

Association of κ -casein, β -lactoglobulin, α -lactalbumin and leptin gene polymorphisms with bovine productivity traits in Western Siberia

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Abstract. The Simmental breed (n=182) and Siberian breed (n=131) cows were genotyped using the CSN3, BLG, LALBA, and LEP genes. In both populations, the frequency of homozygotes did not exceed 10 %, and the proportion of heterozygotes was at the level of 40-45%. The actual genotype distribution corresponded to the theoretically expected Hardy-Weinberg distribution. In terms of milk yield, cows of the «Sibiryachka» breed with the BLG^{AA} genotype significantly outperformed cows with the BLG^{BB} genotype (p<0.05), and in the Simmental breed, cows with the BLG^{AA} genotype had a higher milk yield than BLG^{AB} (p<0.01). According to the LALBA gene in the Simmental breed, cows with the LALBA^{BB} genotype were superior to cows with LALBA^{AA} in fat content (p<0.05), in the «Sibiryachka» breed, higher fat content was observed in cows with the LALBA^{AB} genotype (p<0.05). In both breeds, cows with the LEP^{CC} genotype outperformed cows with the LEP^{TT} genotype in fat content (p<0.05). According to the reproduction indicators, cows with the LALBA^{AA} and LEP^{CT} genotypes had an earlier age of insemination in comparison with cows with the LALBA^{BB} and LEP^{CC} genotypes (p<0.05). Cows with the LEP^{CT} genotype had a longer Calving interval than cows with the LEP^{CC} genotype (p<0.05).

1 Introduction

At this stage of the development of animal husbandry, marker-based selection (MAS), based on the use of DNA markers associated with the characteristics of animal productivity, has become widespread. The use of genetic markers made it possible to predict the potential of the animal at the early stages of ontogenesis and thereby significantly reduce the time of the selection process [1-3]. Due to the fact that in many countries genotyping has become a prerequisite for breeding, scientists conduct a large number of studies on the association of gene polymorphism and milk productivity [4].

The genes of kappa-casein (CSN3), beta-lactoglobulin (BLG), and alpha-lactalbumin (LALBA) are among the most studied protein genes that are studied in connection with

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various economically useful traits. The CSNN3 gene is the most polymorphic of all milk caseins, so it is the most promising object of study in various breeds of cattle [4]. Many studies have shown the association of the CSN3^{BB} genotype with higher milk yield, as well as with more efficient milk coagulation, which is an important criterion in the production of cheese and cottage cheese [5, 6]. Variant B of the CSN3 gene is associated with a smaller micelle size and higher milk coagulation characteristics [7-10]. Studies of the BLG gene polymorphism show that there is a link between the B allele and milk yield indicators, often animals with the BLGAB and BLGBB genotypes have better indicators [11-13]. Information about the relationship of the LALBA gene with indicators of milk productivity is very contradictory. Most often, this gene is associated with the regulation of lactose synthesis in milk [14, 15].

Special attention is also paid to the gene of lipid metabolism-leptin (LEP), which is associated with indicators of functional longevity of cows, marbling of meat, and milk fat content [16-18]. There is evidence that first-born heifers with the LEPT genotype have a significant advantage over animals with alternative genotypes in terms of milk yield and fat content [19].

Research aimed at finding an association of candidate genes with milk productivity indicators should be continued, since the results of research in this area are fragmentary. The aim of this study was to study the relationship between genetic markers and indicators of milk productivity and reproduction in populations of cattle bred in Western Siberia.

2 Materials and methods

Molecular genetic studies were carried out on cows of the Simmental breed (n=182) and the breed «Sibiryachka» (n=131). Studies were conducted in the laboratory of biotechnology of the Siberian Scientific-Research and Design-Technological Institute of Animal Husbandry of the Siberian Federal Scientific Center of Agrobiotechnology of the RAS. Blood sampling was carried out in a volume of 7-10 ml in a vacuum tube for hematological studies containing the anticoagulant EDTA K2. Genomic DNA was isolated from blood using an extraction kit from the clinical material "Ampli Prime DNA-sorb-B" according to the manufacturer's prescription "NextBio" LLC (Moscow). The quality and concentration of the isolated DNA were evaluated in 1% agarose gel by horizontal electrophoresis using the E-Box-CX5.TS-20.M gel-documenting system.

