Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 24, Issue 3, 2024
PRINT ISSN 2284-7995, E-ISSN 2285-3952

EFFICIENCY OF CREATING HERDS OF THE UKRAINIAN RED-AND-WHITE DAIRY BREED CATTLE WITH THE DESIRED BETA- AND KAPPA-CASEIN GENOTYPE

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Abstract

Qualitative characteristics of milk depend on the genotype of cows for beta- and kappa-casein genes. An animal's genotype for the beta-casein gene also has an impact on milk digestibility and human health. The rate of cheese yield depends on the genotype of the animal according to the kappa-casein gene. Genotyping of 349 animals of the Ukrainian Red-and-White dairy breed was carried out in order to establish the features of genotype formation by beta- and kappa- casein. The population of the studied breed (36% of homozygote A2A2 and 46% of heterozygote A1A2) can provide an increase in homozygosity by beta-casein (A2A2), that enables to increase the frequency of desired alleles under the conditions of compliance with the developed recommendations. The term of creation of a herd with genotype A2a2 is 10 years. For this, it is necessary to use breeders with the desired homozygous genotype and to cull cows and heifers with other genotypes. Breeding stock of the same breed (16% of BB homozygotes and 36% of AB heterozygotes) can also provide an increase in kappa-casein (BB) homozygosity in the next generation. Creating a herd with the desired genotype for the kappa-casein gene also requires the use of homozygous breeders. Obtaining animals with genotypes A2A2 for the beta-casein gene and BB for the kappa-casein gene will ensure boosting the economy of dairy farming.

Key words**:** *breed, allele, genotype*

INTRODUCTION

Milk and its various products make up the bulk of food for people around the world. The competitiveness of milk production depends on its quality characteristics [6]. One of the most produced milk products is cheese. It is considered one of the best sources of nutrients for humans, such as proteins, lipids, minerals and vitamins. One of the requirements for producing high-quality cheese is dairy raw materials not only with a high protein content, but also with the appropriate quality [12].

Scientists have noted that for the selection of milk for cheese production, it is essential to pay attention to the genotype of animals for the kappa-casein gene. The B allele is viewed as a more desirable one in cheese production. Milk from animals with the BB genotype has better technological properties in the production of cheese, and the product itself is featured by better physical and chemical qualities [16, 17]. It has been proven that when a person consumes milk from cows with the A2A2 genotype, no negative consequences are observed. Otherwise, milk from cows with the A1A1 genotype can harm human health [7, 21].

The importance of evaluating stud bulls by beta- and kappa-casein genotypes is indicated by the increased interest of livestock breeders in using stud bulls with BB and A2A2 genotypes, respectively. Over the past 2 years there has been an increase in the share of stud bulls in WWS Company with the A2A2 beta-casein genotype and the BB kappa-casein

genotype [17, 22, 25]. Thus, the frequency of such stud bulls in the 2019 Stud Bull Catalogue was 0.51 and 0.29, respectively; and in the 2021 Stud Bull Catalogue, was amounted to 0.68 and 0.31, respectively. Genotyped stud bulls, a significant part of which are imported from abroad, differ in the predominance of the A2 allele of beta-casein (the frequency was 0.63). However, the allele of the B kappa-casein was found in the group of stud bulls owned by Ukrainian enterprises only with the frequency of 0.34, which was 1.6 times lower than among American stud bulls.

Breeders are gradually turning their attention to the genotype of animals for certain caseins. The information about gene evaluation by the proteins becomes more and more common in the catalogs of breeders' semen [15]. The genotype of Holstein animals for the beta-casein gene varies depending on the country of origin. Depending on the country of origin, the A2A2 genotype varied significantly and ranged from 42 to 77%. The frequency of the A2 allele also varied and was 0.6-0.8 [3, 8, 9, 20].

