# Differences between black-and-white cows with alternative genotypes of the alpha-lactalbumin gene in milk features

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**Abstract.** The article presents the study results of the influence of the alpha-lactalbumin gene various genotypes on milk production and the qualitative composition of milk from black-and-white cows bred in the Republic of Bashkortostan. The predominant number of individuals carrying the LALBA$^{AB}$ genotype was found in the analyzed animals in the number of 276 animals (n=175, 63.4%). It was further established that the best characteristics were possessed by the cows containing the allele A in their genotype for the gene under study. These animals were superior to their peers in all indicators of the qualitative composition of milk and milk productivity. The obtained results indicate that genotypes of the milk protein gene under study having the allele A in their composition, including in the heterozygous state, are potentially desirable.

## 1 Introduction

The search for genetic markers capable of predicting economically useful traits in cattle breeds at the core of modern animal husbandry [1]. One of these markers is the alpha-lactalbumin gene, which plays a key role in the formation of milk qualitative characteristics [2]. The expression of this gene increases in response to the hormone prolactin, which leads to the regulation of increased synthesis of lactose and other components of milk, which makes its study especially relevant for breeders and researchers in the field of animal husbandry [3, 4]. The alpha-lactalbumin gene (LALBA) is located on the 5$^{th}$ chromosome of cattle. It encodes an important whey protein and makes up for about 25% of whey proteins and about 5% of all cow's milk proteins. The molecule of the analyzed gene consists of 123 amino acids, which include 1-2 phosphate residues depending on the genotype. The LALBA gene is 10,338 bp long and consists of 9 exons and 8 introns. The molecular weight of alpha-lactalbumin is 14.178 kDa, and its isoelectric point is in the range from 4.2 to 4.5 pH [1]. Alpha-lactalbumin [3] is the most thermostable whey protein. For its coagulation at pH 7.0, milk must be heated to 114°C. In monomeric form, the analyzed protein firmly binds zinc and calcium ions, which determines its bactericidal and antitumor properties. In addition, the alpha-lactalbumin gene is involved in metabolic processes in cattle [5].

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The LALBA gene (Soyudal et al., 2019) is represented by three main allelic variants: A, B and C. Variant B differs from variant A by an amino acid substitution at Arg10 — Gln8 position, caused by a point mutation (A—>G) at 1689 position. This substitution affects the functional features of the protein. Variants B and variant C have the same amino acid sequence. The distinction lies in different point mutations at 1689 position, which can lead to various structural and functional changes in the protein [1, 2]. The scientific literature [3] provides information that the $LALBA^{AA}$ variant is associated with higher milk yield and milk fat content, while the $LALBA^{BB}$ variant is associated with a higher mass fraction of proteins and fat in cow's milk. The $LALBA^{AC}$ variant is less common and has specific characteristics, but detailed information on its influence on milk productivity and technological properties of milk is not provided in the available sources.

In addition, understanding the relationship between LALBA polymorphism and economically useful traits makes it possible to develop more effective breeding strategies aimed at improving the genetic potential of the herd. This may include [6] the creation of breeding programs, selection of parent pairs, and monitoring of genetic variability in cattle populations [3]. LALBA polymorphism and economically useful traits makes it possible to develop more effective breeding strategies aimed at improving the genetic potential of the herd. This may include the creation of breeding programs. Thus, the study of the association of alpha-lactalbumin gene polymorphism with economically useful traits of cattle is of great importance for the development of modern animal husbandry, opening up new horizons for breeders and researchers, allowing them to increase the efficiency of their work and improve the quality of the products obtained.

It is important to note that the information on the effect of different LALBA genotypes on milk productivity cows may be contradictory due to the lack of research on this issue. This highlights the need for further research to fully understand the role of each allelic variant in the formation of milk productivity and milk qualitative composition.

**The aim of the study** is to establish the degree of alpha-lactalbumin gene genotypes effect on the milk qualitative characteristics reflecting the nutritional value of milk from black-and-white cows.

