

## MARKER SELECTION IN ANIMAL HUSBANDRY AND POULTRY FARMING

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### **Abstract**

*This article provides data on candidate genes CS3, CS2, BLG, LALBA as molecular markers for predicting milk productivity, technological properties of milk in cattle of Red Estonian, black-mottled Belarusian and Moldovan breeds, Karakul sheep breed, and also, OV, TFR loci in chickens of various breeds. The marker selection makes it possible to identify animals with valuable genes and preserve them in the population, as well as to look for a relationship with economically useful traits. The disadvantage of this method today is the high cost of research. Analyzing the relationship of candidate genes with productive qualities, it can be seen that the genotypes beneficial for one breed are not those for another breed. This is due to the degree of absorption of the breed - founder/bull in crosses and breeding 'in itself', etc. Nevertheless, in our opinion, valuable kappa-casein alleles are introduced by individuals of the Holstein and Simmental breeds, which are of the greatest value for breeding.*

**Key words:** *genotype, loci QTL, marker genes, productivity, selection.*

### **INTRODUCTION**

The formation of genomics in the 80's of the XX century led to the development of marker selection based on the identification and targeted selection of genes/alleles that influence quantitative traits important for agriculture. Thanks to the development and application of molecular marker technologies and statistical methods of data analysis, a large-scale research was carried out for quantitative trait loci (QTL) - DNA sequences containing genes or regions linked to them that have significance on the effect of a quantitative trait. Such loci were genetically mapped in all farm animals and poultry for a large number of quantitative traits characterizing productivity, fertility and the evolution of various genetic diseases (Kiser et al., 2017; Cai et al., 2019).

Marker selection is a promising branch in breeding, which makes it possible to reliably determine not only the genotype of animals and their productivity, but also to identify pathological mutations in genes, in connection with which it is possible to assess the risk of

hereditary diseases, to make it possible to carry out specific reactions of the body to foreign cells, and to determine the causes of infertility of females (Shushpanova, 2021).

In particular, in cattle, QTL were mapped, which have a significant impact on the development of leukocyte adhesion deficiency syndrome, leading to the death of young animals as a result of immune deficiency, leukemia, scrapes, spongiform encephalopathy encephalopathy and many others (Hu et al., 2019).

In poultry farming, modern breeding technologies use DNA markers (single nucleotide polymorphisms (SNPs), which help identify QTL associated with egg-laying traits. For example, in the publication of Barkova (2021), data obtained using genome-wide association search (GWAS) for the selection of QTL and candidate genes that affect egg production, weight, strength and thickness of eggshell are presented to create a QTL system that is responsible for egg productivity of laying hens and egg quality.

Introducing DNA markers into the practice of animal husbandry were gradually identified

possible growth points in this direction. It shows how the genomic assessment of breeding value is constructed, what are the key conditions necessary for its implementation, as well as the advantages and limitations of genomic and marker selection. Thus, there was a real possibility of rational use of the genetic potential of farm animals (Stolpovsky et al., 2020).

In sheep breeding, for example, it is possible to increase the accuracy of the assessment and selection of animals, and hence the effect of breeding, only if there are reliable methods for determining economically useful traits. (Selionova et al., 2020). It was found that the milk productivity of Lacon sheep of the *GDF9ag* genotype is higher compared to animals carrying homozygous genotypes. The sheep with the homozygous *GDF9GG* genotype surpass sheep carriers of the *GDF9Ag* and *GDF9A* genotypes in terms of the amount of fat and protein in milk (Selionova et al., 2021). The article presents a synthesis of molecular studies for the period 2000-2020 in the research for promising marker genes in cattle breeding, sheep breeding and poultry farming.

## MATERIALS AND METHODS

The studies were conducted on the Holstein, Estonian Red, Belarusian Black and White, and Simmental cows; then in the sheep of the Karakul breed; further in the chickens of the Marans, Orpington, and Plymouth Rock breeds. Polymorphic systems *CS3*, *CS2*, *BLG*, *LALBA*, *OV*, *TFR* were determined by horizontal method of electrophoresis according to Smithies (1955) and Zhebrovsky (1979), and by method PCR.

