



Molecular-genetic aspects of the POU 1 F 1 gene in Holstein reproductive traits

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ABSTRACT

The development of DNA technologies within the framework of the concept of gene marker selection allows us to evaluate animals by allelic variants of genes, directly or indirectly related to productive features. The authors study the polymorphism of the POU1F1 gene and its relation to the milk productivity of Holstein breed cows. As part of the study the samples of biological materials were taken from 50 animals, commercial sets were used to isolate genomic DNA, and polymorphism was analyzed according to a standard procedure. The results of the studies showed that the polymorphism of the POU1F1 gene has a significant effect on the average daily yield of milk yield and milk yield for 305 days of lactation in Holstein breed cows. The authors also found out that alleles, but not genotypes, have the greatest influence on the symptoms. The conducted studies allow to suggest that the diagnosis of allelic variants of the POU1F1 gene can be used in practical work to select heifers genetically predisposed to the highest milk yields.

Keywords: *Holstein breed, POU1F1 gene polymorphism, milk productivity*

INTRODUCTION

The development and functions of the breast are controlled mainly by growth hormones and prolactin secreted by the anterior pituitary gland. Their synthesis is influenced by the pituitary transcription factor-1 (PIT1 or POU1F1). The study of the genetic bases of selection-significant signs of agricultural production. animals, characterized by a polygenic type of inheritance, is of fundamental importance for the effective development of the livestock sector. In selection work, it is necessary to use methods that allow at an early stage to identify animals with the best genetic potential, possessing not only high productivity, but also capable of persistently transmitting these qualities to offspring. In cattle POU1F1 gene is localized in chromosome 1q21-22 and, to date, is considered as a promising genetic marker of milk productivity. A new stage in the analysis of the genetic foundations of quantitative traits is associated with the advent of the method of genome-wide association (GWAS) and the development of next generation sequencing technologies (NGS). In recent years, GWAS has been successfully used in the study of domestic animals: cattle, pigs, horses, dogs, sheep and chickens, etc. [1, p. 221-237]. The high density of SNP markers makes it possible to accurately identify the relationship

between loci, limited SNP markers and traits, taking into account coupling disequilibrium, localize target genes. Despite the high efficiency and productivity of the GWAS method, the high cost of implementing this method today is the limiting factor of its wide distribution in breeding programs. An alternative approach in practical selection can be mononucleotide replacements (SNP, English Single nucleotide polymorphism), defined in genes that are associated with selection-valuable traits of agricultural products. animals. In this connection, the study of polymorphism of genes capable of acting as genetic markers of milk productivity of cows is especially topical in the theoretical and practical sense. One of the genes of interest in this direction is the pituitary transcription factor gene (*POU1F1*). Transcription factors are a group of proteins capable of interacting with regions of DNA located in regulatory regions of genes, initiating a program of increasing or decreasing transcription [2, p. 203-210]. The pituitary transcription factor regulates the expression of growth hormone (*GH*), prolactin (*PRLR*) and thyroid-stimulating hormone (*TSH*) genes [3, P. 523-540]. In cattle, gene *POU1F1* (Gene ID: 282315) is located in 1 chromosome. The aim of the work is to study the polymorphism of the *POU1F1* gene and its relation to the milk productivity of Holstein cows imported to the breeding farms of the Rostov Region of the Russian Federation.

MATERIALS AND METHODS

Molecular genetic studies were carried out in the laboratory of molecular diagnostics and biotechnology of agricultural plants. animals DonGaU. To assess the effect of polymorphism of the *POU1F1* gene on the productive qualities of cows, a group of animals (n = 50) with the same terms of the first calving (January-February 2015) was selected. Genomic DNA was isolated from blood samples. Blood was taken from the stitched vein into vacuum

tubes with EDTA in an amount of 1-2 ml. DIAtomTMDNAPrep reagent kits (IsoGeneLab, Moscow) were used to isolate genomic DNA. The polymorphism of the gene was determined by PCR-RFLP. To amplify a fragment of the *POU1F1* gene 451 bp long, oligonucleotide primers were used: F5'-AAA CCA TCA TCT CCT TTS TT-3' and R 5'-AAT GTA CAA TGT GCC TTC TGA G -3' [4]. Amplification mode: first cycle 94 ° C - 5 min; subsequent 35 cycles - 94 ° C - 30c, annealing 56 ° C - 30c, elongation 72 ° C - 45c; the final cycle is 72 ° C - 10 min. Restriction of the amplified fragment was carried out with the restriction enzyme *HinfI* (OOO SibEnzim-M). The size of the restriction products was determined by electrophoresis in a 2% agarose gel. Genotype AA corresponded to one fragment of 451 bp long; genotype BB - two fragments of 244 and 207 bp. and genotype AB three fragments of 451, 244 and 207 bp. Studies were carried out on cows-primordelkah Holstein breed of the breeding farm of the Rostov region. To assess the milk productivity of cows used data of documents of primary zootechnical accounting, pedigree cards of cows 2-MOL, data of the program "Selax". The following parameters were taken into account: lactation duration (days); milk for 305 days of lactation (kg) and daily average milk yield (kg). A single-factor analysis of variance was used to measure the statistical effect of gene polymorphism on characteristics. The *POU1F1* gene was considered as a factor with three gradations (genotypes AA, AB and BB).