Amplification of the CSN3 gene fragment was performed by standard PCR analysis on a DNA amplifier with 1000 Touch Termal Cycler «BioRad» (Singapore), with two oligonucleotide primers:

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

R: 5' - TTT ATT AAT AAG TCC ATG AAT CTT G - 3'

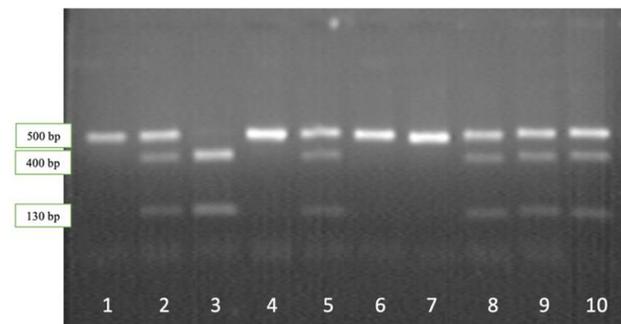


Fig. 1. Results of electrophoresis of restriction fragments of the CSN3 gene: tracks 1,4,6,7 – CSN3^{AA} genotype; tracks 2,5,8-10 - CSN3^{AB} genotype, track 3-CSN3^{BB} genotype.

Amplification of the BLG gene fragment was performed by standard PCR analysis on a DNA amplifier with 1000 Touch Thermal Cycler «BioRad» (Singapore) in the volume of 25 µl, with two oligonucleotide primers:

BLGP3: 5' – GTC CTT GTG CTG GAC ACC GAC TAC A – 3'

BLGP4: 5' – CAG GAC ACC GGC TCC CGG TAT ATG A – 3'

The result of electrophoresis of restriction fragments of amplification products of the BLG gene fragment is shown in Figure 2.

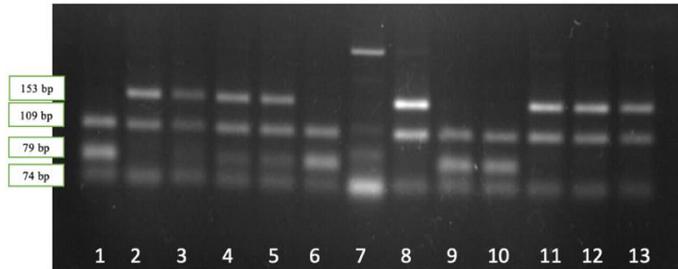


Fig. 2. Results of electrophoresis of restriction fragments of the BLG gene: tracks 2, 3, 8, 11-13 – BLG^{AA} genotype; tracks 4,5-BLG^{AB} genotype; tracks 1, 6, 9, 10-BLG^{BB} genotype, track 7- amplification.

Amplification of the LALBA gene fragment was performed by standard PCR analysis on a DNA amplifier with 1000 Touch Thermal Cycler «BioRad» (Singapore) in the volume of 20 µl, with two oligonucleotide primers:

ALF-LAC1: 5' – AAG AGT TGG ATG GAA TCA CC – 3'

ALF-LAC2: 5' – TTC AAA TTG CTG GCA TCA AGC – 3'

The result of electrophoresis of restriction fragments of amplification products of the L ALBA gene fragment is shown in Figure 3.

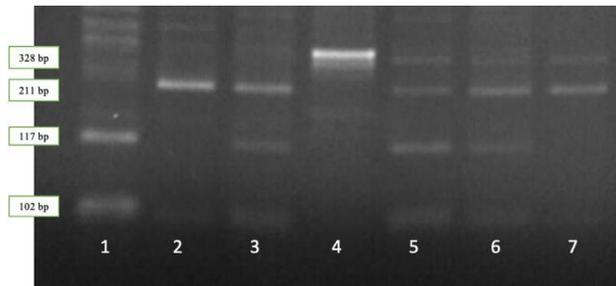


Fig. 3. Results of electrophoresis of restriction fragments of the LALBA gene: track 1 – LALBA^{BB} genotype; tracks 2,7-LALBA^{AA} genotype; tracks 3, 5, 6-LALBA^{AB} genotype, track 4-amplification.

