible to increase the effectiveness of measures to preserve the population of Brown cattle by improving the quality of their milk.

Key words: *genetic polymorphism, beta-casein, kappa- casein, genotype frequency, cattle breeds*

INTRODUCTION

Genetic studies of farm animals mainly focus on identifying genes that determine economically important traits and may be used in breeding programs. In dairy cattle breeding, genes that can determine differences in milk yield and composition are mainly studied. The use of genetic markers makes it possible to predict breeding value based on traits that are difficult to measure by phenotypic manifestation, and therefore, that are not part of the selection criterion. The main goal of our work was to show what happened to the frequencies of alleles of some important QTL, which directly affected the level of manifestation of economically useful traits, but did not have a pronounced phenotypic manifestation (for their determination by phenotype), and therefore were not taken into account by traditional methods of breeding [5].

Identification of genes and their mutations that determine the direction and degree of development of a quantitative trait (for example, the amount of milk yield, the average daily weight gain of fattening animals, the content of fat and protein in milk, etc.) in countries with developed animal husbandry provides significant profits due to the rapid achievement of genetic progress, the main components of which are the intensity of breeding, its accuracy and reduction of the generation interval [11, 9].

Therefore, today molecular genetic research methods are a promising area of fundamental and applied genetics, in particular, the breeding of farm animals. The use of marker-associated breeding makes it possible to increase the effectiveness of traditional methods of breeding work by direct assessment of animals by genotype.

In dairy cattle breeding, the most economically significant genes are those whose products are associated with the nutritional value and technological properties of milk.

According to their properties, all milk proteins can be divided into two groups:

82% are caseins (αS1-, αS2-, β-, k-casein) and 18% are whey proteins (α- and β-

lactoglobulins). Alleles that determine milk protein synthesis are inherited by co-dominance and clearly diagnosed using molecular genetic methods. The vast majority of studies relate to the two most economically important genes – beta-lactoglobulin (β-LG) and kappa-casein (k-casein, CSN3, k-CN), whose polymorphism is associated with milk producing ability.

Unlike the other three casein proteins, kappa-casein is similar in its amino acid composition to fibrin and has the property of forming clots based on the results of proteolysis. This property has long been used in cheese making. The DNA analysis of exon regions of the CSN3 gene has resulted in the identification of several nucleotide substitutions and polymorphic restriction sites and description of 9 allelic variants (A, B, C, E, F, G, H, I, and A1), among which A and B variants are the most common [8]. Much attention in the United States of America, New Zealand, China, and some European countries is paid to the research of the milk protein fraction - β-casein (CSN2) [13]. Scientists and practitioners pay special attention to two allelic variants - A1 and A2. These beta-casein proteins differ in one amino acid, which causes the formation of beta-casomorphin in the human digestive tract (in the case of the A1 beta-casein), which causes a number of pathological effects [1]. In turn, the use of A2 milk significantly reduces the acute symptoms of undigested cow's milk [14, 4]. Products based on A2 milk are gradually spreading in the markets of such countries as New Zealand (2000), Australia (2004), the United States (2003), the United Kingdom (2011), and China (2013). In recent years, where some interest began to appear among our domestic farms regarding the typing of cows by β-casein alleles, it should be noted that the four casein genes (αS1-CN, αS2-CN, β-CN, k-CN) are closely related to and organized on chromosome 6 B. taurus (200-250 kb) in the following order: αS1, β, αS2 i k. This makes this construction interesting from the point of view of studying the nonequilibrium coupling of allelic variants of these genes [5, 11, 12]. In this case, interest arises not only in the polymorphism of individual genes (for example, kappa-casein), but also in the need to analyze complex genotypes and haplotypes of both promising loci – beta- and kappa casein [6, 3].

Different breeds differ significantly in complex genotypes according to beta- and kappa-casein. Thus, the A2A2/AB (22.9%) and A2A2/BB (15.6%) genotypes are more common in Jersey animals. The A1A1/AA та A1A2/AA genotypes are not found. On the contrary, in animals of the Red Danish breed, the A1A2/AA (17.0%) and A2A2/AA (13.1%) genotypes have a higher proportion. The A1A2/BB and A2A2/BB genotypes do not occur [2].

The aim of our research is to study the frequency of composite beta- and kappa-casein genotypes in dairy cattle populations in Ukraine in order to establish the possibility of obtaining milk with the desired qualitative characteristics.

MATERIALS AND METHODS

The genotyping of 193 heads of the following cattle breeds was carried out during the research: Brown cattle (n=145), Simmental (n=41), Black and White cattle (n=26). Experimental animals are bred on the leading breeding farms of Ukraine and purebred. Experimental cows of Lebedyn breed are kept at Mykhailivka Breeding Plant LLC (Lebedyn district of Sumy region) and Komyshanske CAL (Okhtyrka district of Sumy region), Ukrainian Brown dairy breed and Ukrainian Black-and-White dairy breed - at the State Enterprise Research Farm of the Institute of Agriculture of Northern East of the National Academy of Agrarian Sciences (Sumy district of Sumy region), Simmental breed - at Urozhai LLC (Romny district of Sumy region).

