

**Research Article**

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**Analysis of the Relationship between Economic and Useful  
Traits of Cattle with Genetic Polymorphism of Alleles  
of Blood Groups and Dairy Proteins**

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**ABSTRACT.**

The analysis of the allele pool of the black-and-white breed showed a high level of occurrence of such alleles of EAV locus as G2Y2E'1Q' O4E'3G", O2A'2J'1K'O', I2 with a frequency of more than 5 %. Allele G2Y2E'1Q' was characterized by the highest frequency of occurrence and that was 20-24 % of the entire allele pool of the population. A study of the dynamics of the allele pool of cows' population in Moscow and Ryazan regions showed a decrease in the total number of alleles together with simultaneous increase of the frequency of allele G 2Y2E'1Q', which is also characteristic of the allele pool of servicing bulls. Some positive correlation between the level of homozygosity and the milk yield for 305 days of the first lactation, when an initial level of homozygosity in the group of more than 10 %, was revealed.

Assessment of the level of genetic variability and structure of genotypes for two polymorphic loci of milk proteins allowed to establish the absence of targeted breeding and genetic measures aimed at selecting animals with the desired genotypes according to these polymorphic proteins. The level of homozygosity of the population in the kappa-casein locus exceeded 63 %, and it was lower but still significant (43 %) in the beta-lactoglobulin locus. There was a tendency to increase milk yield in the first and third lactation of cows with genotype BB at the beta-lactoglobulin locus, and a reduction in the time of coagulation and gelation of casein in the process of coagulation of milk in animals with genotype BB at this locus.

**Keywords:** polymorphic loci, blood groups, cattle, milk proteins, genetic variability.

## INTRODUCTION

From the beginning of the 20th century to the present day, much attention in animal husbandry has been given to the study of such issues as the basic patterns of inheritance and variability of a number of traits in dairy cattle, theoretical substantiation of methods for evaluating animal genotypes and application in the practice of breeding (Ivanov, V.A., Marzanov, N.S., et al., 2017), investigations on the genetics of biochemical properties and blood groups of farm animals (Berdnikova, N.N., 1996; Nekrasov, A.A., 2017, Eremina, M.A., Ezbekova, I.Yu., 2018), studies of immunogenetics of animals and hereditary polymorphism of blood and milk proteins (Bouman, A.S., 2011) and others.

These areas of study allowed further improvement of animal breeding methods (Mescheryakov, V.Ya., 1983; Glazko, V.I., 2013; Nekrasov, R.V., Anikin, A.S., et al., 2017, Mitrofanova O.V., 2017; Bukarov, N.G., 2018), establishment of the forms of inheritance of a number of proteins and erythrocyte antigens, as well as objective evaluation of genetic shifts in populations under the influence of the selection methods used (Eisner, F.F., 1978, 1980 Samusenko, L.D., 2018). In turn, this ensured the selection of descendants with a greater or lesser degree of homozygosity and similarity with outstanding ancestors, and then determining the difference between individual populations of the same breed.

Currently, due to the intensification of animal husbandry and developments in physiology, biochemistry, genetics and other fields of science, questions of managing the flow of genetic material, obtaining organisms with desirable economically valuable traits, avoiding the appearance of congenital pathologies, and preventing the spread of infectious diseases in artificially created livestock populations are of great importance.

The mechanization of farms and the concentration of the livestock required to breed animals with the given parameters, most adapted to the specified production conditions. However, long-term unilateral selection, aimed at reducing phenotypic variability and obtaining animals of the same type, most often led to a decrease in

productivity, fecundity, viability, and other important properties. Therefore, in dairy cattle breeding, it is necessary to determine and maintain a sufficient level of variability in time. Nowadays DNA technology is the main method for improving farm animals (Pozovnikova, M.V., 2011; Asadollahpour Nanaei, H., 2014, Sermyagin, A.A., Ermilov, A.V. et al., 2017; Glazko, V.I., 2017). The effectiveness of the latter depends largely on the correctness of the chosen directions and the accuracy of the genome evaluation.

One of the main directions in this work is the search for markers that allow to identify genotypes of animals with economically useful traits (Glazko, V.I., 1997; Glazko, V.I., 2001; Sermyagin, A.A. et al. 2016; Glazko, V.I., 2016; Mysik, A.T., Tamaev, I.Sh. et al., 2017; Pozovnikova, M.V., 2017; Kostomakhin, N.M., 2018; Popov, N.A., Marzanova, L.K. et al., 2018).

Raising the level of breeding work requires a detailed study of the gene pool and the characteristics of the breed by polymorphic systems, in particular (Popov, N.A., Eskin, G.V., 2000; Kgwatalala, P.M., 2007; Milanese, E., 2007; Rincon, G., 2012; Perchun, A., 2013; Popov, N.A., Marzanova, L.K., 2017).

Recently, the study of genetic polymorphism has become one of the most important and fruitful areas of both fundamental genetics and applied research.

## AIM AND OBJECTIVES

The aim of our research was to evaluate the genetic variability of blood groups and milk proteins in cattle and to determine the relationship of milk production with different genotypes for the loci under study.