The authors have established the frequency of beta- and kappa-casein genotypes of Ayrshire cattle. In different groups of cattle, the frequency of A2A2 beta-genotype ranges from 0.23 to 0.37 and BB kappa-genotype - from 0.03 to 0.06. The A2A2 and BB genotypes have no negative effect on lactation performance. The authors believe that for Ukrainian breeding enterprises that sell semen products of Ayrshire bulls, it is advisable to increase the share of stud bulls with the A2A2 (beta-casein locus), AB and BB (kappa-casein locus) genotypes. It was found that in different groups of cattle, the frequency of beta-casein loci of the A2A2 genotype ranges from 0.50 to 0.80 and kappa-casein of the BB genotype ranges from 0.66 to 0.88. The A2A2 and BB (beta- and kappa- loci) genotypes do not negatively affect the lactation performance of their carriers [2, 26].

A large sample of Holstein cows (n=8706) showed a low (8.46%) proportion of animals homozygous by the BB kappa-casein gene allele with a high proportion of heterozygotes (44.66%) and homozygotes (46.88%) of AA genotype. Genotyping of stud bulls (n=84) revealed that 7.1% of cattle were homozygous by the desired kappa-casein alleles, and 22.6% by beta-casein. The shortage of domestic Holstein bulls with the desired genotypes today can be compensated by the import of bull semen from countries where breeding is carried out not only by beta- and kappa-casein, but also in general by cheese making. A high proportion (38.87%) of homozygous cows with the A2 allele subject to a significant number of livestock makes it possible to form a specialized herd for the production of "hypoallergenic" milk.

Breeders, used in breeding herds, significantly differ in terms of both beta- and kappa-casein genotypes [1, 19].

The purpose of the article is to assess the polymorphism of beta- and kappa-casein genes in the population of the Ukrainian Red-and-White dairy breed and establish the possibility of creating dairy herds with the desired genotype.

MATERIALS AND METHODS

Genotyping of cattle of the Ukrainian Red-and-White dairy breed was carried out according to the genome of beta-casein (n=235) kept in Ichnianske LLC in Chernihiv Region and kappa-casein (n=114) kept in Khliborob LLC in Kyiv Region.

Blood samples were taken under sterile conditions into 2.7 mL manovettes 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.

The determination of the genotype of the Ukrainian Red-and-White dairy cattle breed by the kappa-casein (k-Cn) gene was carried out in the genetics laboratory of M. V. Zubets Institute of Animal Breeding and Genetics of the National Academy of Sciences of Ukraine. The amplification of gene fragment was carried out with the use of the following primers:

5'-GAAATCCCTACCATCAATACC-3 '; 5'-CCATCTACGCTAGTTTAGATG-3 ' (Pinder

et al., 1991).

The length of the amplified fragment is 273 bp. The amplification product of the k-Cn gene with the use of the above primers includes the region of the 4th exon and 4th intron of the gene. The two allelic variants A and B of the k-Cn gene are detected after restriction of this Hinf1 fragment. The variant B is characterized by point mutations (amino acid replacement of Tyr with Iso in position 136; Ala with Asp in position 148).

The composition of the reaction mixture (10 µL): 4.3 µL of H2O; 2.0 µL of 5-x PCR buffer (15 m Mg-1.0 ML); 0.8 µL of 10-x dNTP mixture (2 mm each); 0.8 µL of two primers (70 ng each); 0.1 µL of Taq polymerase (1 ML /1000 U); 2.0 µL of 50-100 ng DNA. The PCR amplification of kappa-casein gene was carried out under the following temperature regime: initial denaturation –at 94°C for 3 min; 35 cycles: denaturation – at 94°C for 30 s; firing primers –at 61°C for 30 s; synthesis – at 72°C for 30 s; terminal elongation – at 72°C for 5 min.

The Hinf1 restriction was used to restrict the k-Cn gene [11] fragments with a length of 113, 91, 49 bp (cattle of the AA genotype); 224, 113, 91, 49 bp (cattle of the AB genotype); 224 and 49 bp (cattle of the BB genotype) were found after the above restriction [10, 23].

