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АССОЦИАЦИИ ПОЛИМОРФИЗМА ГЕНА СОМАТОТРОПИНА (GH) С ПОКАЗАТЕЛЯМИ МОЛОЧНОЙ ПРОДУКТИВНОСТИ КОРОВ

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Исследования проведены в лаборатории молекулярно-генетической экспертизы в 2018 и 2019 гг. в рамках государственного задания по теме «Разработка селекционно-генетических и теоретических основ сохранения и эффективного использования генофонда крупного рогатого скота в Уральском регионе с применением современных биотехнологий». Объект исследования – крупный рогатый скот голштинизированной черно-пестрой породы. Основная задача – анализ степени влияния полиморфизма гена соматотропина (GH) на показатели молочной продуктивности. В процессе изучения выделено ДНК 270 коров, на основе полученных данных определен полиморфизм гена соматотропина, установлена взаимосвязь различных генотипов с показателями живой массы, удоя, массовой доли белка и массовой доли жира в молоке. Результаты биометрически обработаны при помощи программы IBM SPSS Statistics 23. Выявлено, что *LL* является основным генотипом гена GH среди представителей голштинизированной черно-пестрой породы (частота встречаемости 77,0%). Доля генотипов *LV* и *VV* в популяции составляет 21,9 и 1,1% соответственно. Во время наблюдения коровы – носительницы генотипа *LL* – показывали более высокие удои по первой и третьей лактациям. Разница со сверстницами у них составила 202–2334 кг ($p \leq 0,05$). Зафиксирована тенденция к увеличению содержания белка в молоке у животных с генотипом *VV*. По первой лактации разница с носительницами генотипов *LL* и *LV* достигала 0,03 и 0,06% соответственно. По третьей лактации особи с генотипом *VV* превосходили сверстниц по содержанию белка на 0,07 и 0,12% соответственно.

Ключевые слова: крупный рогатый скот, соматотропин, полиморфизм, удой, массовая доля жира, массовая доля белка, живая масса, первая лактация, третья лактация

ASSOCIATIONS OF SOMATOTROPIN (GH) GENE POLYMORPHISM WITH LACTATION PERFORMANCE OF COWS

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The research was conducted in the laboratory of molecular genetic expertise in 2018 and 2019 within the framework of the state assignment on the topic "Development of genetic-selection and theoretical foundations for the conservation and effective use of the gene pool of cattle in the Ural region with the use of modern biotechnologies". The object of the study is the cattle of Holsteinized black-and-white breed. The main task is to analyze the degree of influence of the somatotropin (GH) gene polymorphism on lactation performance indicators. In the course of the study, DNA from 270 cows was isolated, the polymorphism of the somatotropin gene was determined based on the obtained data, and the relationship between different genotypes and indicators of live weight, milk yield, protein mass fraction and fat mass fraction in milk was established. The results were biometrically processed using IBM SPSS Statistics 23 software program. It was revealed that *LL* is the main genotype of the GH gene among the representatives of the Holsteinized black-and-white breed (frequency of occurrence 77.0%). The proportion of *LV* and *VV* genotypes in the population is 21.9 and 1.1%, respectively. During the observation period, cows carrying the *LL* genotype showed higher milk yields in the first and third lactations. The difference with their peers was 202-2334 kg

($p \leq 0.05$). A tendency towards increased protein content in milk was recorded in animals with the *VV* genotype. In the first lactation, the difference with *LL* and *LV* genotype carriers was 0.03 and 0.06%, respectively. In the third lactation, individuals with the *VV* genotype surpassed their female counterparts in protein content by 0.07 and 0.12%, respectively.

Keywords: cattle, somatotropin, polymorphism, milk yield, MFF, MFP, live weight, first lactation, third lactation

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Conflict of interest
The authors declare no conflict of interest.

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INTRODUCTION

The expansion of marker selection in livestock breeding has contributed to the improvement of dairy and meat product supply in our country. Studying candidate genes such as kappa-casein, lactoglobulin, calpain, and somatotropin allows us to use selection to obtain animals with high milk yields, protein content in milk, meat tenderness, and so on. Additionally, the investigation of the genetic structure of populations of Bos taurus cattle of different breeds is of significant importance[1, 2]. For example, it has been found that the GHLL genotype and GHL allele are predominant in the Holstein and Holsteinized cattle [1], while the L allele is more characteristic of the Ayrshire breed (occurring in 61.5% of individuals). Among the Yaroslavl breed, 65% of animals have the LL genotype [2, 3]. The Hereford breed predominantly exhibits the LL genotype of the somatotropin gene [4]. Furthermore, studies have been conducted on the Simmental and Ukrainian Red Steppe breeds of Bos taurus cattle[3] [5].

