

# Assessment of the genotypes of jersey cows by the molecular markers of the kappa-casein gene

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**Abstract.** The article is devoted to an urgent issue in the era of import substitution – the search for markers that can increase milk productivity. As the obtained research results have shown, genotyping using allelic variants of the kappa-casein gene will make it possible to make and generalize the selection and picking of the desired animal genotypes for practical breeding in order to increase protein content.

## 1 Introduction

In recent years, dairy farming in Russia has been steadily developing in all directions – from automating the processes of breeding, keeping, breeding stable dairy breeds for local economic conditions to processing a wide range of milk to meet the needs of a large target audience of dairy products – from infants to the older generation. Currently, the dairy industry is considered as a priority area of agricultural development, and the main task is to bring it to a level that would allow the country to use it as a competitive advantage in markets around the world. The degree of milk productivity depends on many factors, including heredity, breed, physiological condition, feeding conditions, maintenance and use of animals. Currently, the methods of molecular genetics make it possible to determine codominantly inherited allelic variants of genes associated with dairy productivity [1, 2, 3].

Marker breeding is a program of genetic improvement of animals, which includes the use of information on the results of testing marker genes of selectively significant loci of quantitative traits, it is most justified in addition to traditional breeding. The main direction of marker breeding of farm animals is the development of methods for assessing genotypes by marker loci to increase the effectiveness of early selection of the best animals in the population. Gene screening is necessary for targeted breeding and breeding work, the formation of a highly productive breeding herd based on individuals whose genome contains alleles of genes that allow for high milk yields, the selection of young animals for the formation of a breeding repair herd with high milk quality indicators for the dairy processing industry. One of the genes controlling the yield and quality of milk and dairy products is the kappa-casein gene [4, 5, 6].

In this regard, it is an important selection criterion for dairy cattle breeds. Kappa-casein (k-Cn, CSN3) is considered a fraction of casein – milk protein. Its task is to control the yield and quality of milk and dairy products. It is worth noting that this gene is one of the main selection criteria in dairy cattle breeding. The approach to pet improvement has changed over

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the past thousands of years. As a rule, these studies included long-term observations of the productive qualities of individuals with the identification of the best and their use in breeding. Currently, animal genotyping is increasingly associated with work in the field of DNA technology, which allows the identification of milk protein genotypes in livestock, producers and young animals.

## 2 Research methodology

The purpose of the work carried out at the L.K. Ernst FITZVIZH Moscow Region was to study: 1. estimates of milk productivity by kappa-casein; 2. evaluation of the occurrence in dairy cows with desirable allelic forms of the CSN3 gene using polymerase chain reaction using genetic markers; 3. determination of the effectiveness and practical significance of PCR genotyping; 4. identifying the best forms of genes to increase the productivity of dairy cattle. The data of our study were obtained at the modern agrocomplex of LLC "Vakinskoe Agro", located in the Ryazan region of the Rybnovsky district during the 2020-2021 academic year. The studied objects were 25 Jersey cows, which are distinguished by good quality and fat content of milk, even with small sizes relative to other breeds of cows. The breed has individual external features [7, 8, 9].

They have a narrow forehead and a small head with a concave profile, a cup-shaped udder, a concave spine and oblique ribs with a large chest. The average milk yield for 305 days in liters is 3000-3500. This is not much, in comparison with the indicators of other dairy breeds. The fat content of the product in this breed varies from 5% to 8%, and protein – from 3.5% to 3.7%. In addition, it has a pleasant taste and smell. The blood was obtained from the jugular vein and placed in test tubes with ethylenediaminetetraacetic acid (EDTA), which binds calcium ions and blocks the cascade of blood clotting reactions. PCR is carried out in a final volume of 20  $\mu$ l with 1 x PCR buffer (16.6 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 67.7 mM Tris-HCl, pH=8.8, 0.1% (v/v) Tween 20), 1.5 mM MgCl<sub>2</sub>, 200 mM dNTP, 10 pmol of each of the primers and 1-2 EdTaq polymerases. After initial denaturation (95 °C, 5 minutes), 37 amplification cycles are performed in the following temperature-time mode: 95 °C, 1 minute for denaturation, 57 °C (CSN3); 60 °C (BLG); 1 minute for denaturation, 57 °C (CSN3); 60 °C (BLG); 1 minute for primer annealing, 72 °C, 1 minute for polymerization, and final finishing at 72 °C, 7 minutes.