## 2 Materials and methods

The material for the study was a sample of black-and-white cows bred in the Kalinin Agricultural Production Company LLC in the Sterlitamak District of Bashkortostan Republic (n=276). Animal genotyping on milk protein genes was conducted in the laboratory of molecular genetics of the Bashkir State Agrarian University. DNA was isolated using a set of reagents "DNA-Extran" (NPC "Syntol", Moscow). The LALBA GH gene polymorphism was detected by PCR-RFLP with primers:

LALBA F: 5`-aagagttggatggaatcacct-3`

LALBA R: 5`-ttcaaattgctggcatcaagc-3`;

(restriction enzyme: MhI) followed by electrophoretic separation of restriction fragments in 7.5% PAGE. The allele length was found with the pUC/Msp1 molecular weight marker provided by "Sibenzyme" company. The image of gels after electrophoresis in PAGE was studied with the GelDoc XR gel documentation system and the ImageLab version 2.0 "DNA-analyser" software. The sizes of restriction fragments were: $LALBA^{AA}$ – 328, 102 base pair; $LALBA^{AB}$ – 328, 211, 117, 102 base pair; $ALAA^{BB}$ – 211, 117, 102 base pair. The statistical significance between the average values of the compared groups was assessed according to the Student's criterion using the Microsoft Excel. Milk quality was assessed in the laboratory of milk quality selection control of Bashkirskoye JSC for breeding using the 'Lactan 700M' device. The analysis of the genetic structure of the herd

was performed in the 'Center of Advanced Technologies, Embryology and Genetics of the Bashkir State Agrarian University'.

## 3 Results

The animals were tested for the LALBA gene polymorphism. The frequency of the $LALBA^{B}$ (0.67) is 2 times higher than the frequency of the $LALBA^{A}$ allele (0.33). In the studied sample, the identified genotypes for the studied gene were distributed as follows: $LALBA^{AB}$ – 175 animals (63.4%); $LALBA^{BB}$ – 96 animals (34.8%); $LALBA^{AA}$ – 5 animals (1.8%).

The next stage of the research was to study the influence of the LALBA gene polymorphism on milk productivity and the qualitative composition of milk of the animals under study. For this purpose, detailed analyses aimed at identifying correlations between different genotypes of the LALBA gene and their influence on the productive qualities of cows was conducted. The results of this analysis are presented in Table 1.

**Table 1.** Milk productivity indicators in the studied sample of cows, n=276, M±m.

| Indicator | Genotype (M±m) | | | Genotypes Difference | | |
|---|---|---|---|---|---|---|
| | $LALBA^{AA}$ | $LALBA^{AB}$ | $LALBA^{BB}$ | $LALBA^{AA}$/ $LALBA^{BB}$ | $LALBA^{AA}$/ $LALBA^{AB}$ | $LALBA^{AB}$/ $LALBA^{BB}$ |
| Milk yield for 305 days, kg | 5716.48 ± 149.17 | 5794.72 ± 131.54 | 5754.90 ± 329.14 | –38.42 | –78.24 | +39.82 |
| Live weight, kg | 584.51 ± 81.53 | 589.31 ± 92.59 | 587.44 ± 102.75 | –2.93 | –4.80 | +1.87 |
| Milk production index (MPI) | 978 ± 4.91 | 983 ± 13.83 | 980 ± 5.60 | –2 | –5 | +3 |
| Biological effectiveness of cows (BEC) | 212.83± 1.72 | 210.52± 1.30 | 209.72± 1.75 | +3.11 | +2.31 | +0.8 |
| Biological full-value coefficient (BFC) | 84.30± 4.22 | 83.68± 1.14 | 83.56± 3.20 | +0.74 | +0.62 | +0.12 |

It was found that under similar feeding and housing conditions, heterozygous cows were the leaders in terms of abundant milk production and live weight with an insignificant difference. These individuals also slightly exceeded other genotype variants in terms of milk production index. The lowest milk production index was found in cows with the $LALBA^{AA}$ genotype (978). In terms of biological efficiency, cows with a homozygous genotype for the A allele were 2.3 - 3.1 units ahead of their peers. In addition, the animals of this group have a predominant coefficient of biological full-value of milk. The difference between the BFC values in cows of the above-mentioned genotype compared to $LALBA^{AB}$ is 0.62 (0.74%), with $LALBA^{BB}$ - 0.74 (0.88%), which is less than 1%. No significant differences were found between the indicators in different genotypes.

