ТЕХНОЛОГІЯ ВИРОБНИЦТВА І ПЕРЕРОБКИ ПРОДУКЦІЇ ТВАРИННИЦТВА

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Features of herd formation based on beta- and kappa-casein of different dairy cattle breeds

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In dairy cattle breeding, much attention is paid to indicators of the milk quality and technological properties. These indicators are significantly influenced by milk proteins. Therefore, the goal of our research is to evaluate the effectiveness of creating dairy herds of domestic dairy cattle breeds that are characterized by the desired beta- and kappa-casein genotypes. To fulfill these tasks, the genotyping of cows of the Ukrainian Brown dairy, Sumy intrabreed type of the Ukrainian Black-and-White dairy, Simmental and Lebedyn breeds was carried out. The beta- and kappa-casein gene polymorphism was determined in the genetic laboratory of the Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine using real-time molecular biological analysis of allele recognition by polymerase chain reaction (PCR).

As a result of the conducted studies, it is found that cattle of Lebedyn and Ukrainian Brown dairy breeds have a higher frequency of the desired kappa-casein BB genotype. As a result, they are also characterized by a higher frequency of the B allele. More than 50 % of cattle of Simmental breed have a heterozygous AB genotype. Cattle of the Sumy intrabreed type of the Ukrainian Black-and-White dairy breed have a higher frequency of the homozygous AA genotype and the A allele. Cattle of the Ukrainian Brown dairy breed are characterized by the highest frequency of the A2A2 genotype and the A2 allele of beta-casein. Cattle of the Simmental breed, which are also characterized by the highest frequency of the A1A2 heterozygous genotype, have a slightly lower frequency. Homozygous A1A1genotype is more common in cattle of the Sumy intrabreed type of the Ukrainian Black-and-White dairy breed.

Using the principles of the model for creating a herd with the desired genotype, which is proposed by Mencarini IR et all, it is found that when testing the entire breeding stock, inseminating it with semen of homozygous stud bulls with the A2A2 genotype, random cow disposal at the level of 22 %, it takes 9–10 years to create herds with the desired genotypes of the Ukrainian Brown dairy, Simmental and Lebedyn breeds, respectively. To reduce the period for creating herds, it is essential to increase the percentage of cow disposal and simultaneously disposecattle with undesirable genotypes.

Key words: genotype, casein, stud bull, breeding, allele.

Problem statement and analysis of recent research. High-quality and technological indicators of milk are given great attention in dairy cattle breeding. The composition of milk proteins significantly affects the physical and chemical properties of milk and, as a result, its technological indicators.

In cheese manufacture, much attention is paid to the quality of dairy raw materials, namely the type of kappa-casein that is part of these raw materials. The kappa-casein gene polymorphism has been studied for more than 40 years. To date, thirteen genetic variants of bovine kappa-casein have been described. The most common genetic vari-

ants are A and B [6]. It is found that the B allele is associated with the production of milk that is most suitable for cheese manufacture in terms of technological properties. This explains the fact that cattle with the homozygous BB genotype have better cheese making of milk [4, 5, 20].

Different breeds of cattle and the same breed differ significantly in the frequency of kappa-casein genotypes and alleles. The Jersey cattle are characterized by a high frequency of the B allele (0.69) and BB genotype (0.45) [9, 13]. The Holstein breed is characterized by significant differentiation on this basis, depending on the country of origin (breeding). The frequency of the desired B allele, depending on the country of origin, was 41–49 % (Serbia, Iraq) and 14–17 % (Poland, China). Accordingly, the BB genotype was more common in the Serbian population – 23 %, the least – in the Canadian population (2.7 %) [1, 2, 8, 12, 15].

Recently, beta-casein polymorphism has attracted great interest from both the scientific community and the dairy sector due to its impact on milk quality. The two main subvariants of beta-casein (A1 and A2) are encoded by the CSN2 gene. It is found that the consumption of A1 variant, in comparison with A2 variant, potentially negatively affects human health after its digestion, but at the same time its availability improves the technological properties of milk [7, 16, 18].