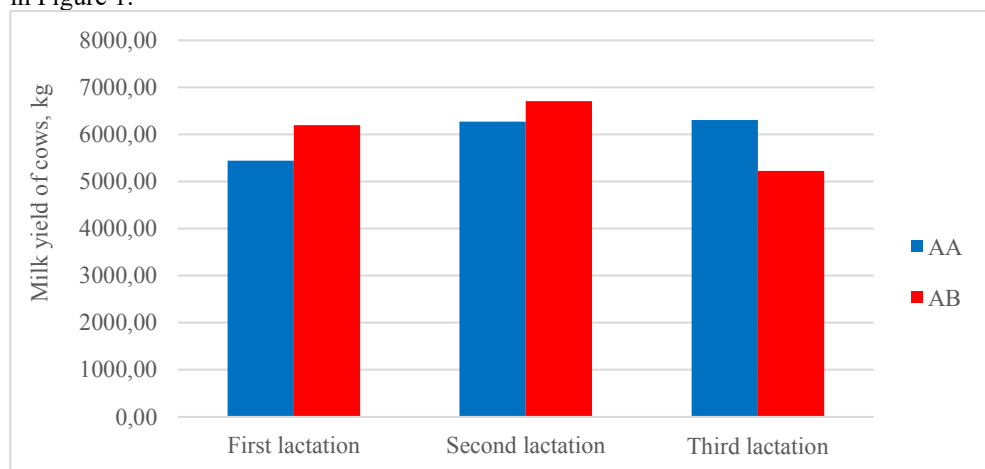
Upon completion of PCR, 5  $\mu$ l of the reaction is applied to the agarose gel in order to control the presence of fragments. In case of successful amplification, the remaining reaction mixture is divided into aliquots and treated with restrictases, depending on the gene under study. PCR-PDRF analysis of the CSN3 (kappa-casein) gene makes it possible to identify its 2 main variants – A and B. Upon completion of PCR with primers VAR5, VAR3, amplification control is performed, with successful PCR, an aliquot of 10  $\mu$ l is taken from the reaction mixture and treated with restriction enzymes TagI. After 9-10 hours, separation (electrophoretic) is carried out. This is carried out in an agarose gel, with a concentration of 2%, in a TAE buffer at 130V. For a final concentration of 30 ng/ml, dimidium bromide is added. Fragments are visualized using ultraviolet light [10, 11, 12, 13].

## 3 The results of the study

The frequency of the gene does not change with the change of generations, because the distribution of genotypes in each of them obeys the formula of the Newton biome, which is expressed as the square of the sum of the frequencies of two alleles:  $(pA+qB)^2=p^2AA+2pAqB+q^2BB$ ;  $p+q=1$  (1) According to the results of the study, out of 25 Jersey cows, we received 15 cows with the AA genotype and 10 cows with the AB genotype,

cows with the BB genotype were not identified. The frequency of alleles was calculated by the formula:  $p2AA+2pAqB+q2BB$  (2)  $p=3,87$   $q=1,29$  Thus, the frequency of allele A is 3.87, the frequency of allele B is 1.29. The frequency of allele A exceeds the frequency of allele B by 3 times. Most likely, this is caused by the purposeful culling of cows with the BB genotype, since it causes an increase in the mass fraction of protein in milk, which makes it possible to make high-quality dairy products from it, and this farm is aimed at obtaining high milk yields [14, 15, 16, 17].

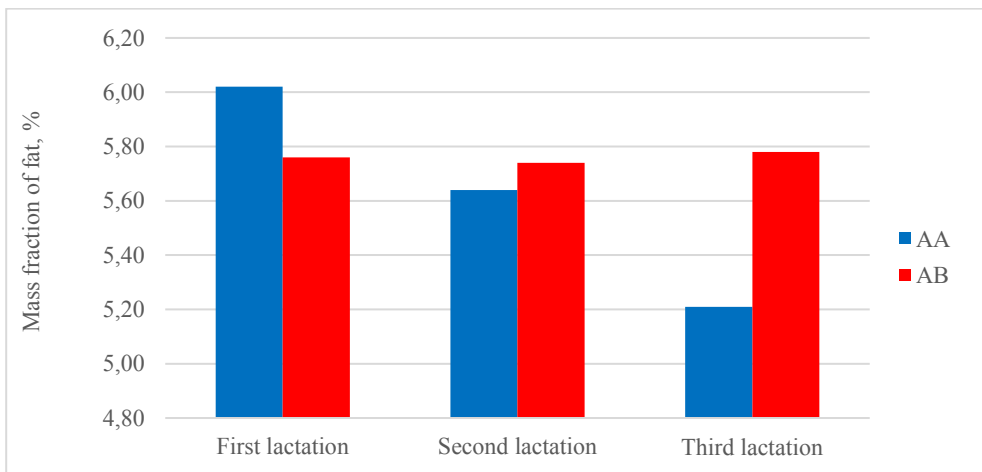
In order for the desired BB genotype to appear in subsequent generations of the herd, it is necessary to start breeding animals with a heterozygous AB genotype. The milk yield of Jersey cows of different genotypes according to kappa-casein (for three lactations) is shown in Figure 1.