The molecular weight marker Fermentas# SM0373, GeneRuler 50-bp DNA Ladder was used during the research.

The electrophoretic separation of DNA restriction fragments was performed according to the methodological recommendations [5] in 2 %, agarose gel in tris borate electrophoresis buffer (TBE: 0.0879 m Tris, 0.089 m boric acid, 0.002 m EDTA pH 8.0) for 1-3 hours (voltage 2 v/cm of gel). The buffer composition for sample application: 0.25% bromphenol blue, 0.25% xylene cyanol, 30% glycerin. The gel staining was performed for 10 minutes subject to the use of ethidium bromide (0.5 mcg/ml) followed by washing with distilled water. Visualization was performed in UV light (ʎ = 380 nm) after staining the gel with ethidium bromide.

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′-GGTTTGAGTAAGAGGAGGGATGTTT -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 DISCUSSIONS

To improve the economic efficiency of milk production, a promising task is to take measures to form a dairy herd with programmable productivity. In two farms for breeding the Ukrainian Red-and-White dairy breed, the task was to create a herd with a certain genotype by different casein loci. In the first farm of Khrystynivske State-Owned Farm it is planned to create a herd for obtaining milk suitable for the production of hard cheeses. For this purpose, the dairy herd was assessed according to the kappa-casein gene. It was established that the desired BB genotype had an insignificant share of 16% (Table 1).

Table 1. Distribution of allelic and genotypic frequencies of the kappa-casein gene and the results of Hardy-Weinberg equilibrium statistical test

Distribution	Genotypes, %			Allele, pcs.		χ^2
	AA	AB	BB	A	B	
Actual	48	36	16	0.662	0.338	4.379
Theoretical	44	45	11			

Source: The authors' own research.

The majority of animals had a different homozygous AA genotype (almost 50%). Allele B had a low frequency (0.338). It should be noted that the actual genotype frequencies and theoretically calculated ones do not coincide and there is a statistically significant difference between them. We also mention a lack of heterozygous genotypes.

In another farm (Ichnianske LLC), it is planned to obtain dairy raw materials for the production of "gentle" milk (from animals with the A2A2 genotype). According to the results of our genetic research, it was established that the A1A2 genotype had the largest share. The share of homozygous A1A1 and A2A2 genotypes differed twofold in favor of the latter genotype. The frequency of the desired A2 allele is 0.589, which is quite high, given the widespread use of Holstein stud bulls in the breeding stock of the herd (Table 2).

Table 2. Distribution of allelic and genotypic frequencies of the beta-casein gene and the results of the Hardy-Weinberg equilibrium statistical test

Distribution	Genotypes, %			Allele, pcs.		χ^2
	A1A1	A1A2	A2A2	A1	A2	
Actual	18	46	36	0.411	0.589	0.409
Theoretical	17	48	35			

Source: The authors' own research.

There was no difference between the actual frequency of genotypes and the theoretically calculated one.

Research results have indicated the superiority of theoretical heterozygosity over actual heterozygosity (Table 3). More significant difference was found in the kappa-casein gene.

Table 3. Values of the main volatility indicators

Gene	Ho	He	Fis
CSN2	0.464	0.484	0.042
CSN3	0.360	0.447	0.195

Ho – actual heterozygosity, He – expected heterozygosity, Fis – fixation index
Source: The authors' own research.

Using genetic and statistical methods of analysis, by determining the digital values of such genetic constants as the degree of homozygosity (Ca), the level of polymorphism (Na), we tried to assess the prospects and investigate the effectiveness of creating herds with the desired genotype (Table 4).

It was established that the CA indicator for the studied genes varied from 51.6 to 55.3. The Na indicator prevailed over the beta-casein gene.