The assessment of gene polymorphism was carried out by amplification of gene fragments *CSN3* using primers:

CAS1 5'-ATA GCC AAA TAT ATC CCA ATT CAG T-3'

CAS2 5'-TTT ATT AAT AAG TCC ATG AAT CTT G-3';

gene *LALBA*:

LAC 1: 5'- AAGAGTTGGATGGAATCACC - 3';

LAC 2: 5'- TTCAAATTGCTGGCATCAAGC- 3'

gene *BLG*:

LG 1: 5'-TGTGCTGGACACCGACTACAAAAAG - 3'

LG 2: 5'-GCTCCCGGTATATGACCACCCTCT- 3'

## RESULTS AND DISCUSSIONS

Among the many genes that control milk productivity and milk quality, we can single out

a group of major genes that make the greatest contribution to the formation and functioning of this quantitative trait. Between such genes include the kappa casein gene *CSN3*, which is one of the genetic markers associated with qualitative and quantitative signs of dairy productivity of animals.

It is known that animals with the *CSN3<sup>BB</sup>* genotype are characterized by high protein-milk content, their milk has good cheese-making qualities (Zyryanova, 2021).

In our experiments, a *B*-allele variant of kappa casein was established in all cattle populations, which is associated with higher protein content in milk and with higher cheese yield, as well as better coagulation properties of milk. This is due to the different levels of glycolysis and the smaller diameter of micelles in the milk of animals with *BB* genotype. Cheese made from the milk of animals with the *BB* genotype contains more protein and less fat (24.7% and 33.18%, respectively), compared with *AA* homozygotes (24.22 and 33.71%).

The *CSN3* locus is correlated with the amount of milk produced, fat content and milk fat yield. The productivity per lactation in heterozygous *CSN3<sup>AB</sup>* cows of the red Estonian breed is higher (with 4746.3 liters of milk) than in homozygous *CSN3<sup>AA</sup>* (with 4229.3 liters). The average fat content is also higher in heterozygous individuals - 4.03%, and exceeds, on average, by 0.11% homozygous relatives ( $\approx 3.92\%$ ). The heterozygous genotype was also predominant in terms of milk fat yield by 25.5 kg.

In cows of the Belarusian Black and White breed, the desired genotype in terms of protein content *CSN3<sup>BB</sup>* exceeded the genotypes *CSN3<sup>AA</sup>* and *CSN3<sup>AB</sup>* in terms of fat content by an average of 0.2 %, and in terms of protein by 0.03 % - 0.11 %, respectively. It should be noted that this genotype is quite rare, in the populations of cows of the Belarusian black-and-white breed, its frequency is 1.1% (Ganja et al., 2019), and in Simmental cows it may be absent altogether (Zyryanova, 2021).

Numerous studies have shown that the frequency of occurrence of the desired *CSN3B* allele in black-and-white cattle populations is very low and systematically continues to decline, according to some experts, due to the widespread use of the Holstein breed.

Genetic variants of alpha-S1-casein (*LALBA*) influence the technological properties of milk. (Tyulkin, 2019). Association analysis showed that polymorphism g.-1001T > C in the promoter region of the *LALBA* gene has an effect on milk productivity in Polish Holstein-Friesian cows. High daily milk yield and dry matter yield, as well as high lactose yield and concentration are associated with the *TT* genotype. Cows of the *TT* genotype also had a smaller number of somatic cells in milk, which can be considered as an indicator of healthy state of udder (Ostrowska et al., 2021).

According to the *LALBA* gene in the Simmental breed, cows with the *LALBA<sup>BB</sup>* genotype were superior to cows with *LALBA<sup>AA</sup>* in fat content ( $p < 0.05$ ), in the «Sibiryachka» breed, higher fat content was observed in cows with the *LALBA<sup>AB</sup>* genotype (Unzhakova et al., 2021).

In other studies, no significant effect of *LALBA* on milk production was found on Holstein Friesian cows (Soyudal et al., 2019).

*LALBA* polymorphism and association with signs of milk lactation were also found in Chinese Holstein dairy cows (Yang, 2020).

In our studies, this locus has shown the presence of correlations with the amount of milk produced, the fat content in it and the yield of milk fat. It was found that animals with the homozygous *LALBA<sup>BB</sup>* genotype (with 3332.5 and 9368.2 liters of milk per lactation) have higher productivity in Moldovan and Belarusian Black and White cows, whereas the *LALBA<sup>BC</sup>* genotype (of 4648 liters) has an advantage in the Estonian Red breed. The most advantageous in terms of fat content in milk in black-and-white individuals of the Moldavian type is the heterozygous *LALBA<sup>AB</sup>* genotype (of 3.66 %), which exceeds the *BB* genotype by 0.02 %. In the Estonian Red breed, a higher fat content was found in cows with the heterozygous *LALBA<sup>BC</sup>* genotype - 4.02%, and in Belarusian black-and-white with the heterozygous *LALBA<sup>AA</sup>* - 4.17%. According to the yield of milk fat in black-and-white cows of the Moldavian type, the *LALBA<sup>BB</sup>* genotype turned out to be a priority and amounted to 121.5 kg, which was 1.2 kg higher than in the cows of the *LALBA<sup>AB</sup>* genotype.