Since somatotropin (GH) is a growth hormone, it can be assumed that its gene is associated with the body weight of cattle[4, 5]. There is also evidence of a correlation between the somatotropin gene and milk productivity [6–9]. Similar studies have been conducted in Russia,

[1]*Tyulkin S.V., Akhmetov T.M., Valiullina E.F., Vafin R.R.* Polymorphism on the genes of somatotropin, prolactin, leptin, thyroglobulin bulls-producers // Vavilov Journal of Genetics and Breeding. 2012. N 4 (16). pp. 1008–1012.

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[3] *Dolmatova I.Yu., Ilyasov A.G.* Relation of polymorphism of somatotropin gene polymorphism of Simmental cattle with productivity // Zootechniya. 2008. N 5. pp. 6–8.

[4]*Soloshenko V.A., Goncharenko G.M., Inerbaev B.A., Khramtsova I.A., Goryacheva T.S., Grishina N.B.* Influence of polymorphisms of thyroglobulin and somatotropin genes on the intensity of growth in cattle // Problems of productive animal biology. 2011. N 1. pp. 55–58.

[5]*Grala T.M., Phyn C.V., Sheahan A.J., Lee J.M., Roche J.R., Lucy M.C.* Somatotropic axis and concentrate supplementation in grazing dairy cows of genetically diverse origin // Journal of dairy science. 2011. N 1 (94). pp. 303–315.

Indonesia, Turkey, China, and India[6-9] [10-12].

The purpose of our research was to determine the extent of the influence of somatotropin gene polymorphism on milk productivity and body weight of the Holsteinized Black-and-White cattle. The objectives included analyzing the prevalence of different genotypes and alleles of somatotropin in the population, as well as assessing the specific genotypes' impact on milk yield, milk fat content (MFC), and milk protein content (MPC).

MATERIAL AND METHODS

For the first time in the Urals region, genetic material from Holsteinized Black-and-White cattle was used to study the polymorphism of the somatotropin gene and its association with economically valuable traits. The research was conducted in 2018 and 2019 at the Laboratory of Molecular Genetic Expertise. Blood samples were collected from 270 representatives of the Black-and-White Holsteinized cattle for DNA diagnostics. Blood samples were taken from the tail vein and collected in tubes containing 100 mM EDTA (ethylenediaminetetraacetic acid) to a final concentration of 10 mM. DNA extraction was performed according to the protocol of "Sintol" company (Russia). Genotyping of the animals for the GH gene was carried out using the PCR-RFLP method based on the technique developed by S.V. Tyulkina et al. (see footnote 1) with the Bio-Rad PTC-225 DNA Engine Tetrad Cycler (Bio-Rad Lab., USA).

The list of economically valuable traits (milk yield, MFC, MPC, body weight) was compiled based on the "SELEX. Dairy Cattle" program. The study utilized the data from the first and third lactations (305 days each), as cows typically have an average productive lifespan of three lactations. This approach allows for observing the influence of the genotypes throughout the entire period of animal use.

The frequency of the genotype occurrence was calculated using the formula

$$p = n/N,$$

where p is the frequency of the genotype, n is the number of individuals with a specific genotype, and N is the total number of the animals. Mean values of the studied traits and their standard errors were determined. The results were analyzed using the IBM SPSS Statistics 23 software.

RESULTS AND DISCUSSION

First, the genotypes of the somatotropin gene present in the population were determined (see Figure 1). It was found that the *LV* polymorphism of the growth hormone gene is represented by two alleles - *L* and *V*, resulting in three genotypes: *LL*, *LV*, and *VV*. The *LL* genotype is represented by a single lower band at position 159 bp, the *VV* genotype is represented by a single upper band at position 211 bp, and the *LV* genotype is represented by two bands at positions 159 bp and 211 bp.

Next, the frequency of occurrence of the *LV* polymorphism of the somatotropin gene in the studied animals was determined (see Figure 2).