To achieve the aim, we set the following objectives:

1. to evaluate the allele pool of servicing bulls and cows of the black-and-white breed by the alleles of the EAV locus of blood groups;
2. to determine the level of genetic variability in the studied population by alleles of the EAV locus;

3. to estimate the relationship between the level of genetic and phenotypic variability of the population for some economically useful traits;
4. to determine the strength of the influence of the turnover of selection types on the level of genealogical homogeneity of the population;
5. to study the relationship of the level of homozygosity for alleles of the EAV locus with economically useful traits and to determine the optimal level of population variability to maximize the positive relationship;
6. to estimate the frequency of occurrence of allelic variants of kappa-casein and beta-lactoglobulin using DNA diagnostics;
7. to evaluate the marker effect of polymorphic loci of milk proteins;
8. to analyze milk production in the context of combinations of beta-lactoglobulin and kappa-casein genotypes;
9. to determine the technological suitability of milk for cheese production.

#### MATERIALS AND METHODS

Experimental studies were performed on 646 cows of the black-and-white breed of Ryazan and Moscow regions in the period from 2006 to 2017. The black-and-white cows were selected according to the principle of analogues, taking into account the breed, productivity, age and live mass. The average milk yield per cow according to the rating was 6,456 kg: the yield of fresh cows was 6,056 kg with a fat content of 3.78 %, the yield of the second lactation cows was 6,516 kg with a fat content of 3.78 % and that of full-aged cows was 6,816 kg with fat mass fraction of 3.82 %. The diet for the animals was prepared of local feed, taking into account productivity and physiological state in accordance with the norms of All-Russian Research Institute of Livestock and Russian Academy of Science.

Groups of servicing bulls ( $n = 215$ ) having immunogenetic certification were formed according to their lineage using catalogs of stations for artificial insemination, taking into account blood groups, productivity of maternal ancestors, and assessment of the quality of offspring.

The formation of groups of first-calf cows belonging to 4 lines and 355 families was

carried out taking into account the parameters of the exterior and the genealogical homogeneity of the cows of the leading group, which included cows with milk yield exceeding the herd parameters by  $+ \sigma$  (standard deviation).

The first experiment was to study the effect of allelic forms of the genes of the blood group of the EAV locus on the productive qualities and homogeneity in related groups of animals.

The second experiment was connected with studying the effect of allele variability in the loci of blood groups on the economic and breeding value of representatives of lines. These studies were carried out in the laboratory of animal genetics of the All-Russian State Research Institute of Livestock.

The authenticity of the descendants of the experimental groups was determined by the method of Matoushek, Y. (1964), based on the blood group systems. Alleles were isolated in a family analysis according to the methods of Sorokovskiy, P.F. (1981, 1984).

The coefficients of regression ( $R$ ) and correlation ( $r$ ) were determined by Merkur'yeva, E.K. (1971).

To evaluate the genealogical structure of the studied groups of livestock, the genealogical homogeneity formula proposed by Eisner, F.F. and modified by Alexandrova, A.I., Terebilo, N.A. and Filimonova, S.S. was used:

$$V = \frac{7n - x}{7n} \times 100\% \quad [1]$$

where  $V$  – is the coefficient of genealogical homogeneity for a group of animals,  
 $n$  is the number of animals in the group,  
 $x$  is the number of different bull nicknames in pedigrees.

And the formula of the relative share of one bull in total genealogical homogeneity was also used:

$$D = \frac{n}{x} \times 100\% \quad [2]$$

where  $D$  is the proportion of one bull in the total genealogical homogeneity,  
 $n$  is the number of animals in the group,  
 $x$  is the number of different bull nicknames in pedigrees.

The level of homozygosity in the group was calculated based on Hardy-Weinberg algorithm (1908):

$$Ca = q_1^2 + q_2^2 + q_3^2 + \dots + q_n^2, \quad [3]$$

where  $q_1, q_2, q_3, q_n$  are allele frequencies.

Student's criterion was used to compare the productivity of animals of specific alleles and antigens.

The third experiment included genotyping of cows for kappa-casein and beta-lactoglobulin, which was carried out in the laboratory of DNA technologies of the All-Russian Research Institute of Breeding. Nuclear DNA was determined in the cattle blood by the conventional phenol-detergent method (Blin, N., Stafford, D.W., 1976). The following primers were used to amplify a fragment of the 4th exon of the kappa-casein gene (Denicourt, D. et al., 1990):

Bocas A: 5' –

ATAGCCAAATATATCCCAATTCAGT 3'

Bocas B: 5' –

TTTATTAATAAGTCCATGAATCCTG 3'

## RESULTS AND DISCUSSION

Several related European breeds actively participated in the formation of the genotype of the investigated population of cattle. An assessment of the allele type of the servicing bulls, carried out on the basis of FSUE "Moskovskoe" and FSUE Central Station of Artificial Insemination (CSAI), showed the presence of 62 different alleles in the genotype, including alleles of German black-and-white, British-Friesian and Dutch breeds. It should also be noted that according to data obtained by other researchers (Popov, N.A., Eskin G.V., 2000, Popov N.A., Marzanova L.K., 2017; Popov N.A., Marzanova L. K., 2018), allele type of this population is somewhat wider than that found by us. Thus, they allocated 108 alleles of the EAV locus in the population of the black-and-white breed, and 71 alleles in the Holstein black-and-white breed. The level of homozygosity of the population ( $Ca$ ), calculated by the alleles of the EAV-locus, ranged from 7.6 to 9.1 %.