Amplification of the LEP gene fragment was performed by standard PCR analysis on a DNA amplifier with 1000 Touch Thermal Cycler «BioRad» (Singapore) in the volume of 20 µl, with four oligonucleotide primers:

LEP-F1: 5' – GAC GAT GTG CCA CGT GTG GTT TCT TCT GT – 3'

LEP-R1: 5' – CGG TTC TAC CTC GTC TCC CAG TCC CTC C – 3'

LEP-F2: 5' – TGT CTT ACG TGG AGG CTG TGC CCA GCT – 3'

LEP-R2: 5' – AGG GTT TTG GTG TCA TCC TGG ACC TTT CG – 3'

The result of electrophoresis of restriction fragments of amplification products of the LEP gene fragment is shown in Figure 4.

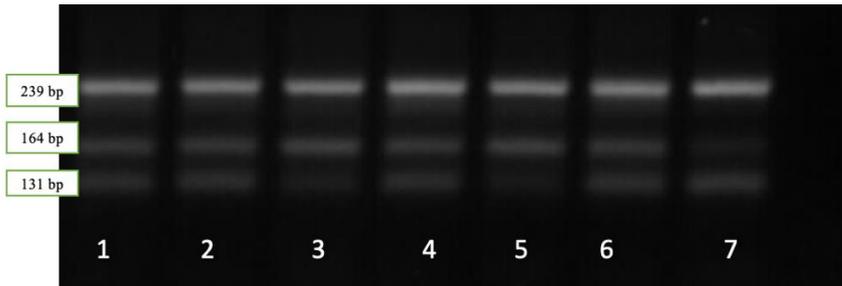


Fig. 4. Results of electrophoresis of restriction fragments of the LEP gene: track 3, 5 – LEP^{CC}; genotype of track 1, 2, 4, 6 – LEP^{CT} genotype; track 7-LEP^{TT} genotype.

Statistical processing of the obtained data was carried out using computer programs R-Studio and Microsoft Excel, as well as using generally accepted methods. For multiple comparison of samples, the Student's t-test was calculated with the Bonferroni correction.

The object of the study was the genes of milk productivity - kappa-casein (CSN3), beta-lactoglobulin (BLG), alpha-lactalbumin (LALBA) and leptin (LEP).

3 Research results

Earlier, we described the genetic structure of the population of the Simmental breed and the «Sibiryachka» breed. It was found that the populations are in a state of gene balance, which indicates that there is no influence of selection factors, migration and gene drift. The level of homozygosity in all populations was at the level of 60%, the level of heterozygosity-about 40%. The relatively high level of heterozygosity indicates the genetic variability of the population. The CSN3 gene has the lowest level of polymorphism, while the BLG, LALBA, and LEP genes have the highest level [20]. In the next stage of the work, molecular genetic studies of animals were carried out on the basis of genes associated with indicators of milk productivity.

Data on the milk productivity of animals of the two breeds are presented in Figures 5-8. Comparison of the average values of indicators between different genotypic groups of cows for the CSN3 gene revealed significant differences. At the same time, in both breeds, cows with BLG^{AA} genotypes had higher milk yield compared to other genotypes ($p < 0.05-0.01$). Fat content was significantly higher in simmentals with the LALBA^{BB} genotype in comparison with cows carrying the LALBA^{AA} genotype ($p < 0.05$), while in the group of cows of the «Sibiryachka» breed, a higher content of milk fat was observed in heterozygotes ($p < 0.001$). According to Figure 6, the protein-milk content of cows of the «Sibiryachka» breed with the LEP^{CC} genotype is higher than that of LEP^{TT} ($p < 0.001$), and in the Simmental breed the opposite pattern is observed ($T/T > C/C$; $p < 0.05$).