The beta-casein locus *CSN2* can also serve as a marker in animal selection for milk productivity. Analysis of the effect of this gene on the productivity of Estonian Red cows showed the

superiority of the *AB* genotype (4977 liters of milk) over the *AA* genotype (4210.5 liters). In terms of fat content, there is a slight superiority - by 0.07% of the heterozygous genotype *AB* over the homozygous *AA*.

The inclusion in the traditional breeding rules for the selection of cows with another marker - betalactoglobuline (*BLG*) makes it possible to achieve significant success in increasing milk yields and improving its quality in specific herds of black-and-white cattle in a shorter time.

The *BLG* locus on cows of the red Estonian and black-mottled breed of the Moldavian type showed that animals with homozygous genotype *BB* have the highest productivity (4303 liters of milk per lactation and 3339.2 liters, respectively). The heterozygous *AB* genotype (of 3.69 %), which exceeds the *AA* genotype by 0.08 %, is advantageous in terms of fat content in milk in Black and White cattle of the Moldavian type.

In the Estonian Red breed, a higher fat content was found in *AA* homozygotes - 4.08%. According to the yield of milk fat in Black and White cows of the Moldavian type, the *AB* genotype is predominant (123.09 kg), whereas for the red Estonian breed, the homozygous *BLG<sup>AA</sup>* (169.36 kg).

Thus, analyzing the influence of the genetic polymorphism of QTL loci on the milk productivity of Estonian Red cows, it can be concluded that heterozygous genotypes in *LALBA*, *CSN2*, *CSN3* loci are advantageous for the selection of animals according to such traits as the amount of milk produced, fat content and milk fat yield. At the *BLG* locus, a homozygous *BB* genotype is desirable in terms of milk production, whereas the *AA* genotype is desirable in terms of fat content.

In the course of the research, the features of protein systems were studied in connection with the association with leukemia and mastitis, which cause great economic damage to animal husbandry, especially to the breeding genetic fund of highly productive livestock.

Diagnosis of infection of cattle with bovine leukemia virus (BLV - Bovine Leukemia Virus) is mainly performed by assessing the presence of antibodies to BLV antigens in animals using REED diagnostics. The method is characterized by low accuracy, it is known that a READ-

positive analysis is confirmed only by a pathoanatomic analysis in 1-8% of cases.

It is proved that the sensitivity to leukemia in cattle is determined by postalbumins of type *BB* and transferrins of type *AD*. It follows from this that during breeding work it is necessary to constantly monitor the accumulation of these alleles in the population, and breeding bulls should be typed according to these genes.

In sheep, the locus that controls the synthesis of *BLG* is localized on the second chromosome and it is believed that it gives taste to milk. The data on the relationship of  $\beta$ -lactoglobulin with the milk productivity of sheep are contradictory (Klimanova & Tarasenko, 2021).

In our studies, in Karakul sheep, the *BLG<sup>AA</sup>* genotype is interrelated with high milk productivity, they produce 0.24 % more dry matter compared to heterozygous *AB* individuals. *BB* homozygotes contain 0.79 % more dry matter in milk than heterozygotes. Comparing the percentage of fat, it was found that 0.13% more fatty milk ( $8.55 \pm 2.14$  %) can be obtained from sheep with the genotype *BLG<sup>BB</sup>*, compared with the genotype *BLG<sup>AA</sup>* ( $8.07 \pm 1.041$ %) and 0.61 % more, compared with heterozygotes *BLG<sup>AB</sup>* ( $7.94 \pm 1.14$  %).

The highest percentage of 4.82 % of protein was found in the milk of individuals with the *AB* genotype, which is 0.16 % higher than in the *AA* genotype and 0.45 % higher than in heterozygous animals.

As for the percentage of casein in the milk of Karakul sheep, it turned out that individuals with the genotype *BLG<sup>BB</sup>* have the lowest casein content of 3.45 %, which is 0.25 % less than the casein content in the milk of homozygous *AA* individuals 3.7 % and 0.28 % lower than in heterozygous animals 3.73 %. As you can see, the most advantageous genotype for such traits as: the protein and casein content in the *BLG* locus is the heterozygous ones.