Analysis of the gene pool of servicing bulls showed a high level of occurrence of such alleles of the EAV locus as  $G_2Y_2E_1Q'$  (0.2488),

$O_4E_3G''$  (0.0698) and  $O_2A_2J_1K'O'$  (0.0512) in bulls of FSUE "Moskovskoe" and  $G_2Y_2E_1Q'$  (0.2033),  $I_2$  (0.0933) and  $O_4E_3G''$  (0.0633) in bulls of CSAI. Allele  $G_2Y_2E_1Q'$  had the highest frequency of occurrence – 20-24 % of the total population allele pool (Table 1).

**Table 1** – Frequency of occurrence of the most common EAV-locus alleles of the servicing bulls

No.	Alleles of EAV-locus	Herd frequency	
		$q, n=215$	$q^2$
FSUE "Moskovskoe"			
1	$G_2Y_2E_1Q'$	0.2488	0.06192
2	$O_4E_3G''$	0.0698	0.00487
3	$O_2A_2J_1K'O'$	0.0512	0.00262
FSUE CSAI			
1	$G_2Y_2E_1Q'$	0.2033	0.04134
2	$I_2$	0.0933	0.00871
3	$O_4E_3G''$	0.0633	0.00401

Therefore, there is a high probability of a sharp increase in the level of this allele in breeding and commercial herds of Moscow Region, which in turn, may lead to a decrease in the overall genetic diversity of the herd gene pool.

To confirm this assumption, studies were conducted in several farms of Ryazan and Moscow regions. The most typical situation for the region is noted in the State Unitary Enterprise, Production and Scientific Association "Poyma" and APC Named after Lenin, , Moscow Region.

So, when evaluating the allele pool of first-calf cows in the State Unitary Enterprise "Poyma", Moscow Region, 61 alleles of the EAV locus were found, among which alleles characteristic mainly of Holstein and Dutch black-and-white cattle breeds were found. The average group homozygosity was within 10 %, which was a little more than in the population of servicing bulls. Studying the dynamics of the herd allele pool showed a decrease in the total number of alleles, which was an indirect confirmation of our assumption about a decrease in the genetic diversity of the allele pool of the studied population. It is necessary to note the high frequency of occurrence of the  $G_2Y_2E_1Q'$  allele, which is characteristic of the allele pool of the servicing bulls of the Moscow population. The level of this allele in first-calf cows at the farm was 22.3 %, which was quite comparable with its level in the servicing bulls' population.

Similar studies conducted in APC Named after Lenin, reflected a similar situation. Thus, in the genotype of first-calf cows, only 43 alleles were detected, which was also less than found in earlier studies.

The fathers of the studied population of first-calf cows in APC Named after Lenin (n = 109) were 13 servicing bulls, while the fathers of their contemporaries from the state unitary enterprise "Poyma" (n = 390) were only 7 bulls. Such a relatively large number of bulls used for a relatively small population of cows in APC Named after Lenin was the main reason for the average level of homozygosity in the group of heifers of this farm was lower - only about 6.7 %, and the frequency of occurrence of the most common allele G<sub>2</sub>Y<sub>2</sub>E<sub>1</sub>Q' was only 18.9 %. These data suggest a fairly large herd uniformity. Therefore, without a targeted selection of servicing bulls, the level of herd uniformity will increase, and the frequency of occurrence of common alleles, such as G<sub>2</sub>Y<sub>2</sub>E<sub>1</sub>Q', O<sub>4</sub>Y<sub>2</sub>A<sub>1</sub>I', I<sub>2</sub>, O<sub>4</sub>D'E<sub>3</sub>F<sub>2</sub>G'O', O<sub>2</sub>A<sub>2</sub>J<sub>1</sub>K'O', O<sub>4</sub>E<sub>3</sub>G" and Q'I" will steadily increase. To check the results, the studies were conducted in other farms, where the situation was characterized by a very similar tendency.

The decrease in genetic variability resulted in an increase in the phenotypic homogeneity of the herd according to its economically beneficial traits (Table 2).

**Table 2** – The level of homozygosity and phenotypic variability of economically useful traits

Ca	Ratios of variability, %	
	Milk yield for 305 days of lactation	Fat content in milk
0.100	15.90	2.58
0.067	17.62	7.93

Parameters of genetic and phenotypic variability are characterized by one trend. Artificial restriction of the spectrum of alleles and a decrease in the overall genetic variability of the herd have led to an increase in the phenotypic homogeneity of the herd, while prolonged linear breeding without genetic variability in the blood group alleles develops a tendency to decrease the level of homogeneity.