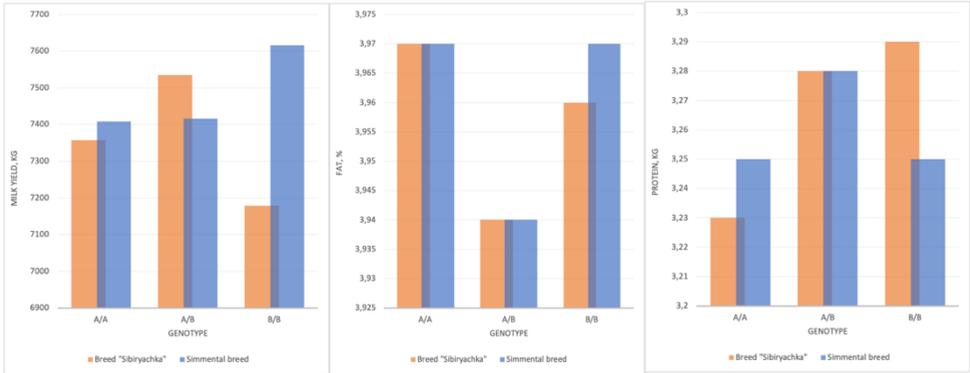


Fig. 5. Milk productivity of cows of the first lactation of two breeds according to the CSN3 gene.

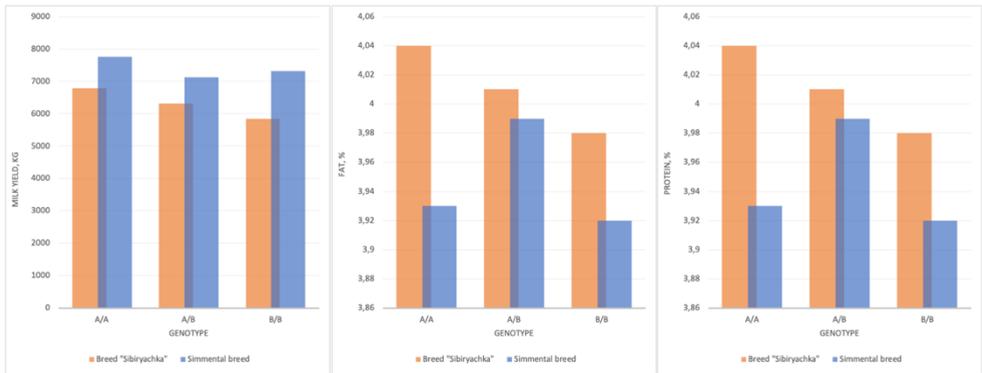


Fig. 6. Milk productivity of cows of the first lactation of two breeds according to the BLG gene.

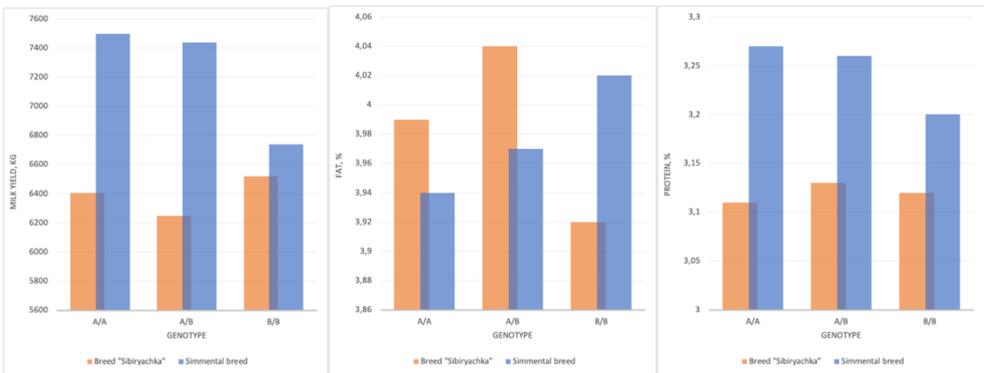


Fig. 7. Milk productivity of cows of the first lactation of two breeds according to the LALBA gene.

