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A Study on Milk Productivity of Black-and-white Cows Considering Genotypes of DNA Markers CSN2, LGB, CRH, STAT1, TFAM1, and TFAM2

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Abstract

The work on forecasting the level of dairy productivity of cows leads to an increase in the efficiency of breeding improvement of dairy herds of our country. The search for DNA markers associated with the dairy productivity of cattle is one of the promising areas of the industry. The purpose of our research was to study the polymorphism of DNA markers and search for associations with signs of dairy productivity of black-and-white cows. The animals were genotyped using Bovine GGP 150K biochips ("Illumina/Neogen", USA) with a coverage density of 138974 SNP. The article presents the results of a study of associations of genotypes for the genes CSN2, LGB, CRH, STAT1, TFAM1, and TFAM2 with signs of milk productivity. It was found that in most cases, animals with homozygous genotypes were superior in milk yield. Milk yield, the mass fraction of fat and protein in milk, the amount of milk fat and milk protein are higher in animals carrying the CC genotype by CSN2, with the heterozygous AG genotype by LGB, with the AA genotype by CRH, the STAT1 gene - with the AA genotype, with the GG genotype by TFAM1 and the CC genotype by TFAM2. The results obtained make it possible to assume that DNA diagnostic methods can be used by breeding enterprises specializing in cattle breeding at the stages of development and implementation of breeding programs.

Disciplinary: Biotechnology & Genetics, Animal Sciences.

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1 Introduction.

The forecast of dairy productivity of cattle is an integral part of effective breeding work in breeding enterprises of our country [1]. When implementing this direction, specialists use both traditional methods of obtaining products "with specified properties" and develop new ones. The use of DNA markers associated with the level of productivity and quality of cow milk is gaining increasing interest in dairy cattle breeding [2-7].

Identification of DNA markers associated with livestock dairy productivity is one of the relevant directions mainly in connection with the process of industry intensification. The outcome of breeding work depends on the level of accuracy of the assessment and prediction of animal breeding value. In the process of industrial milk production, the use of high-intensity technologies does not always allow specialists to identify the advantages or disadvantages of dairy cows, or the potential of animals is evaluated directly during their operation [8, 9].

One of the main advantages of using DNA markers is an early assessment of animal genotype, which is not interrelated with gender, age and physiological state. It is not necessary to wait a lot of time to evaluate a dairy herd by the level of its own productivity [10].

The results of scientists' research indicate that markers in close relationship with the loci of productive traits are transmitted to offspring, which makes it possible to mark indicators characterizing the productive qualities of animals. Using the coupling of markers with productivity, breeders can select cows with great accuracy and reliability that have the necessary gene alleles [11, 12].

Thus, the genes of kappa-casein (CSN3), beta-lactoglobulin (BLG), leptin (LEP), prolactin hormones (PRL), somatotropin (GH), etc., according to scientists, are markers of milk productivity, qualitative and technological characteristics of milk [13, 14]. The results of the authors' research proved that the first heifers with the TT genotype of the TG5 gene (thyroglobulin) had a higher milk yield and mass fractions of fat and protein compared to animals of the CC genotype [15-17].

Scientists claim that quantitative traits, such as milk yield, depend not only on environmental conditions but are controlled by many genes. The scientific results obtained in the field of molecular genetics are used in cattle breeding and allow influence several economically important characteristics of cattle. Studies of the gene-hormone somatotropin (GH - growth hormone) have shown that its biological effect consists in stimulating postnatal growth and metabolism, as well as influencing the level of milk productivity and the qualitative composition of milk [18].

The diacylglycerol_O_acyltransferase 1 (DGATI1) gene, according to some data, influences the processes of lipoprotein binding, adipose tissue formation, lactation period. Cows of the black-and-white breed of the first lactation with the AK genotype of the DGATI1 gene surpassed their peers in protein-milk content, with the KK genotype - in fat-milk content, with the AA genotype - in milk yield [19]. The leptin gene (LEP) serves as a marker that determines the fat and protein

content in milk. The PCR analysis of Holstein cows during the first lactation carried out by scientists indicated polymorphism of the LEP gene. Animals with homozygous TT genotype had higher milk productivity [20].

It should be noted that at present the process of marker-directed animal breeding development is undergoing a crisis phase. The fact is that the DNA markers identified to date based on the results of associative studies do not, for the most part, provide a large-scale improvement in the breeding process. Over the past decades, genome-wide studies have not revealed new results of work with the genetic apparatus responsible for the level of dairy productivity of animals. And at the same time, the mechanisms for the formation of milk productivity indicators at the molecular level are poorly studied, the approaches currently used are unable to provide quick solutions to these problems.

In this regard, the comprehensive study and application of DNA technologies in conjunction with cow productivity indicators has clear prospects. There is a clear need for a more systematic analysis of the molecular mechanisms of the formation of milk productivity parameters of breeding herds of animals. This will make it possible to make a rational choice of candidate genes for associative research and their further use in the breeding.