Then, the qualitative composition of black-and-white cattle sample milk with different genotypes for the LALBA gene was analyzed. The data obtained are presented in Table 2.

**Table 2.** Qualitative composition of milk from cows with different genotypes for the LALBA gene, M±m.

| Indicator | Genotype | | | Average |
|---|---|---|---|---|
| | $LALBA^{AA}$ | $LALBA^{AB}$ | $LALBA^{BB}$ | |
| Number, animals | 5 | 175 | 96 | 276 |
| Mass fraction of fat, % | 3.82±0.07 | 3.89±0.04* | 3.79±0.03 | 3.83±0.05 |
| Mass fraction of protein, % | 2.97±0.03 | 3.02±0.02 | 2.99±0.03 | 2.99±0.03 |
| Milk fat yield, kg | 218.37±15.05 | 225.41±31.55 | 218.11±26.40 | 220.63±24.33 |
| Milk protein yield, kg | 159.50±13.08 | 173.64±20.57 | 172.07±14.49 | 168.40±16.47 |
| MSNF, % | 8.62±0.04* | 8.51±0.03 | 8.53±0.1 | 8.55±0.03 |
| Milk density, $A^0$ | 1030.75±0.28* | 1030.1±0.16 | 1030.32±0.36 | 1030.39±0.27 |
| Dry matter, % | 12.44±0.14 | 12.40±0.12 | 12.32±0.18 | 12.39±0.15 |
| Lactose, % | 4.74±0.04 | 4.68±0.04 | 4.69±0.06 | 4.70±0.05 |
| Energy value, kJ | 2689.8±37.78 | 2714.0±24.78 | 2673.87±44.71 | 2692.56±35.76 |

* P≤0,05

Based on the data presented in Table 1, it can be concluded that the qualitative composition of milk in black-and-white cattle with different genotypes for the LALBA gene has certain differences. The highest percentage of fat mass fraction was recorded in the $LALBA^{AB}$ genotype (3.89%). According to this indicator, the animals of this genotype exceeded their peers by 0.07 - 0.1% (P ≤ 0.05). In addition, the milk of these animals was characterized by an increased percentage of protein content, the best results in the yield of milk fat and milk protein, and a higher energy value.

The milk of the homozygous genotype for the $LALBA^{AA}$ gene has the highest mass fraction of MSNF (8.62%, P≤0.05) and density (1030.75 kg/$m^3$, P≤0.05), which indicates a high content of dry nonfat milk residue and the peculiarities of the composition and structure of milk. Also, the $LALBA^{AA}$ genotype demonstrates the highest indicators for the mass fraction of DM and lactose (12.44% and 4.74%, respectively), which is of significant importance for the production of dairy products.

The $LALBA^{AB}$ genotype is a balanced variant with moderate performance in most parameters, making it a universal choice for various production purposes in the dairy industry.

## 4 Discussion

The study revealed a positive correlation between the $LALBA^{A}$ allele and the productive qualities of the analyzed group of animals. Milk from cows carrying this locus has increased nutritional properties. Animals with the $LALBA^{AA}$ genotype were superior to their peers with the $LALBA^{BB}$ genotype in terms of fat, MSNF, dry matter and lactose content in milk, but in terms of milk protein content they were slightly inferior to milk samples from cows with an alternative homozygous genotype. The best productive qualities of cows are manifested in the heterozygous state for the studied gene. Similar studies were conducted by a group of scientists from the Kazan State Veterinary Academy named after N. E. Bauman together with researchers from the Federal State Budgetary Scientific Institution "Federal Center for Toxicological, Radiation and Biological Safety" and the Chuvash State Agricultural Academy. The given research was aimed to study black-and-white cows of the Republic of Tatarstan. The genotype incidence rate in the studied groups of animals varied. The colleagues from Tatarstan revealed more AA genotype (50.6%), while the BB genotype for the analyzed gene showed the lowest results. As the results of our studies showed, cows with the LALBAAB genotype had the highest fat rate (3.87%).

## 5 Conclusion

Thus, our study confirmed the prospects for further studies of LALBA gene for associations with milk features of the cattle breed.

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