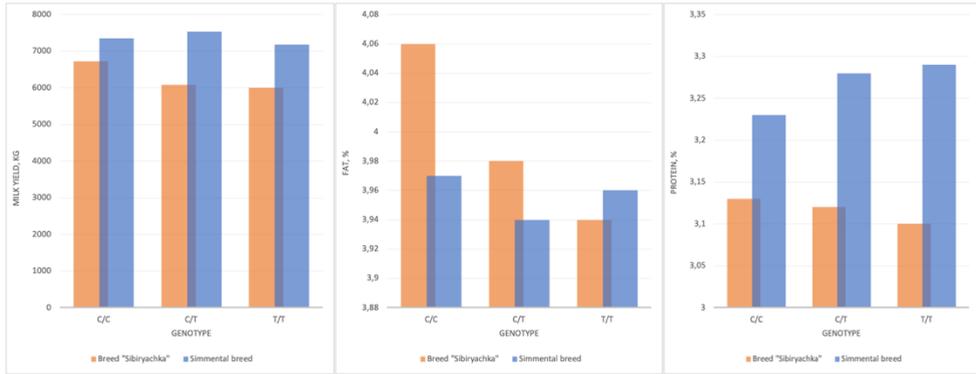


Fig. 8. Milk productivity of cows of the first lactation of two breeds according to the LEP gene.

The obtained results indicate a possible genetic coupling of polymorphic genes with loci of quantitative traits. At the same time, it should be taken into account that the contribution of individual genes to the formation of complex deterministic traits may be insignificant. In any case, the influence of the genotype on the genes associated with the characteristics of milk productivity is due to the genetic constitution of an individual, and the population sample reflects the general genotypic component in the formation of various phenotypic characteristics.

Indicators of reproductive qualities of Simmental cows with different genotypes are presented in Table 1. Animals with the LALBA^{AA} and LEP^{CT} genotypes had an earlier insemination age compared to LALBA^{BB} and LEP^{CC} ($p < 0.05$). Cows with the LEP^{CT} genotype had an average calving interval of 21 days longer than the LEP^{CC} genotype ($p < 0.05$). There were no significant differences in reproduction rates between groups of animals with other genotypes.

Table 1. Reproductive qualities of Simmental cows depending on the genotype for the CSN3, BLG, LALBA and LEP genes.

Genotype	n	Age of first insemination. months.	Service period. days	Calving interval. days	Interlactation period. days
CSN ^{AA}	110	13.5±0.17	97.7±7.03	383.4±6.84	65.9±2.41
CSN ^{AB}	66	13.5±0.18	112.2±9.13	387.2±9.39	71.4±4.85
CSN ^{BB}	6	13.8±0.87	110.0±40.0	392.0±18.86	61.0±2.92
BLG ^{AA}	84	13.5±0.16	100.1±7.2	394.7±9.35	66.2±3.61
BLG ^{AB}	79	13.5±0.21	109.6±10.0	381.6±7.4	69.6±3.74
BLG ^{BB}	19	13.6±0.39	90.8±13.75	368.3±10.92	67.1±5.30
LALBA ^{AA}	91	13.2±0.13 ^a	99.9±6.95	389.4±9.41	64.5±2.83
LALBA ^{AB}	80	13.7±0.21	105.3±9.33	378.9±5.66	70.6±3.95
LALBA ^{BB}	11	14.6±0.58 ^a	114.2±31.07	400.7±24.98	70.7±8.74
LEP ^{CC}	78	13.8±0.2 ^a	104.5±8.24	372.7±5.59 ^a	67.2±3.11
LEP ^{CT}	87	13.2±0.17 ^a	104.5±8.17	399.8±10.11 ^a	67.1±3.78
LEP ^{TT}	1	13.6±0.31	83.6±12.19	389.4±18.03	75.4±10.38

The letter a indicates the groups that have significant differences in the frequency of genotypes, the level of confidence is indicated in the text.