The researchers have concluded that the amino acid content, fatty acid content, and milk color may be affected by the CSN2 A1A1, A1A2, and A2A2 genotypes. As a result of selective breeding of genotypes with the desired qualities, milk and dairy products can be improved [19].

The researchers state that the simplest process for creating a herd of cattle with the A2A2 genotype is to use bull semen with the A2A2 genotype by beta-casein. However, the transformation function is curved and asymptotic, and this strategy itself cannot achieve herd purity. The conversion rate can be significantly increased by genotyping calves and/or cows. This is required for completing the conversion process. The mating of heifers with semen with the A2A2 genotype by beta-casein can have a significant impact, especially in connection with an increase in the level of cow disposal. Combining these strategies can lead to pure herds with the A2A2 genotype within 5–8 years, depending on the initial structure of the herd. The use of sexed semen can further accelerate the conversion period [14].

The results of studies to estimate the frequency of beta-casein variants in the offspring of pre-genotyped cows inseminated with homozygous A2A2 semen indicate that the frequency of cattle with the A2A2 genotype has almost doubled – from 37 to 69 % [17].

Similar work may be carried out on the creation of dairy herds with the BB genotype by kappa-casein.

The goal of the research is to evaluate the prospect of creating dairy herds with the desired genotypes by beta- and kappa-casein of domestic dairy cattle breeds.

Material and methods of research. The genotyping of cows of the Ukrainian Brown Dairy breed (n=44), Sumy intra-breed type of the Ukrainian Black-and-White Dairy breed (n=26), Simmental (n=30), Lebedyn (n=59) breeds was carried out.

The kappa-casein gene polymorphism was determined in the genetic laboratory of the Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine using real-time molecular biological analysis of allele recognition by polymerase chain reaction (PCR). Blood samples were taken to the 2.7 ml Monovette (Sarstedt, Germany), followed by freezing the samples and storing them at -20ºC. DNA for genotyping was obtained from samples using the Monarch® New England BioLabGenomic DNA Purification Kit (USA) in accordance with the manufacturer's protocol.

Allele frequencies were calculated taking into account the number of homozygotes and heterozygotes found for the corresponding allele using the formula:

$$P(A)=\frac{2N1+N2}{2n}$$

where N_1 and N_2 are the number of homozygotes and heterozygotes for the allele under study, respectively;
n is the sample number.

In order to assess the statistical reliability of the discrepancy between the distributions of the obtained results, the Pearson criterion was used as follows:

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

where: A is the actual number of genotypes;
T is the theoretical number of genotypes.

Theactual (available) heterozygosity was determined by direct calculation using the formula:

$$H_O=\frac{N2}{n}$$

The expected heterozygosity was determined by the formula:

$$H_E=1-\sum_{i=1}^{n} p_i^2$$

where p_1, p_2,….$p_n$are allele frequencies.

The research results were processed using mathematical statistics using the Statistica-6.1Package in the Windows PC environment.

Results and discussion. According to the frequency of the desired genotype of BB kappa-casein, cattle of the Lebedyn and Ukrainian Brown dairy breeds have an advantage. They also predominate among representatives of other breeds in terms of the frequency of the B allele. The Simmental breed has a higher frequency of heterozygous (AB) genotypes, in which it is almost 50 %. Cattle of the Sumy intrabreed type of the Ukrainian Black-and-White Dairy breed have the highest frequency of the homozygous AA genotype and Aallele. In all the studied breeds, except Simmental, there is an advantage of the expected heterozygosity over the actual one (Table 1).

Studying the beta-casein polymorphism, it is found that the highest frequency of both the A2A2 genotype and the A2 allele is found in cattle of the Ukrainian Brown Dairy breed. Half of them have this genotype. Cattle of the Simmental breed have a slightly lower frequency of the A2A2 genotype (40 %). At the same time, cattle of this breed have the highest frequency of heterozygous A1A2 genotype (57 %). Cattle of the Sumy intrabreed type of the Ukrainian Black-and-White Dairy breed have the highest frequency of homozygous A1A1type (38 %). They are also characterized by the advantage of the expected heterozygosity over the actual one (Table 2).