To characterize the level of herd uniformity, it is helpful to study the pedigrees and determine the

level of genealogical homogeneity of the studied populations. Thus, in the course of the study, the level of homogeneity and the proportion of one bull in the total genealogical homogeneity in the groups according to the turnover of maternal types of selection were evaluated (Table 3).

**Table 3** – The impact of changing the types of selection on the level of genealogical homogeneity of the population

Group of animals by change of species selection	Genealogical homogeneity, %	The proportion of a bull in the total genealogical homogeneity of the group, %
Double linear	82.9	83.3
Double cross	88.3	122.1

The level of genealogical homogeneity with double cross of lines is about 5 % higher than with interlinear selection. The same tendency was found when calculating the relative homogeneity of the group in the proportion of one bull, since the number of descendants in the group of first-calf cows obtained by the double cross method exceeded the number of bulls' names in three rows of their pedigrees. These findings were also confirmed by the results of determining homogeneity by genetic markers: the level of homogeneity for the first-calf cows of this group was 11.8 % higher than parameters of herd mates.

The highest degree of genealogical homogeneity was characteristic for the groups of cows of alleles G<sub>2</sub>Y<sub>2</sub>E<sub>1</sub>Q' (86 %), O<sub>4</sub>D'E<sub>3</sub>F<sub>2</sub>G'O' (84 %) and O<sub>4</sub>E<sub>3</sub>G" (81 %), which also confirmed the previously presented conclusions about the relationship of genetic and genealogical homogeneity in the group (Table 4).

**Table 4** – The level of genealogical homogeneity of cows of the most common alleles of the EAV locus

Alleles of EAV-locus	F	Genealogical homogeneity, %	The proportion of a bull in the total genealogical homogeneity, %
G <sub>2</sub> Y <sub>2</sub> E <sub>1</sub> Q'	130	86.3	81.8
O <sub>4</sub> D'E <sub>3</sub> F <sub>2</sub> G'O'	93	84.5	58.5
O <sub>4</sub> E <sub>3</sub> G"	79	80.7	49.7
O <sub>2</sub> A <sub>2</sub> J <sub>1</sub> K'O'	42	75.5	26.4

The calculation of the correlation coefficient showed a strong positive relationship between these parameters (r = 0.935).

For cattle breeding it is desirable to know the optimal level of homogeneity when breeding a group of animals, in which you can get the maximum return, expressed in an increase in parameters of economically useful traits. To do this, you first need to track the relationship of the level of genealogical and genetic homogeneity with the production characteristics of animals.

When studying the relationship of the level of homozygosity in the EAV locus alleles with economically useful traits, a positive relationship was found between an increase in the level of homozygosity and milk yield in the group of first-calf cows of SUE PNO "Poyma" ( $r = 0.9114$ ) with a population homozygosity of 0.100. In contrast to SUE PNO "Poyma", in farms characterized by a lower level of homozygosity of the herd, there was a slight negative relationship between these parameters ( $r = -0.221$ ).

Thus, a trend of positive correlation between the level of homozygosity and the amount of milk yield for 305 days of the first lactation when the initial level of homozygosity in the group of more than 10 % was revealed. When the level of homozygosity was below 10 %, the correlation between these parameters was negative.

For a more detailed study of the influence of genetic homogeneity on the economically valuable traits, 4 groups of first-calf cows were formed, depending on the degree of prevalence of EAV locus alleles. Significant differences in the productivity of both mothers and first-calf cows were not identified, but, nevertheless, we can note a tendency to decrease in the level of productivity when a decrease in the level of homozygosity in the group. In addition, there was a tendency to decrease the height at the withers and the length of the posterior third of the body in the group with a high level of homozygosity.

The optimal parameters of lactation usefulness and the duration of the service period were found in the group with an average level of homozygosity close to the average of the herd ( $Ca = 0.110$ ), which suggests that at this level of selection homozygosity of 11 % is close to the optimal one. At the same level of homozygosity,

the greatest correlation was noted for the yield of mothers and daughters, which characterizes this group as the most adapted to the existing conditions of breeding.

With an increase in the level of homozygosity, the relationship with productivity for 305 days of lactation decreased. The correlation coefficient ( $r$ ) was 0.678 when  $Ca = 0.098$  and  $r = -0.6402$  when  $Ca = 0.304$ . The same tendency was observed in the yield of milk fat for lactation.

Determining the division of cows according to the phenotype expressed in terms of productivity, it was intended to identify different levels of homozygosity in groups of animals with different productivity, as well as its different relationship with the economically useful traits of the cattle. To do that correlation coefficients between groups of cows with different productivity and main economic traits were determined.

With an increase in productivity, the relationship between the level of homozygosity and such traits as lactation usefulness, milk yield per lactation and for the first 305 days, the amount of milk fat, as well as such linear measurements as the width and depth of the chest and girth behind the shoulder blades, increased.

In the group of cows characterized by relatively low productivity, a positive relationship was determined between milk yield and height measurements and the development of the rear part of the body.