**Fig. 1.** Milk yield of Jersey cows of different genotypes by k-Cn.

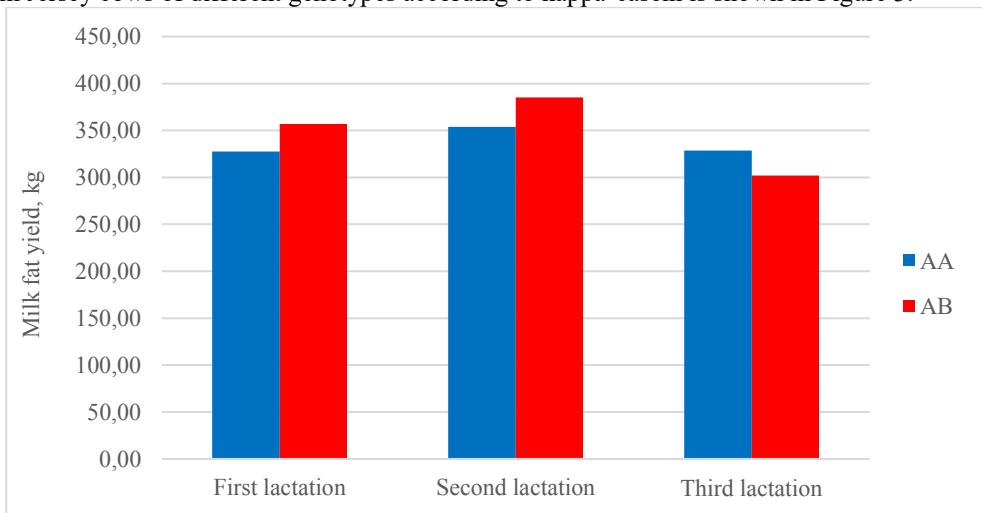
The milk protein yield of Jersey cows with the AA genotype was inferior in the first lactation by 29.05 kg, in the second lactation by 19.87 kg to the group of cows with the AB genotype. However, the difference in indicators for the third lactation of Jersey cows of the AA genotype has grown significantly and amounted to 211.25 kg, which is 37.85 kg more than cows with the AB genotype.

The milk yield of a group of Jersey cows with the CSN3 AA genotype was 754 kg lower for the first lactation, and 433 kg lower for the second lactation for a group of cows with the CSN3 AB genotype. However, the difference in indicators for the third lactation of Jersey cows with the CSN3 AA genotype increased significantly and amounted to 6306 kg, which is 1083 kg more than cows with the CSN3 AB genotype. The mass fraction of milk fat of Jersey cows of different genotypes according to kappa-casein is shown in Figure 2.



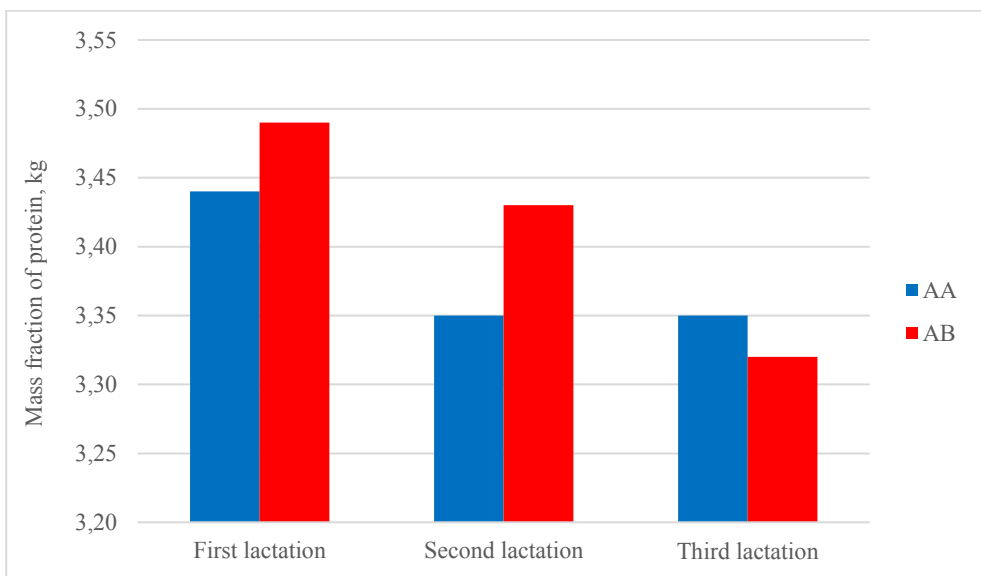
**Fig. 2.** The mass fraction of fat in the milk of Jersey cows of different genotypes according to k-Cn.