The most common genotype was found to be *LL*, with a frequency of occurrence of 77.0%. The *LV* genotype ranked second with a frequency of 21.9%. The *VV* genotype was extremely rare, observed only in 1.1% of the animals. The frequency of the *L* allele was 0.883%, while the *V* allele frequency was 0.117%. Similar data have been reported by other researchers [2, 12].

Table 1 presents the results of studying the association between somatotropin genotypes and economically valuable traits of cows based on data from the first lactation.

Thus, cows with the *LL* genotype produced 602 kg more milk compared to the animals with the *LV* genotype ($p \leq 0.01$) and 2334 kg more

[6]*Pawar R.S., Tajane K.R., Joshi C.G., Rank D.N., Bramkshtri B.P.* Growth hormone gene polymorphism and its association with lactation yield in dairy cattle // Indian journal of animal sciences. 2007. N 9 (77). pp. 884–888.

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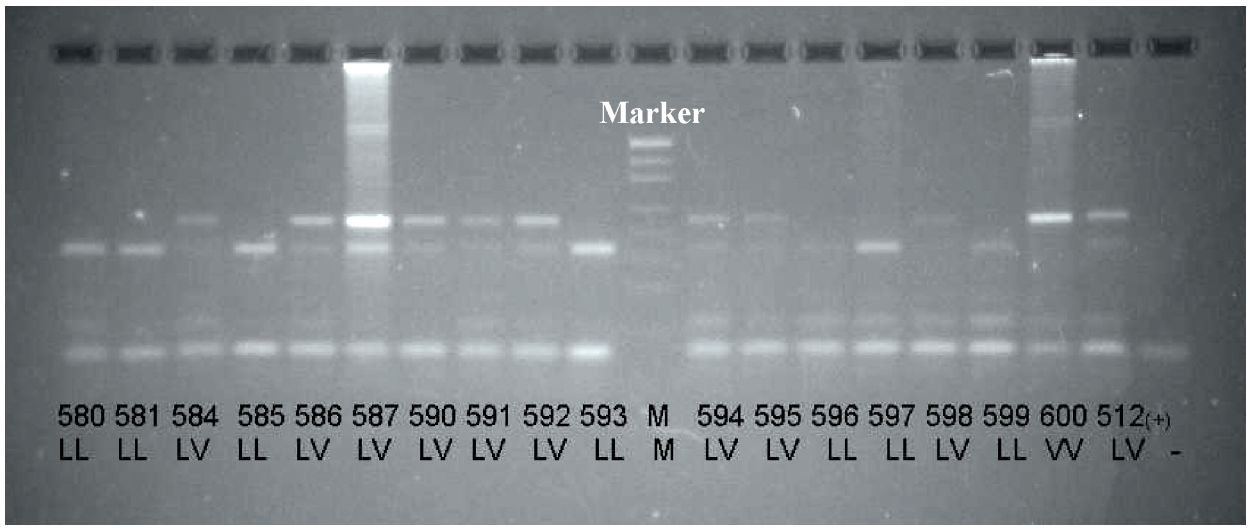

Рис. 1. Фореграмма *LV*-полиморфизма гена соматотропина:
М – маркер молекулярного веса; 580, 581, 585, 593, 596, 597, 599 – генотип *LL*; 584, 586–592, 594, 595, 598, 512 (положительный контроль) – генотип *LV*

Fig. 1. Foregram of *LV*-polymorphism of the somatotropin gene:
M – molecular weight marker; 580, 581, 585, 593, 596, 597, 599 – *LL* genotype; 584, 586-592, 594, 595, 598, 512 (positive control) – *LV* genotype

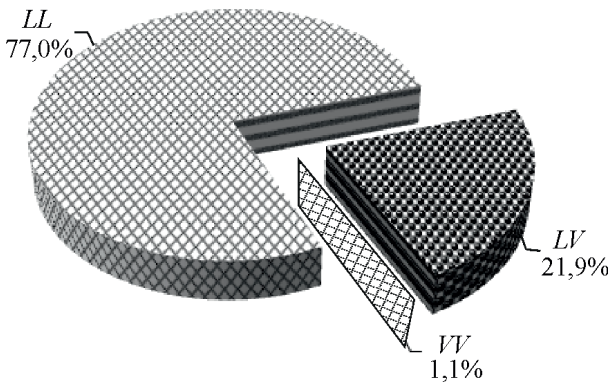

Рис. 2. Частота встречаемости различных генотипов гормона роста в выборке

Fig. 2. The occurrence of growth hormone genotypes in the sample

milk compared to individuals with the VV genotype ($p \leq 0.05$). In terms of the body weight, individuals with the *LL* genotype exceeded carriers of the *LV* genotype by 17 kg ($p \leq 0.01$) and carriers of the *VV* genotype by 2 kg.