The number of cattle with the desired homozygous A2A2 genotype by beta-casein and BB genotype by kappa-caseincan be increased by selecting parent pairs. Complete penetrance, i.e. 100 % of the frequency and probability of phenotypic gene expression, can be achieved if:

♂ ♀
A2A2 x A2A2 = 100 % penetrance;
A2A2 x A1A2 = 50–70 % penetrance;
A1A2x A2A2 = 50–70 % penetrance;
A2A2 x A1A1 = 50–70 % penetrance;
A1A2 x A1A2 = 25–50 % penetrance;
A1A1 x A1A2= 25–50 % penetrance;
A1A2 xA1A1= 25–50 % penetrance.

Table 1 – **Frequency of alleles and genotypes by kappa-casein genelocus**

Distribution	Genotypes, %			Allele,pcs.		χ^2
	AA	AB	BB	A	B	
Ukrainian Brown Dairy						
Actual	30	40	30	0,500	0,500	1,455
Theoretical	25	50	25			
Ukrainian Black-and-White Dairy						
Actual	58	27	15	0,712	0,288	3,079
Theoretical	51	41	8			
Simmental						
Actual	43	47	10	0,667	0,333	0,075
Theoretical	44	44	11			
Lebedyn						
Actual	15	36	49	0,328	0,672	2,014
Theoretical	11	44	45			

Table 2 – **Frequency of alleles and genotypes by beta-casein genelocus**

Distribution	Genotypes, %			Allele, pcs.		χ^2
	A1A1	A1A2	A2A2	A1	A2	
Ukrainian Brown Dairy						
Actual	7	43	50	0,284	0,716	0,167
Theoretical	8	41	51			
Ukrainian Black-and-White Dairy						
Actual	38	35	27	0,558	0,442	2,314
Theoretical	31	49	20			
Simmental						
Actual	3	57	40	0,317	0,683	2,871
Theoretical	10	43	47			
Lebedyn						
Actual	19	51	30	0,447	0,553	0,048
Theoretical	20	49	31			

An individual approach to the issues of selection, control of the processes of gene transfer from parents to offspring using DNA diagnostics methods will make it possible to replenish herds with carriers of the A2 or B gene in a relatively short time [10, 11].Taking into account the initial frequency of genotypes and alleles of the studied casein fractions in the studied breeds, using the principles of the herd creation model with the desired genotype [14], it is found that when using bull semen with the BB or A2A2 genotype on a number of cows and heifers without testing them, random cow disposal at the level of 22 % to create herds with the desired genotypes takes more than 15 years. Similar results will be obtained when using cow testing. It is possible to reduce the time required to create a herd by using genotype testing of heifers or cows and heifers. The best indicators regarding the timing of herd creation can be obtained by testing cows and heifers in combination with the use of sexed semen of stud bulls with the A2A2, BB genotypes. At the same time, over a 10-year period, such a herd can be created using genetic testing of heifers. In the case of using genetic testing of cows and heifers, such a herd can be created in nine years. Thus, in 9 years it is possible to create a herd of cattle of the Lebedyn breed with the BB genotype by kappa-casein and cattle of the Ukrainian Brown dairy and Simmental breeds with the A2A2 genotype by beta-casein. More time is needed to create similar herds of cattle of other studied breeds (Table 3).

It should be note that it is possible to reduce the time required to create such herds by increasing the percentage of accidental cow disposal and disposal of cattle with undesirable genotypes.

Conclusion. Results of the conducted studies indicate prospects for creating dairy herds with cattle of the desired genotypes by kappa-casein (BB) and beta-casein (A2A2). Given that the frequency of desired genotypes is higher in cattle of the Lebedyn and Ukrainian Brown Dairy breeds, this work is more promising with these breeds. In 9 years, the breeders can create a herd of cattle of the Lebedyn breed with the BB genotype by kappa-casein and cattle of the Ukrainian Brown Dairy and Simmental breeds with the A2A2 genotype by beta-casein. To reduce the time frame for creating herds, it is essential to increase the percentage of cow disposal and simultaneously dispose cattle with undesirable genotypes.