This research determined the frequency of occurrence of DNA marker genotypes and conducted an associative analysis of polymorphic variants with milk productivity traits of black-and-white cows.

2 Materials and Methods

The research was carried out on Holstein cows of the black-and-white breed of a breeding herd of PJSC Kamenskoye in the Sverdlovsk region. PJSC Kamenskoye has four dairy farms, which contain 7700 heads. At the same time, 3,300 heads are animals of their own breeding. The volume of milk production at the enterprise is sufficient to occupy 36th place in the Russian Federation and ensure the supply of raw milk to dairy processing enterprises. This enterprise actively conducts breeding work with cattle, has more than 15,000 hectares of acreage, including grain crops for animal feeding.

The animals were genotyped using Bovine GGP 150K biochips ("Illumina/Neogen", USA) with a coverage density of 138974 SNP. The main characteristics of GGP Bovine 150K include the following: SNPS are specially selected for the high frequency of minor alleles and uniform genome coverage for most beef and dairy cattle breeds; includes both new effective markers of productivity and reproduction of cattle, and all widely used markers of the USDA and ISAG origin. For analysis, data on genes with polymorphic genotypes were processed: *CSN2, LGB*, *CRH, STAT1, TFAM1*, and *TFAM2*.

The CRH gene refers to markers that determine the term of pregnancy, as well as the time of calving, stimulating the release of adrenocorticotropic hormone from the hypophysis. The STAT1 gene is a member of the transcription factor family of signal transducers and transcription activators. The TFAM1 and TFAM2 genes have been identified as components of the mitochondrial

transcription initiation complex required for the basic transcription of mitochondrial DNA. The CSN2 gene is aimed at encoding β-casein, it is associated with an increase in the yield of milk and milk protein, which once again confirms the main role of the casein gene cluster in the effectiveness of milk productivity. The LGB gene encodes β-lactoglobulin and affects milk coagulability, milk yield, as well as protein and fat content [21].

The occurrence frequency of genotypes of the analyzed genes was calculated by the ratio of the number of cows from among the genotype carriers to the total number of animals in the study group.

Indicators of dairy productivity of animals were evaluated for 305 days of the first lactation in accordance with the "Procedure and conditions for carrying out bonitation of breeding cattle of dairy and dairy-meat productivity directions" (order of the Ministry of Agriculture of the Russian Federation No. 379 dd 28.10.2010).

The data obtained in the experiment were processed in the programs Microsoft Excel, Biostatistics when calculating the main statistical and biometric indicators. At the same time, the thresholds of statistically significant differences were determined at *p<0.05; **p<0.01; ***p<0.001.

3 Results and Discussion

The frequency of genotype occurrence in the cattle population may vary under the influence of various factors. Some processes (mutations, selection, gene drift) change the frequencies of genotypes. Considering the frequency of genotype occurrence allows an individual approach to the selection of animals, to increase the efficiency of breeding work in general.

Table 1: Frequency of occurrence of black-and-white cow genotypes

Gene	Genotype	Frequency of genotype occurrence, %
CSN2	AA	12
	AC	57
	CC	31
BLG	AA	6
	AG	47
	GG	47
CRH	AA	20
	AG	47
	GG	33
STAT1	AA	8
	AG	41
	GG	51
TFAM1	AA	14
	AG	59
	GG	27
TFAM2	AA	47
	AC	43
	CC	10

When determining the frequency of genotypes associated with indicators of milk productivity of cows, it was found that genotype AC (57.0 %) by gene *CSN2* was more frequent in animals; by gene *BLG* – genotype AG (47.0 %) and GG (47.0 %), by gene CRH – AG (47.0 %), STAT1 – GG (51.0 %), TFAM1 – AG (59.0 %), TFAM2 – AA (47.0 %) (Table 1).

When studying the *CSN2* gene and its conjugation with cow milk, it was found (Table 2) that in animals from among carriers of the C allele at the locus of this gene in the homozygous variant (CC), milk yield is higher compared with animal milk yield and the number of carriers of the AA genotype by 1048.0 kg (12.9%) (at p< 0,05) and genotype AC by 366.0 kg (4.5%). In addition, the C allele in the homo- and a heterozygous variant of the *CSN2* gene is associated with the fat mass fraction in milk (FMF) of the cows studied. Thus, the FMF of animals with the CC and AC genotype is higher by 0.12% (at p < 0.05) and by 0.9% (at p < 0.05) than the FMF of animals with the AA genotype for the analyzed gene. The difference between the indicators of protein mass fraction in the milk of cows carrying the *CSN2* gene has no significant differences. At the same time, the amount of milk protein in animals of different genotypes differed. In the owners of the homozygous CC genotype for the *CSN2* gene, the milk protein content is 36.8 kg more (at p<0.05) than in the group of cows with the AA genotype; 12.7 kg more than in cows with the AC genotype.