The conducted studies suggest that the relationship between the level of genetic and genealogical variability with economically valuable traits is not straightforward. It largely depends on the level of milking of the considered groups and the characteristics of the trait itself. This implies the assumption that, under these conditions and at a given level of selection, a further increase in the level of homogeneity in the group can lead to the separation of a number of characters and their further independent selection. This assumption is confirmed by the calculated regression coefficients between the level of homogeneity in the group and the main breeding characteristics of first-calf cows. Calculations showed that a

further increase in the level of genealogical homogeneity in the group of first-calf cows differing in relatively low productivity can lead to 1 % increase in milk yield by 38.2 kg of milk per lactation, in the group of cows characterized by an average productivity of 23.9 kg, and in the group of highly productive cows, it may decrease by 78.9 kg. The same situation was noted in the study of parameter "lactation evenness". The optimal level for this parameter was a level of genealogical homogeneity equal to 51.2 %, which is much lower than in the first-calf group under study (79.6 %). The influence of the level of genealogical homogeneity on other economically useful traits was not so significant.

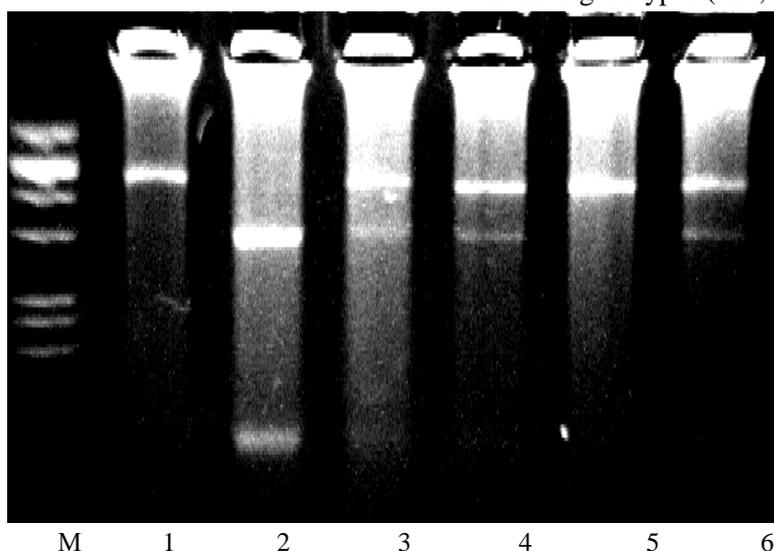
Not only blood types, but also polymorphic loci of milk proteins, such as kappa-casein and beta-lactoglobulin can be viewed as economic characters of the cattle (Glazko, V.I., Zhuravel,

Ye. V., 1997; Gallyamova, A., 2008; Bouwan, AC, Bovenhuis, H., 2011).

To analyze the polymorphism of allelic variants of the kappa-casein gene, blood was taken from 79 of cows in this population, and DNA fragments were recovered from the material under study.

To amplify the kappa-casein gene containing mutation, primers Bocas A and Bocas B were taken (Denicourt, D. et al., 1990), synthesized on the basis of the DNA nucleotide sequence of the kappa-casein gene in cattle. They were homologous to the highly conserved kappa-casein gene. Fragments of the kappa-casein gene with a length of 530 para nucleotides were obtained from all DNA preparations. The length of all experimental amplicate samples was identical, which indicates high specificity.

In the experimental DNA samples, 2 alleles of the kappa-casein gene were detected: A and B with three genotypes (AA, AB, BB) (Figure 1)



**Figure 1** - Restriction of amplicates (track number 1, 2, 3, 4, 5, 6)

No. 1 — AA genotype, No. 2 — BB genotype, No. 3, 4 — AB genotype, No. 5 — AA genotype, No. 6 — AB genotype.

The AA genotype was distinguished by the highest frequency of occurrence of the kappa-casein gene in the polymorphic locus. It was observed in approximately 58 % of the animals examined. The rarest genotype in this locus was the BB genotype — only about 5 % of the animals examined (Table 5). Thus, the level of homozygous animals as for the kappa-casein locus varied within 63 %, which constitutes a significant part of the studied population. The level of heterozygous genotypes was only 36-37 %, which indicates a high level of population uniformity as for this polymorphic locus.

Calculation of the frequency of occurrence of individual marker genes of kappa-casein showed a high homogeneity of the population of allele A and its frequency of occurrence was in the range of 0.77.

**Table 5** – Polymorphism of the kappa-casein gene of the studied population

Distribution	Genotype frequency, %			Allele frequency		$\chi^2$
	AA	AB	BB	A	B	
Observed distribution	58.23	36.71	5.06	0.77	0.23	0.06
Expected distribution	59.49	35.45	5.06			

To identify deviations of the empirical frequency distribution from the theoretical one, the Hardy-Weinberg law was applied, as well as the  $\chi^2$  method, which led to the conclusion that in the studied population of animals there was no statistically significant genetic equilibrium shift in any of the 3 genotypes. This means that in this population there was no artificial selection affecting the genotypes of animals as for the kappa-casein gene locus.