One of the important indicators of the reproductive qualities of cows is the ease of calving. We conducted a comparative assessment of this trait depending on the genotype of the animals. There were no associative relationships between groups of animals, taking into account the different degree of manifestation of the trait (Table 2).

Table 2. Ease of calving depending on the genotype for the CSN3, BLG, LALBA and LEP genes, %.

Genotype	n	Ease of calving			
		Normal	Mild pathology	Moderate pathology	Severe pathology
CSN ^{AA}	109	73.4±4.23	6.4±2.34	19.3±3.78	0.9±0.9
CSN ^{AB}	64	78.1±5.17	6.3±3.04	15.6±4.54	0±1.48
CSN ^{BB}	6	66.7±19.24	33.3±19.24	0±11.02	0±11.02
BLG ^{AA}	83	80.7±4.33	4.8±2.35	14.5±3.86	0±1.16
BLG ^{AB}	78	69.2±5.23	6.4±2.77	23.1±4.77	1.3±1.28
BLG ^{BB}	17	76.5±10.28	23.5±10.28	0±4.99	0±4.99
LALBA ^{AA}	90	73.3±4.66	7.8±2.83	18.9±4.13	0±1.08
LALBA ^{AB}	78	78.2±4.68	6.4±2.77	14.1±3.94	1.3±1.28
LALBA ^{BB}	11	63.6±14.51	9.1±8.67	27.3±13.43	0±7.12
LEP ^{CC}	77	79.2±4.63	6.5±2.81	14.3±3.99	0±1.25
LEP ^{CT}	85	72.9±4.82	8.2±2.98	17.6±4.13	1.2±1.18
LEP ^{TT}	17	64.7±11.59	5.9±5.71	29.4±11.05	0±4.99

4 Discussion

The use of genetic markers in breeding programs requires a deep and comprehensive study. Since most productivity traits have a complex polygenic inheritance pattern, the search for the involvement of individual allelic gene variants becomes more complicated. The results of many studies confirm the contradictory nature of the relationship of known markers with certain signs of productivity. Our research was devoted to the study of genetic polymorphism of the most studied genes of milk proteins and the hormone leptin. Genotyping of animals based on these genes and studying the selectivity of their use as productivity markers based on a comparative analysis of phenotypic manifestations revealed a number of significant differences in breed indicators. It is obvious that these genes are part of a complex of loci of quantitative traits and represent functional genes, different variants of which have different gene expression, manifested in different degrees of manifestation of traits. Different breeds show the same patterns in some genes. Thus, the fat content in both breeds for the LEP gene was higher for the LEP^{CC} genotype, and for the BLG gene in animals with the BLG^{AA} genotype, there is a higher milk yield. But at the same time, according to the LALBA gene, animals of different breeds do not have the same patterns in terms of protein-milk content and fat-milk content. Perhaps this is due to the fact that high productivity in animals eliminates genotypic differences, so to study the influence of the genotype on these genes, the most preferred object of research will be animals with average productivity and relatively high phenotypic variability of the trait. There were no significant differences in reproductive qualities, because these genes are less involved in the formation of the trait.

5 Conclusion

1. The influence of the genotype on milk productivity was established. In both breeds, cows with the BLG^{AA} genotype outperformed animals with alternative genotypes in milk yield. According to the LALBA gene, the fat content was higher in Simmentals with the LALBA^{BB} genotype, while in the group of animals of the «Sibiryachka» breed, a higher content of milk

fat was observed in LALBA^{AB}. Cows with the LEP^{CC} genotype in the «Sibiryachka» breed are superior to animals with the LEP^{TT} genotype in terms of fat content and protein content; in the second breed, the reverse pattern is observed in terms of protein content.

2. Significant differences between groups of cows with different genotypes in reproductive qualities were found in the study of the LALBA and LEP genes: cows with the LALBA^{AA} and LEP^{CT} genotypes had an earlier insemination age compared to LALBA^{BB} and LEP^{CC}. The calving interval in cows with the LEP^{CT} genotype was longer compared to the LEP^{CC} genotype.

3. Animals with different genotypes did not differ in the ease of calving.

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