The genotyping of 23 bullocks of Brown cattle grown in Mykhailivka Breeding Plant LLC (Lebedyn district of Sumy region) and Komyshanske CAL (Okhtyrka district of Sumy region) and the State Enterprise Research Farm of the Institute of Agriculture of Northern East of the National Academy of

Agrarian Sciences (Sumy district of Sumy region) and 12 breeders of Brown cattle (Sumy State Breeding Center).

Blood samples were taken under sterile conditions into 2.7 mL monovettes containing 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 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.

The obtained samples were tested by analyzing the obtained PCR curves. SDS software version 4.2. was used by us to analyze the amplification products.

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

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

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

where:

N1 and N2 – number of homozygotes and heterozygotes for the studied allele, respectively;

n – sample number.

RESULTS AND DISCUSSIONS

The studies to determine the proportion of composite genotypes for beta- and kappa-casein showed that Brown cattle were characterized by the all possible genotypes. In most cases, the Simmental breed was characterized by the availability of three composite genotypes: A2A2/AA (22.0%), A1A2/AB (26.8%), A2A2/AB (19.5%). Cows of the Black and White cattle were characterized by the following three composite genotypes: A1A2/AA (26.9%),

A2A2/AA (23.1%) and A1A1/AB (23.1%) (Table 1).

Table 1. Frequency distribution of the studied combinations of CSN2 and CSN3 milk protein genotypes

Populations	CSN3	Frequency of composite genotypes, %		
		CSN2		
		A1A1	A1A2	A2A2
Brown cattle	AA	4.7	9.6	8.2
Simmental		7.3	14.6	22.0
Black and White cattle		7.7	26.9	23.1
Brown cattle	AB	3.4	22.0	22.7
Simmental		0.0	26.8	19.5
Black and White cattle		23.1	0.0	3.8
Brown cattle	BB	2.6	4.8	22.0
Simmental		0.0	9.8	0.0
Black and White cattle		7.7	7.7	0.0

Source: Own research.

Research results indicate the absence of complex genotypes A1A1/AB, A1A1/BB and A2A2/BB in animals of Simmental breed, and A1A2/AB and A2A2/BB in animals of Black and White breed. The combination of the two desired A2A2 beta-casein genotypes and BB kappa-casein is only found only in cattle of the Brown cattle.

The formation of cattle genotype is equally influenced by the genotypes of the male and female parents. Based on the results of our previous studies, it is found that among the breeding bulls of the Holstein breed, which are widely used in the breeding stock of Black and White cattle, only 43% were evaluated by the polymorphism of the beta- and kappa-casein genes. 9 combinations of such genotypes were identified. Most stud bulls had the combined A2/AB genotype. The share of stud bulls of the desired A2A2/BB composite genotype was only 12.4%. Breeding bulls of the Brown Swiss breed have only two variants of the complex genotype. The desired complex genotype A2A2/BB was found in them with a frequency of 75%. This determines their further use in Brown cattle in Ukraine.

No cattle with the desired A2A2/BB composite genotype have been found among the Simmental stud bulls [10].

In the period from 2017 to 2019, the scientists of Sumy National Agrarian University implemented the scientific project "Justification of the Methodology for Improving and Conserving the Brown Cattle Population in the conditions of the North-Eastern Region of Ukraine". The project resulted in the development and practical implementation of a fundamentally new scheme for reproducing the genealogical structure of local (native) brown breed herds, namely, work with the application of the population reciprocal crossing method. The original breeds involved in creating the Brown cattle were the Gray Ukrainian and various offspring of the Swiss breed. Therefore, the use of stud bulls of these breeds will expand the genealogical structure of the Brown cattle to avoid undesirable inbreeding in further work with stud bulls, whose semen is stored. At the initiative of the Rector of Sumy NAU in 2019, the semen of three stud bulls of the original German Brown breed (Julex DE 814660509, Urano SN 110027139002, Nimrod DE 814720783) was imported to the farms of the region. The expected result of using these stud bulls was: the conservation of the Brown cattle in micropopulations; the formation of dairy herds with the A2A2 beta-casein genotype and BB kappa-casein [9]. We performed a genetic assessment of the obtained stud bulls and stud bulls whose semen is stored in a deep frozen state, according to the complex genotypes of beta-and kappa casein (Table 2).

Among the obtained bulls, the majority was animals with such composite genotypes as A1A2/AA, A1A2/AB, A2A2/AB, A2A2/BB.

The small proportion of homozygotes in the A1A12/AA composite genotype indicates prospects for creating micropopulations with the desired A2A2/BB composite genotype.

This is also evidenced by the presence of stud bulls with the A2A2/BB composite genotype, and a significant proportion of heterozygous animals. Although it should be noted that it is essential to increase the proportion of cattle with the A2A2/BB genotype.