For a clearer understanding of the genetic structure of the population as for the kappa-casein locus, a comparative study of the frequency of occurrence of genotypes of this locus was conducted, depending on the selection methods used. Purebred and crossbred animals of the studied farms were to be compared (Table 6).

**Table 6** – Polymorphism of the kappa-casein gene depending on the selection methods used

Groups of cows studied	Genotype frequency, %			Allele frequency	
	AA	AB	BB	A	B
Purebred	63.16	31.58	5.26	0.79	0.21
Crossbred (from bulls-improvers)	56.67	38.33	5.00	0.76	0.24
d	-6.49	+6.75	-0.26	-0.03	+0.03

When using interbreeding with bulls-improvers, there was a tendency to increase the incidence of heterozygous genotypes by 6.8 %, which directly indicates an increase in the genetic variability of the population, as this should occur with this type of crossing. At the same time, the frequency of kappa-casein alleles slightly changed and there was a tendency to an increase in the frequency of gene B, which varied within 0.24, which reflects the situation characteristic of Holstein cattle. So, according to Schmidt, R.G. (1964), the frequency of the B allele in Holstein cattle is 0.15, according to Woychik, J.H. (1965) it is 0.26 and according to Pinder, S.J. with co-authors (1991) it is 0.20. But, nevertheless, the allelic variant A of the kappa-casein gene predominates in both crossbred and purebred cattle, and the frequency of the kappa-casein B allele was significantly lower.

The beta-lactoglobulin locus identified two allelic genes A and B and 3 genotypes (AA, AB and BB) (Table 7).

**Table 7** – Beta-lactoglobulin gene polymorphism

Distribution	Genotype frequency, %			Allele frequency		$\chi^2$
	AA	AB	BB	A	B	
Observed distribution	3.45	56.89	39.66	0.32	0.68	5.82
Expected distribution	10.34	43.11	46.55			

The frequency of homozygous genotypes in this polymorphic locus of milk proteins is much lower than in the kappa-casein - only about 43 % versus 63 %. This indicates a greater degree of genetic variability in the population for this locus. Allele B was the most common one with a frequency of 0.68.

However, no statistically significant shift in genetic equilibrium was found for any of the 3 locus genotypes, which also indicates the absence of directed selection for this polymorphic locus.

Thus, the assessment of the level of genetic variability and the structure of genotypes for two polymorphic milk protein loci made it possible to establish the absence of targeted selection and genetic measures aimed at selection and breeding of animals with the desired. Nevertheless, such work is currently an extremely important task, since numerous studies have established the interrelation and the

marker effect of these loci and the main economically useful traits (Woychik, J.H. 1965; Threadgill, D.W., Womack, J.E., 1990; S.J. Pinder, B.N. Perry, C.J. Skidmore, D. Sawa, 1991; Ng-Kwai-Hang, K.F., 1993; Gallyamova, A., 2008; Nekrasov, A.A., Popov, N.A., et al., 2017; Eremina, M.A., Ezdakova, I.Yu., 2018;).

Evaluation of the marker effect of polymorphic loci of milk proteins was carried out according to several productive signs. There was a tendency to increase the milk yield for the first lactation of cows with the genotype BB at the beta-lactoglobulin locus. The animals exceeded their peers with the AA and AB genotypes by 232 and 178 kg, respectively. In lactation 3, the superiority in milk yield of cows with the BB genotype was 179 kg compared to AA and 167 kg - with AB. On the other hand, heterozygous animals were characterized by the stability of the mass fraction of fat during three lactations - from 3.68 to 3.70 %, and with an increase in milk yield, there was also a slight increase in milk fat content. According to the results of lactation 3 in homozygote cows, the fat content in milk decreased by 0.01– 0.32 %, respectively. The dynamics of milk fat in experimental animals was similar to the change in milk yield.

Analysis of milk production in the context of combinations of beta-lactoglobulin and kappa-casein genotypes (Tables 8-9) showed that animals with a combination of the kappa-casein AA genotype were the ones, that most intensely began to yield more milk. They exceeded their peers by milk yield for three lactations by 61-444 kg, respectively, but they were less than 0.06-0.09 % in mass fraction of fat (MFF) in milk, while milk fat yield (MFY) was also higher in them. In cows that combined the beta-lactoglobulin AB genotype and kappa-casein AA, the fat content in milk increased with milk yield.

**Table 8** – Milk productivity of cows with different combinations of AB beta-lactoglobulin and kappa-casein genotypes

Parameter	Kappa-casein genotype		
	AA	AB	BB
Lactation 1			
Yield, kg	3,393±119	3,332±238	2,850
MFF, %	3.64±0.04	3.72±0.05	3.80
MFY, кг	123.4±4.1	123.9±8.8	108
Lactation 2			
Yield, kg	3,941±105	3,844±269	-
MFF, %	3.67±0.05	3.76±0.06	-
MFY, кг	144.7±4.6	143.9±8.9	-
Lactation 3			
Yield, kg	4,146±104	3,702±108	-
MFF, %	3.71±0.05	3.77±0.05	-
MFY, кг	153.7±4.2	139.3±4.0	-

A slightly different dynamics of productivity parameters was observed in cows with a combination of beta-lactoglobulin AB and kappa-casein genotypes.