Thus, the highest percentage of fat content in the *BLG* locus is noted for the *BLG<sup>BB</sup>* genotype, and in the case of protein and casein, the heterozygous *AB* animals are prioritized.

To assess the gene pool of agricultural chickens, 5-6 systems can be used, in which a stronger genetically determined polymorphism is detected. These include albumins, globulins G3 and G2, transferrins, etc.

In our studies on populations of chickens in the Marans and Orpington breeds, the *OV* locus showed a pleiotropic effect, influencing the fertilization and hatchability of eggs. The homozygous *AA* chickens had increased egg production, with the lowest live weight, egg weight and low hatchability of chickens, compared with the heterozygotes birds by 3-10 %. Chickens carrying the *OV<sup>A</sup>* allele outperformed chickens with the *OV<sup>B</sup>* allele in live weight and egg weight. In homozygous *OV<sup>BB</sup>* hens, the chickens are more viable compared to heterozygous ones.

The transferrins are associated with the egg mass. The homozygous *BB* genotype in Marans chickens determined the lowest egg mass of  $46.54 \pm 0.55$  g. The *AA* genotype had the highest embryonic mortality of 4.9% in the Orpington breed. The *AB* genotype is interrelated with the live weight of ♀ Orpington × ♂ Moldavian Naked Neck hybrids ( $2.58 \pm 0.09$  kg).

Immunogenetic indicators can also be used as markers to study the genetic structure in order to determine the common origin of breeds. For example, the genetic similarity of cows of the Estonian Red and Red Steppe breeds was  $r = 0.9976$  and between Jersey and Black and White Cow breeds  $r = 0.8907$ . The Moldavian type of Black and White cattle is genetically similar with the Holstein breed by 77.14%, which indicates their commonality (the common ancestor of the Holstein) in lactoproteins. At the same time, a genetic change was established between them ( $D = 0.2595$ ), i.e. on average, for every 26 out of 100 loci, complete allele substitutions occurred. This is due to the reproductive and isolating mechanism, namely, the breeding of a population of Black and White breed of cows "in itself".

## CONCLUSIONS

Analyzing the relationship of candidate genes with productive qualities, it can be seen that the genotypes beneficial for one breed are not those for another breed. This is due to the degree of absorption of the breed - founder/bull in crosses and breeding "in itself", etc. Nevertheless, in our opinion, valuable kappa-casein alleles are introduced by individuals of the Holstein and Simmental breeds, which are of the greatest value for breeding.

Thus, the obtained research results allow us to draw the following conclusions.

In cattle breeding:

For the production of protein products with a high protein content, we recommend considering the *CSN3* locus of the BB genotype as an economically important selection criterion for dairy cattle of the Belarusian Black-and-White breed.

The *LALBA* gene can be used as a genetic marker in the selection of animals according to the following indicators: the amount of milk produced, the fat content in milk, the yield of milk fat (for the Estonian red breed of the BC genotype; for black-and-white cows of the Moldavian type and Belarusian black-and-white breed - genotype BB).

The *BLG* locus is associated with milk yields (the advantage of the BB haplotype in the populations of the Estonian red breed and the Moldovan black-mottled cattle breed has been proven; the AA haplotype in cows of the Belarusian black-mottled breed).

To increase the fat content of milk in the *BLG* locus, a heterozygous genotype AB is desirable in cows of the Moldavian Black-and-White type, genotype AA in the Estonian Red breed, homozygous BB in the Belarusian Black-and-White breed.

To constantly monitor the accumulation of alleles that determine leukemia and mastitis, we suggest using marker-dependent proteins, postalbumins and transferrins.

In sheep breeding:

The *BLG* locus is recommended as a genetic marker for increasing milk the productivity and fat content in Karakul sheep (genotype AA for high milk productivity and genotype BB for increasing the percentage of fat in milk).

To evaluate the gene pool of agricultural chickens, we recommend using ovalbumin proteins as markers. To stabilize the lines by quantitative characteristics of productivity, it is recommended to give preference to homozygous AA genotypes, as well as to select chickens with homozygous  $OV^{BB}$  genotype for the production of the next generation and increase their viability.

Thus, it is necessary to continue monitoring the allele found of local breeds in order to preservation of optimal genetic diversity (polymorphism of the level of 5%) for further

search of the most representative markers for each breed of animals separately.

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