The heterozygosity test (HT), which indicates the level of genetic diversity of the population, is negative in the studied herds, indicating a lower proportion of actual heterozygotes

compared to the proportion of theoretical heterozygotes by the corresponding casein genes. As for the excess coefficient (D), which characterizes the ratio of actual heterozygosity to theoretical heterozygosity, we note a deviation of actual heterozygosity from the expected one with left-sided excess, which also indicates a deficiency of heterozygotes. This is especially true for the kappa-casein gene. In general, it can be stated that the data of genetic and statistical analysis indicate a slight excess in the beta-casein locus of homozygous A1A1 and A2A2 variants, and a lack of heterozygous A1A2 and a significant excess of homozygous AA and BB and a lack of heterozygous AB.

Table 4. Frequency Genetic structure of herds by CSN2 and CSN3

Indicators	Genes			
	CSN2		CSN3	
	actual	theoretical	actual	theoretical
Heterozygotes	109	114	41	51
Homozygotes	126	121	73	63
Coefficient hetero/homozygotes	0.87	0.94	0.56	0.81
Heterozygosity test	-0.073	-	-0.248	-
Degree of homozygosity, Ca, %	51.6	-	55.3	-
Polymorphism level, Na	1.93	-	1.81	-
Excess coefficient D	-0.042	-	-0.196	-
Proportion of homozygotes, %	53.60	-	64.04	-

Source: The authors' own research.

According to the analysis of the Dairy and Dairy-Meat Breed Bull Catalogue showing stud bulls allowed to be reproduced in Ukraine, it was found that among the Holsteins allowed to be used and evaluated by the quality of offspring and genomically, 47% of animals had a score based on the kappa-casein genotype (Fig. 1).

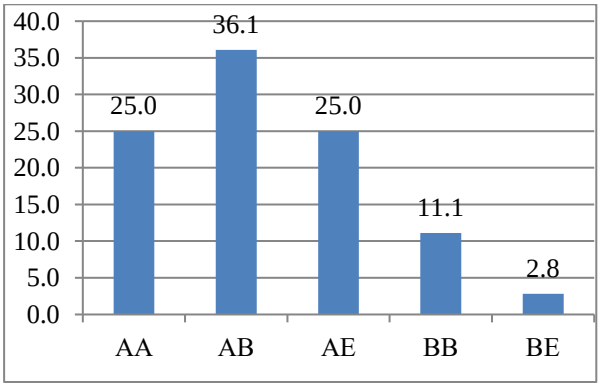

Fig. 1. Evaluation of breeders according to the frequency of the kappa-casein gene, %
Source: The authors' own research.

The frequency of the desired homozygous BB genotype among them was equal to 11%.

Solely 33% of the breeders, evaluated for the beta-casein gene, had the homozygous A2A2 genotype (Fig. 2).

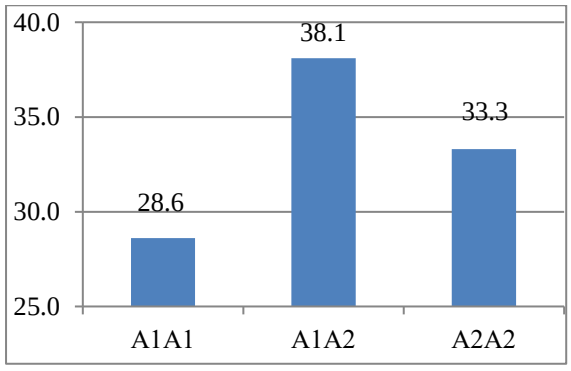

Fig. 2. Evaluation of breeders according to the frequency of the beta-casein gene, %
Source: The authors' own research.

The availability of breeders with the desired genotypes for the studied genes suggests the possibility of creating herds with the recommended genotypes.
According to the scheme of creating a herd (Table 5) only stud bulls that are homozygous by the desired genotypes (A2A2 – by beta-casein and BB – by kappa-casein) should be used [13, 14, 24].