By the third lactation, animals that combined the BB beta-lactoglobulin genotype and kappa-casein AB began to yield more milk. Their milk yield was 1,380 kg, and the milk fat yield was 42 kg, but the mass fraction of fat in milk decreased by 0.19 %.

The herdsmates with the kappa-casein BB genotype due to their low milk yield were distinguished by the lowest milk fat yield. At the same time, they exceeded the analogues with the AA genotype in the mass fraction of fat in milk for the first and second lactations by 0.09-0.10 %, and by the third lactation, they showed the best result - 3.71 %.

Animals with the kappa-casein AA genotype, having high milk yield with low milk fat, were intermediate in total milk fat yield.

**Table 9** – Milk productivity of cows with different combinations of genotypes of beta-lactoglobulin BB and kappa-casein

Parameter	Kappa-casein genotype		
	AA	AB	BB
Lactation 1			
Yield, kg	3,653 ± 183	3,080 ± 238	3,066 ± 249
MFF, %	3.60 ± 0.03	3.74 ± 0.04	3.69 ± 0.02
MFY, кг	131.6 ± 6.2	115.0 ± 8.1	113.0 ± 9.5
Lactation 2			
Yield, kg	3,839 ± 217	3,976 ± 481	3,478 ± 200
MFF, %	3.64 ± 0.03	3.75 ± 0.15	3.74 ± 0.01
MFY, кг	144.0 ± 7.6	148.0 ± 14.5	130.0 ± 7.0
Lactation 3			
Yield, kg	4,295 ± 160	4,460 ± 560	3,859 ± 8.0
MFF, %	3.56 ± 0.06	3.55 ± 0.11	3.71 ± 0.07
MFY, кг	152.8 ± 5.4	157.0 ± 14.7	143.0 ± 3.0

Thus, to obtain whole-milk products, preference should be given to animals combining the genotype of beta-lactoglobulin BB and kappa-casein AB. Milk of cows with AB genotypes for beta-lactoglobulin and kappa-casein is more expedient to use for fat-and-milk products.

Technological suitability of milk for cheese production is often determined by the duration of its coagulation under the action of rennet, which, according to numerous studies, ranges from 15 to 35 minutes (Ovsyannikova, GV, Kopyrina, L.Yu., 2014). A decrease in clotting time indicates greater technological suitability of milk for cheese making (Table 10).

**Table 10** – Genetic dependence of the duration of milk coagulation stages

Genotype of cows on the kappa casein locus	The duration of the phase, min		
	Coagulation	Gelation	Clotting
AA	7.0 ± 0.6	26.7 ± 1.4	33.7 ± 2.03
AB	5.7 ± 0.3	21.7 ± 1.2	27.3 ± 1.45
BB	5.0 ± 1.0	20.0 ± 2.0	25.0 ± 1.99
AA to AB	+ 1.3	+ 5.0*	+ 6.3*
AA to BB	+ 2.0	+ 6.7*	+ 8.7*

Thus, some reduction in the casein coagulation time in animals with the BB genotype at this locus was detected, and in the gelation phase, the genetic dependence of the technological trait manifested itself most clearly: this phase in cows with the BB genotype was 6.7 minutes shorter ( $P < 0.05$ ), than in animals with the AA genotype. The level of clotting of milk from heterozygous for the kappa-casein locus cows occupied an intermediate position (27.3 min), which suggests the inheritance of this trait on the principle of incomplete dominance.

Thus, the conducted studies have once again proved the marker effect of polymorphic loci of blood groups and milk proteins in relation to the main production characteristics of cattle, as well as the effect of the level of genetic variation of the population on the degree of their manifestation.

## CONCLUSIONS

1. Several related European breeds actively participated in the formation of the genotype of the investigated population of cattle. An evaluation of the allele type of the servicing bulls of the Moscow region population showed the presence of 62 different alleles in the genotype, including the alleles of the German black-and-white, British-Friesian and Dutch breeds.

2. The level of homozygosity of the population (Ca), calculated by the alleles of the EAB locus, ranged from 7.6 to 9.1 %. The analysis of the allele pool of the black-and-white breed of the Moscow cattle population showed a high level of occurrence of such alleles of the EAB locus as  $G_2Y_2E'_1Q' O_4E'3G''$ ,  $O_2A'_2J'_1K'O'$ ,  $I_2$  with a frequency of more than 5 %. The  $G_2Y_2E'_1Q'$  allele was characterized by the highest

frequency of occurrence - 20-24 % of the total allele pool of the population.

3. Parameters of genetic and phenotypic variability are characterized by one trend. Artificial restriction of the spectrum of alleles and a decrease in the total genetic variability of the herd led to an increase in the phenotypic homogeneity of the herd, while prolonged linear breeding without considering genetic variability in the alleles of blood groups develops a tendency to reduce the level of herd homogeneity.