Table 2. Characteristics of the frequency of composite genotypes for the conservation of Brown cattle

CSN3	Frequency of composite genotypes, %		
	CSN2		
	A1A1	A1A2	A2A2
AA	2.9	22.9	11.4
AB	5.7	20.0	17.1
BB	0.0	5.7	14.3

Source: The authors' own research.

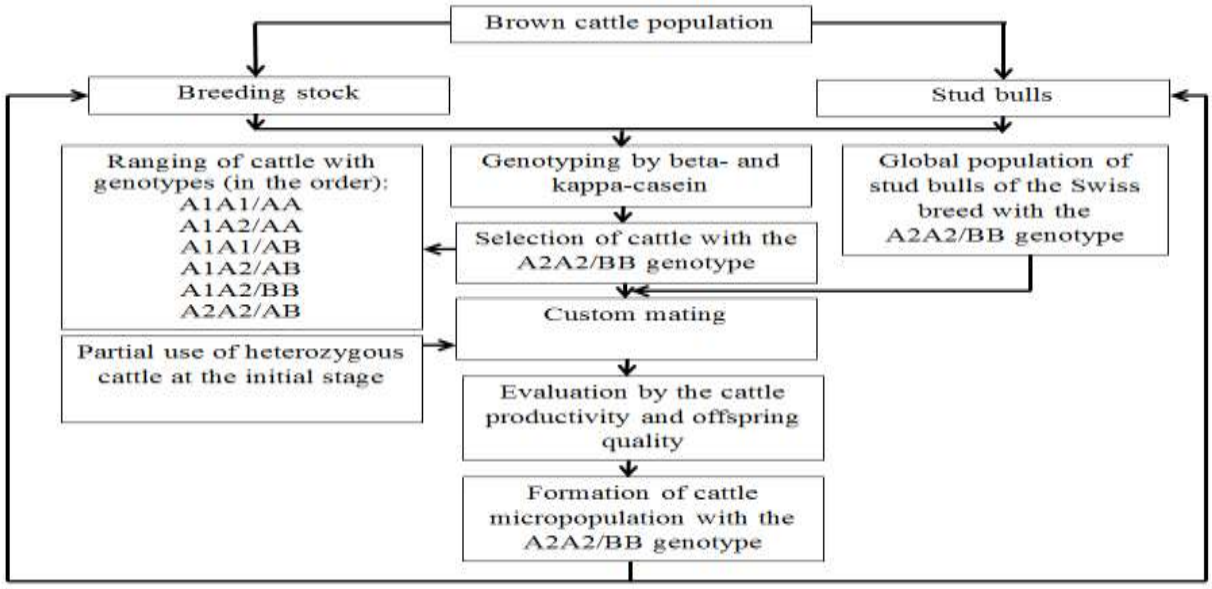

Fig. 1. Scheme of population formation with the desired A2A2/BB composite genotype
Source: The authors' own research.

In order to preserve the population of Brown cattle and form micropopulations with the desired genotype by beta-and kappa-casein, we propose an appropriate scheme (Fig. 1).

Comparing our results with those of other researchers, we note that the frequency distribution of complex genotypes in Black and White cattle had similar results to the studies of Vallas M. et al [15]. The proportion of A2A2/AA and A1A2/AA composite genotypes almost coincided (27.4% and 23.1%, respectively; 23.1 and 26.9%). The proportion of the desired A2A2/BB genotype was equally low (0.0 and 0.1%, respectively). Comparing the research results of Kyselová, J. et al [7] on Simmental cattle, we note a similarity in the frequency distribution by the A1A2/AB (26.8 and 17.8%, respectively), A2A2/AA (22.0 and 18.1%, respectively), and A1A2/AA (14.6% and 16.65%, respectively) genotypes. The desired A2A2/BB genotype had a low frequency (0.0 and 1.3%, respectively).

CONCLUSIONS

The conducted work has resulted in determining the frequency of alleles and genotypes by the beta- and kappa-casein loci, composite genotype. It is established that the breeds of dairy cattle bred in the North-East of Ukraine differ significantly in these traits.

At this stage of breeding, the formation of herds (micropopulations) with the A2A2/BB composite genotype is only possible among the Brown cattle.

In the future, exclusively by order of processing enterprises (the market), it is possible to breed herds of the Simmental

breed with the specified genetic parameters quite quickly.

Among Black and White populations, the above genetic combinations may only appear as a result of implementing special breeding programs, where the primary task will be to breed bulls of the A2A2/BB genotype that requires much more time to increase the occurrence frequency of among breeding stock.

The results obtained make it possible to increase the effectiveness of measures to preserve the population of Brown cattle in Ukraine. In addition, the formation of a population of Brown cattle with the A2A2/BB genotype enables to provide the population with safe and high-quality dairy raw materials, which will inevitably improve people's health. Increased cheese suitability of milk content will increase the economic efficiency of milk production.

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