Table 3 – **Analysis of mechanisms for creating herds with the desired genotype**

Optionsforcarryingoutthework	Genotype fractionin 10 years in cattle of the mainherd		Required time, years	
	CSN2	CSN3	for creating a herd of cows with a genotype	
	A2A2	BB	CSN2 (A2A2)	CSN3 (BB)
Ukrainian Brown Dairy				
Lack of testing of cows and calves	0,78	0,66	>15	>15
Genetictestingofcows	0,85	0,71	>15	>15
Genetictestingofcalves	1,00	0,99	10	12
Genetic testing of cows and calves	1,00	1,00	9	10
Genetic testing of cows and calves and the use of sexed semen (A2A2) or (BB)	1,00	1,00	9	10
Ukrainian Black-and-White Dairy				
Lack of testing of cows and calves	0,66	0,54	>15	>15
Genetic testingofcows	0,71	0,57	>15	>15
Genetic testingofcalves	0,99	0,93	12	14
Genetic testing of cows and calves	1,00	0,95	10	11
Genetic testing of cows and calves and the use of sexed semen (A2A2) or (BB)	1,00	1,00	10	10
Simmental				
Lack of testing of cows and calves	0,78	0,54	>15	>15
Genetic testingofcows	0,85	0,57	>15	>15
Genetic testingofcalves	1,00	0,93	10	14
Genetic testing of cows and calves	1,00	0,95	9	11
Genetic testing of cows and calves and the use of sexed semen (A2A2) or (BB)	1,00	1,00	9	10
Lebedyn				
Lack of testing of cows and calves	0,71	0,78	>15	>15
Genetic testingofcows	0,99	0,85	>15	>15
Genetic testingofcalves	1,00	1,00	12	10
Genetic testing of cows and calves	1,00	1,00	10	9
Genetic testing of cows and calves and the use of sexed semen (A2A2) or (BB)	1,00	1,00	10	9

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Особливості формування стад за бета- та капа-казеїном молочної худоби різних порід

Ладика В. І., Павленко Ю.М., Скляренко Ю.І.

У молочному скотарстві велику увагу приділяють показникам якості та технологічності молока. На ці показники істотний вплив мають білки молока. Тому метою цих досліджень є оцінка ефективності створення молочних стад вітчизняних порід молочної худоби, які характеризуються бажаними генотипами за бета- та капа-казеїном. Для виконання поставлених завдань проведено генотипування корів української бурої молочної, сумського внутрішньопородного типу української чорно-рябої молочної, симентальської та лебединської порід. Визначення поліморфізму гену капа-бета-казеїну проводили в генетичній лабораторії Інституту фізіології ім. Богомольця НАН за допомогою молекулярно-біологічного аналізу розпізнавання алелів методом полімеразної ланцюгової реакції (ПЛР) у реальному часі.

У результаті проведених досліджень встановлено, що тварини лебединської та української бурої молочної порід мають вищі значення частоти бажаного

генотипу ВВ капа-казеїну. Як наслідок, для них характерна і вища частота алеля В. Понад 50 % тварин симентальської породи мають гетерозиготний генотип АВ. Більшу частоту гомозиготного генотипу АА та алеля А мають тварини сумського внутрішньопородного типу української чорно-рябої молочної породи. Для тварин української бурої молочної породи характерна найбільша частота генотипу А2А2 та алеля А2 бета-казеїну. Дещо нижчу частоту мають тварини симентальської породи, для яких також характерна найбільша частота гетерозиготного генотипу А1А2. Гомозиготний генотип А1А1 частіше зустрічається у тварин сумського внутрішньопородного типу української чорно-рябої молочної породи.

Використовуючи принципи моделі створення стада з бажаним генотипом, яку запропоновано Mencarini IRetall, встановлено, що при тестуванні всього маточного поголів'я, осіменіння його спермою гомозиготних плідників з генотипом А2А2, випадковому вибракуванні корів на рівні 22 % для створення стад з бажаними генотипами української бурої молочної, симентальської та лебединської порід, відповідно необхідно 9–10 років. Для скорочення строків створення стад необхідно збільшити відсоток вибракування корів та паралельно вибраковувати тварин з небажаними генотипами.

Ключові слова: генотип, казеїн, плідник, селекція, алель.

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