4. The level of genealogical homogeneity with a double cross of lines is about 5 % higher than with interlinear selection. The same tendency was found when calculating the relative homogeneity of the group in the proportion of one bull, since the number of descendants in the group of first-calf cows obtained by the double cross method exceeded the number of bulls' names in three rows of their pedigrees. These findings were also confirmed by the results of determining homogeneity by genetic markers. The level of homogeneity in the first-calf cows of this group was higher, than that of their herdmates by 11.8 %.

5. The tendency of positive correlation between the level of homozygosity and the milk yield for 305 days of the first lactation at the initial level of homozygosity in the group of more than 10 % was revealed. When the level of homozygosity was below 10 %, the correlation between these parameters was negative. Optimal parameters of the usefulness of lactation and the duration of the service period were found in the group with an average level of homozygosity close to the average one of the herd ( $C_a = 0.110$ ), which suggests that at this level of selection homozygosity of 11 % is close to optimum. At the same level of homozygosity, the greatest correlation was noted for the milk yield of mothers and daughters, which characterizes this group as the most adapted to the existing conditions of breeding. With an increase in the level of homozygosity, the relationship with productivity for 305 days of lactation decreased. When  $C_a = 0.098$ , the correlation coefficient was  $r = 0.678$ , and when  $C_a = 0.304$ ,  $r = -$

0.6402. The same tendency was observed in milk fat yield for lactation.

6. The method of DNA diagnostics in the cow population revealed three genotypes for the kappa-casein gene locus - AA, AB and BB. The AA genotype was distinguished by the highest frequency of occurrence of the kappa-casein gene in the polymorphic locus. It was observed in approximately 58 % of the animals examined. The rarest genotype in this locus was the BB genotype — only about 5 % of the animals examined. The level of heterozygous genotypes was only 36-37 %, which indicates a high level of population homogeneity in this polymorphic locus. The calculation of the frequency of occurrence of individual marker genes of kappa-casein showed a high homogeneity of the population of allele A. Its frequency of occurrence was in the range of 0.77. The beta-lactoglobulin locus also identified two allelic genes A and B and 3 genotypes (AA, AB and BB). The frequency of homozygous genotypes in this polymorphic locus of milk proteins was much lower than in the kappa-casein locus - only about 43 % versus 63 % in the kappa-casein locus. This indicates a greater degree of genetic variability in the population for this locus. The most common allele was B with a frequency of 0.68.

7. Evaluation of the marker effect of polymorphic loci of milk proteins was carried out according to several productive signs. There was a tendency to increase the milk yield for the first lactation of cows with the genotype BB at the beta-lactoglobulin locus. The animals exceeded their herdmates with the AA and AB genotypes by 232 and 178 kg, respectively. In lactation 3, the superiority in milk yield of cows with the BB genotype was 179 kg as compared to AA and 167 kg as compared to AB. Heterozygous animals were characterized by the stability of parameters of the mass fraction of fat during three lactations - from 3.68 to 3.70 %, and with an increase in milk yield, there was also a slight increase in milk fat content. According to the results of 3 lactations, the fat content in milk of homozygote cows decreased by 0.01– 0.32 %, respectively. The dynamics of

milk fat in experimental animals was similar to the change in milk yield.

8. The analysis of milk productivity in the context of combinations of beta-lactoglobulin and kappa-casein genotypes showed that animals with a combination of the kappa-casein AA genotype began to yield more milk most intensively. They exceeded their herdmates by milk yield for three lactations by 61-444 kg, respectively, but they had 0.06-0.09 % less in mass fraction of fat in milk, while milk fat yield was also higher in them. In cows that combined the beta-lactoglobulin AB genotype and kappa-casein AA, the fat content in milk increased with milk yield.

A slightly different dynamics of productivity parameters was observed in cows with a combination of beta-lactoglobulin AB and kappa-casein genotypes. By the third lactation, animals that combined the genotype of beta-lactoglobulin BB and kappa-casein AB began to yield more milk. Their milk yield was 1,380 kg, and the milk fat yield was 42 kg, but the mass fraction of fat in milk decreased by 0.19 %. The herdmates with the kappa-casein BB genotype due to their low milk yield were distinguished by the lowest milk fat yield. At the same time, by the mass fraction of fat in milk for the first and second lactations, they exceeded the analogues with the AA genotype by 0.09-0.10 %, and by the third lactation they showed the best result equal to 3.71 %. Animals with the kappa-casein AA genotype, combining high milk yield with low milk fat, were intermediate in total milk fat yield.

9. Some reduction in the coagulation time of casein in animals with the BB genotype at this locus was detected, and in the gelation phase the genetic dependence of the technological trait manifested itself most clearly. So, this phase in cows with the BB genotype was 6.7 minutes shorter ( $P < 0.05$ ), than in animals with AA genotype. The level of clotting of milk from heterozygous for the kappa-casein locus cows occupied an intermediate position (27.3 min), which suggests the inheritance of this trait on the principle of incomplete dominance.

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