Table 2: Frequency of occurrence of genotypes and indicators of milk productivity of black-and-white cows, $\overline{X} \pm S_{\overline{x}}$

Gene	Genotype	Milk yield, kg	Fat mass fraction, %	Milk fat amount, kg	Mass fraction of protein, %	Milk protein amount, kg
CSN2	AA	7085±268.5	3.87±0.04	274.3±11.86	3.18±0.03	225.8±9.63
	AC	7767±273.9	3.96±0.02*	307.5±11.27*	3.22±0.02	249.97±8.71
	CC	8133±345.9*	3.99±0.03*	324.6±14.23*	3.23±0.02	262.6±10.8*
BLG	AA	8238	4.00	331.6	3.24	265.8
	AG	8059±267.4	3.94±0.02	317.7±10.75	3.23±0.02	259.9±8.26
	GG	7490±277.3	3.97±0.03	297.4±11.67	3.21±0.02	240.4±9.18
CRH	AA	7922±421.1	3.98±0.04	314.9±16.49	3.25±0.02	256.7±13.32
	AG	7665±258.0	3.97±0.03	304.1±10.71	3.22±0.02	247.1±8.38
	GG	7924.6±398.2	3.93±0.03	312.4±16.97	3.20±0.02	253.3±12.60
STAT1	AA	8605±247.3	4.02±0.06	345.6±5.52	3.21±0.04	276.9±11.52
	AG	7738±344.7	3.99±0.04	308.7±14.12*	3.22±0.02	248.8±10.64
	GG	7729±258.0*	3.92±0.01	303.6±10.89**	3.22±0.02	248.9±8.47
TFAM1	AA	6543±429.2	3.93±0.06	256.9±16.00	3.25±0.04	212.2±14.35
	AG	7780±257.6*	3.96±0.03	308.6±11.00*	3.21±0.01	249.9±8.19*
	GG	8478±265.1***	3.96±0.03	335.8±10.65***	3.22±0.02	273.1±8.57**
TFAM2	AA	7446±293.4	3.96±0.03	295.2±12.54	3.22±0.02	239.5±9.10
	AC	8043±290.9	3.94±0.02	317.6±11.85	3.22±0.02	259.9±9.66
	CC	8444±430.3	4.00±0.06	337.3±16.27*	3.17±0.02	267.8±13.39

Animals - carriers of homozygous AA genotype for the *STAT1* gene significantly (at $p<0.05$) surpassed cows with the GG genotype in terms of the amount of milk obtained in 305 days of the first lactation, by 876.0 kg (10.2%). The difference between the amount of milk fat, in this case, was 42.0 kg (at $p<0.01$). In addition, the milk fat content in the milk of cows of the AG genotype (STAT1 gene) was 36.9 kg less (at $p<0.05$) than in animals with the AA genotype for this gene.

The studies established the superiority of cows with the GG and AG genotypes of the TFAM1 gene over animals with the AA genotype in milk yield by 1935.0 kg (22.8%) (at $p<0.001$) and 1237.0 kg (15.9%) (at $p<0.05$), respectively; in milk fat amount – by 78.9 kg (at $p < 0.001$) and 51.7 kg (at $p < 0.05$), respectively; in milk protein amount – by 60.9 kg (at $p < 0.01$) and 37.7 kg (at $p < 0.05$), respectively.

The analyzed indicators of milk productivity of black-and-white cows also differed in the group of animals from among the carriers of the TFAM2 gene. Thus, the milk fat content in cows with the homozygous CC genotype was higher by 42.1 kg (at $p<0.05$) compared to animals of the AA genotype; 19.7 kg more - compared with animals of the AC genotype.

In our studies, as in the works of other authors [15, 18, 20], in most cases, animals with homozygous genotypes were superior in the amount of milk obtained. The difference in milk yield, fat content and protein content were not significant in all groups, nevertheless, there was a tendency for homozygous genotypes to be superior to heterozygous genotypes.

4 Conclusion

Thus, the animal genotype, or rather, the profile of polymorphic variants of many genes contributes to the development of qualitative and quantitative characteristics of milk. The use of DNA markers of milk productivity in marker-directed breeding will improve the productivity of animals, as well as the efficiency of production of high-quality dairy products. Cattle genotyping by candidate genes for controlling parameters of milk productivity with subsequent selection will strengthen positive marker-directed breeding in relation to increasing economically valuable milk indicators.

Milk yield, fat, and protein content in milk, as well as the amount of milk fat and milk protein is higher in animals from the number of carriers of the gene *CSN2* with the homozygous CC genotype, gene *BLG* – with heterozygous AG genotype, gene *CRH* – with AA genotype, gene *STAT1* – with AA genotype, gene *TFAM1* – with GG genotype and gene *TFAM2* – CC. The results obtained in our studies allow us to judge that DNA diagnostic methods provide objective information about the presence of the genetic potential of individual animals and breeding herds. Purposeful selection of dairy cows from among the carriers of the most preferred genotypes contributes to increasing the efficiency of breeding work.

The data obtained in the future can be used by specialists in breeding work in the development and implementation of programs for the breeding improvement of black-and-white cattle herds with the use of DNA technologies.

5 Availability of Data and Material

Data can be made available by contacting the corresponding author.

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