The mass fraction of milk fat of Jersey cows of the AA genotype exceeded the group of cows with the AB genotype by 0.26% after the first lactation. However, the difference in indicators for the second and third lactation according to the AA genotype decreased significantly. The difference was 0.1% for the second lactation and 0.57% for the third lactation, relative to the group of Jersey cows with the AB genotype. The yield of milk fat from Jersey cows of different genotypes according to kappa-casein is shown in Figure 3.



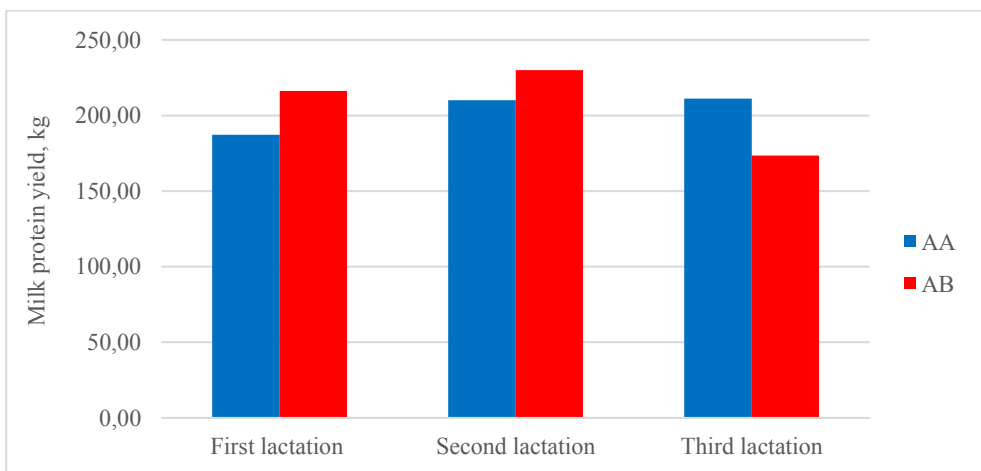
**Fig. 3.** The yield of milk fat in the milk of Jersey cows of different genotypes according to k-Cn.

The yield of milk fat in Jersey cows with the CSN3 AA genotype was inferior in the first lactation by 29.28 kg, in the second lactation – by 31.13 kg to the group of cows with the CSN3 AB genotype. However, the difference in indicators for the third lactation of Jersey cows with the CSN3 AA genotype increased significantly and amounted to 328.54 kg, which is 26.65 kg more than that of analogues with the CSN3 AB genotype. The mass fraction of milk protein of Jersey cows of different genotypes according to kappa-casein is shown in Figure 4.



**Fig. 4.** The mass fraction of protein in the milk of Jersey cows of different genotypes according to k-Cn.

The mass fraction of milk protein of Jersey breed cows with the CSN3 AA genotype was 0.05% lower in the first lactation, and 0.08% lower in the second lactation for the group of cows with the CSN3 AB genotype. However, the difference in indicators for the third lactation of Jersey cows of the CSN3 AA genotype increased significantly and amounted to 3.35%, which is 0.03 kg more than cows with the CSN3 AB genotype. The yield of milk protein of Jersey cows of different genotypes according to kappa-casein is shown in Figure 5.



**Fig. 5.** Milk protein yield in Jersey cows of different genotypes according to k-Cn.

The milk protein yield of Jersey cows with the AA genotype was inferior in the first lactation by 29.05 kg, in the second lactation by 19.87 kg to the group of cows with the AB genotype. However, the difference in indicators for the third lactation of Jersey cows of the AA genotype has grown significantly and amounted to 211.25 kg, which is 37.85 kg more than cows with the AB genotype.

## 4 Discussion of the results

Based on the data obtained, we found out that out of 25 Jersey cows, 15 have the AA genotype, the rest have the AB genotype. The desired BB genotype was not identified. In order to obtain high-quality dairy products and high milk yield, the agrocomplex purposefully improves the breed of cattle we are studying and brings out a more desirable genotype with the help of breeding. Jersey cows with the kappa-casein BB genotype have higher milk protein content and yield. But it's not easy to get it, so first they breed cattle with the AB genotype.

Conclusion. Genotyping using allelic variants of the k-Cn gene will allow in the future to offer a scientifically based selection and selection of the desired genotypes of animals for practical breeding in order to increase the content of milk protein in the herd.

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