Table 5. Possible combinations when crossing cattle of different genotypes by beta-or kappa-casein

	Parent 1			Parent 1			Parent 1			Parent 1			Parent 1		
		A1 or A	A2 or B		A1 or A	A2 or B		A1or A	A2 or B		A2 or B	A2 or B		A2 or B	A2 or B
Parent 2	A1 or A	A1A1 or AA	A1A2 or AB	A2 or B	A1A2 or AB	A2A2 or BB	A1 or A	A1A1 or AA	A1A2 or AB	A1 or A	A1A2 or AB	A1A2 or AB	A2 or B	A2A2 or BB	A2A2 or BB
	A1 or A	A1A1 or AA	A1A2 or AB	A2 or B	A1A2 or AB	A2A2 or BB	A2 or B	A1A2 or AB	A2A2 or BB	A1 or A	A1A2 or AB	A1A2 or AB	A2 or B	A2A2 or BB	A2A2 or BB
	50% A1A1 or AA 50% A1A2 or AB			50% A1A2 or AB 50% A2A2 or BB			25% A1A1 or AA 50% A1A2 or AB 25% A2A2 or BB			100% A1A2 or AB			100% A2A2 or BB		

Source: (Ladyka V., Pavlenko Y., 2021; Ladyka, V. et al., 2019; Sklyarenko, Y. et al., 2018) [13, 14, 24].

Taking into account the initial frequency of genotypes and alleles of the investigated casein fractions in both farms, we recommend to utilize the selection model [18]. According to it, creating a herd of animals with the A2A2 genotype will take less time than herds with the BB genotype (Table 6). At the same time, during a 10-year period, such a herd can be created using genetic testing of heifers. In the case of genetic testing of cows and heifers, such a herd can be created in nine years.

Table 6. Duration of selection work

Varants for carrying out the work	Proportion of genotype after 10 years in animals of the main herd		required time, years	
	CSN2	CSN3	for creating a herd of cows with a genotype	
	A2A2	BB	CSN2 (A2A2)	CSN3 (BB)
Lack of testing of cows and calves	0.78	0.54	>15 years	>15 years
Genetic testing of cows	0.85	0.57	>15 years	>15 years
Genetic testing of calves	1.00	0.93	10 years	14 years
Genetic testing of cows and calves	1.00	0.95	9 years	11 years
Genetic testing of cows and calves and the use of sexed semen (A2A2) or (BB)	1.00	1.00	9 years	10 years

Source: The authors' own research.

One can also significantly speed up the process of creating a herd by using the following measures:

- transplantation of female embryos with the desired homozygous genotype;
- increase in the proportion of culling of cows with genotypes (A1A1 and A1A2 by beta-casein or AA and AB by CAPA-casein) with the introduction of first-born cows with the desired homozygous genotypes (A2A2 or BB) into the herd;
- purchase of cattle with the desired genotype.

CONCLUSIONS

Cows of the Ukrainian Red-and-White dairy breed (36% of homozygote A2A2 and 46% of heterozygote A1A2) can provide an increase in homozygosity by beta-casein (A2A2) in the next generation subject to the use of homozygous (A2A2) stud bulls. The genetic structure of Holstein Red-and-White breed (33.3% of homozygotes A2A2) which may be reproduced in Ukraine enables to form populations homozygous for this trait in subsequent generations. To increase the frequency of the desired A2A2 genotype, it is necessary to cull animals with the A1A1 and A1A2 genotypes, using breeders' semen with the A2A2 genotype. Such work will take about 10 years. Using the genetic testing of cows and

calves or genetic testing of cows and calves using sexed semen (A2A2) speeds up this process by a year.

Breeding stock of the same breed (16% of BB homozygotes and 36% of AB heterozygotes) can also provide an increase in kappa-casein (BB) homozygosity in the next generation. As with beta-casein, the condition is the use of homozygous (BB) stud bulls. Their genetic structure (11.1% of BB homozygotes) also enables to form populations homozygous by this trait in subsequent generations. To create herds with the desired genotype for the kappa-casein gene, it is necessary to carry out a similar work.

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