Regarding milk fat content, cows with the *VV* genotype outperformed animals with the *LL* genotype by 0.16%. Carrier cows of the *LL* genotype had a 0.03% higher milk protein content compared to carriers of the *LV* genotype ($p \leq 0.05$). Additionally, cows with the *VV* genotype had the highest protein content at 3.12%.

Table 2 presents the results of studying the association between cow body weight, milk

Табл. 1. Молочная продуктивность и живая масса коров с различными генотипами соматотропина в период первой лактации ($n = 270$; $X \pm S_x$)

Table 1. Indicators of milk productivity and live weight of cows for the first lactation with different genotypes of somatotropin ($n = 270$; $X \pm S_x$)

Genotype	Number of heads	Milk yield, kg	MFF, %	MFP, %	Live weight, kg
LL	206	8008 ± 103	3,99 ± 0,01	3,09 ± 0,01	577 ± 2
LV	62	7406 ± 165	3,96 ± 0,03	3,06 ± 0,01	560 ± 5
VV	2	5674 ± 875	4,15 ± 0,20	3,12 ± 0,10	575 ± 177
LV–LL	–	−602**	−0,03	−0,03*	−17**
VV–LL	–	−2334*	0,16	0,03	−2

*$p \leq 0,05$.
**$p \leq 0,01$.

Табл. 2. Молочная продуктивность и живая масса коров с различными генотипами соматотропина в период третьей лактации ($n = 270$; $X \pm S_x$)

Table 2. Indicators of milk productivity and live weight of cows for the third lactation with different genotypes of somatotropin ($n = 270$; $X \pm S_x$)

Genotype	Number of heads	Milk yield, kg	MFF, %	MFP, %	Live weight, kg
LL	206	9297 ± 132	3,95 ± 0,01	3,17 ± 0,01	644 ± 4
LV	62	9095 ± 264	3,98 ± 0,03	3,12 ± 0,02	644 ± 9
VV	2	7106 ± 537	3,96 ± 0,52	3,24 ± 0,15	661 ± 69
LV–LL	–	−202	0,03*	−0,05	0
VV–LL	–	−2191*	0,01	0,07	17

*$p \leq 0{,}001$.

productivity during the third lactation, and the *LV* polymorphism of the somatotropin gene.

It was found that animals with the *LL* genotype had a milk yield that was 202 kg higher than animals with the *LV* genotype and 2191 kg higher than animals with the *VV* genotype ($p \leq 0.001$). Cows carrying the *VV* genotype had a higher milk protein content, with a difference of 0.07% to 0.12% compared to their counterparts. In terms of milk fat content, individuals with the *LV* genotype surpassed carriers of the *LL* and *VV* genotypes by 0.03% and 0.02%, respectively. Animals with the *VV* genotype had a higher body weight compared to animals with the *LL* and *LV* genotypes, with a difference of 17 kg. Due to the low prevalence of the *VV* genotype, it is difficult to draw conclusions about its influence on body weight and economically valuable traits.

CONCLUSION

Thus, it has been established that in the population of Holsteinized Black and White cattle carriers of the *LL* genotype significantly predominate, while individuals with the *VV* genotype are rare. The *L* allele is present in 88% of the animals.

Based on the results of the first lactation, milk productivity of cows with the *LL* genotype was found to be 600-2000 kg higher compared to the carriers of other genotypes. Differences in milk fat and protein content were observed in the carriers of the *VV* genotype, which is associated with low milk productivity.

In the results of the third lactation, cows with the *LL* genotype produced 200-2000 kg more milk compared to the carriers of other genotypes. Animals with the *VV* genotype had lower milk yield but higher protein content in the milk.

Based on the conducted research, it can be concluded that the *LL* genotype contributes to high milk production in Holsteinized Black and White cows, while animals with the *VV* genotype tend to have higher protein content in the milk. Therefore, it is recommended for agricultural organizations to conduct selective breeding work to increase the number of the cows carrying the *LL* genotype in order to enhance the milk productivity of their herds.

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