RESULTS OF THE STUDY

Molecular genetic studies of Holstein cows showed polymorphism of the *POU1F1* gene, caused by the alleles A and B. The allele B and the BB genotype had the highest frequency in the studied group of cows (Fig. 1).

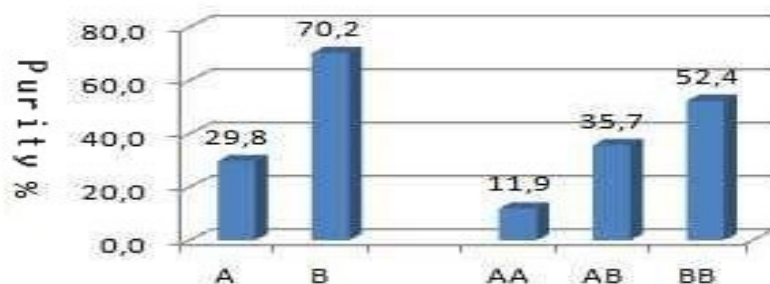


Fig. 1 - Frequency of alleles and genotypes of the *POU1F1* gene

During the lactation period, the amount of daily milk yield is subject to change. After calving, daily milk yields increase and reach the maximum values (the end of the first - the beginning of the second month) and gradually decrease to start. The graphical representation of the lactation stroke reflects the features of the lactation curve, which depend on

individual indices. When studying the lactational curves of cows, taking into account the genotypes of the *POU1F1* gene, the results showed that over the entire lactational period, cows with AA and AB genotypes had higher daily average milk yields compared to cows with the AA

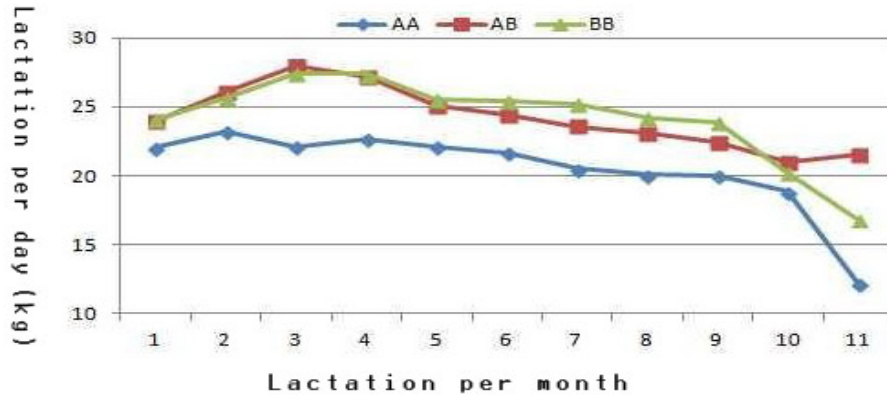


Fig. 2 - Lactational curves of cows of different genotypes of the POU1F1 gene

The results of a single-factor analysis showed that the factor of polymorphism of the *POU1F1* gene significantly affects the average daily yield of cows and milk over 305 days of lactation (Table). The presence of genotype BB in cows, in comparison with the AA genotype, is associated with the best indicators of daily average milk yield by 3.8 kg and milk yield for 305 days of lactation by 1158.2 kg. It should be noted that there was no difference between the cows of the genotypes BB and AB. We assumed that in this case the greatest influence on the signs is

not on genotypes, but on alleles. To test our hypothesis, the *POU1F1* gene factor was divided into two grades (the presence of allele B and the absence of B allele) and as a result, the reliability of the effect of the factor on the analyzed traits (daily average yield and milk yield for 305 days of lactation) reached $P = 0.009$. There were no significant differences for the lactation period and total milk yield for lactation associated with the polymorphism of the *POU1F1* gene in the study group.

Table 1 - Indicators of milk productivity of cows of different genotypes of the gene *POU1F1*

Genotype	Lactation period, days.	Total milk yield for lactation, kg	Average daily milk yield, kg	Done for 305 days. lactation, kg
AA	380.00 ± 44.95	7484.61 ± 631.66	20.01 ± 0.75	6104.73 ± 228.98
AB	391.11 ± 22.97	9357.13 ± 600.31	23.93 ± 0.74	7299.5 ± 224.50
BB	375.64 ± 16.92	8949.81 ± 475.79	23.81 ± 0.69	7262.92 ± 212.17
P-value	0.85	0.26	0.035	0.035

Studies have shown that the presence of allele B of the *POU1F1* gene is associated with higher yields. Similar results were obtained in studying the effect of polymorphism of the *POU1F1* gene on the milk productivity of red-steppe cows [4, pp. 142-146], [5, pp. 29-33]. The results of studies of Holstein cows bred in Iran also showed the effect of *POU1F1* gene polymorphism on milk yield over 305 days. lactation, but with the best performance indicators, the

heterozygous genotype AB was associated [6, P. 503-507]. In their work, Jia X. et al. [7, pp. 1359-1365] attempted to evaluate the joint effect of the *POU1F1* and *PRL* genes on the productivity of dairy cows.

The results showed effects separately for each gene, but when combined, their effect was not summarized and the *POU1F1* gene effect was more significant.

CONCLUSIONS

As a result of the studies conducted, the effect of genotypes on the mass fraction of fat and protein in milk was not established, but the relationship of polymorphism of the *POU1F1* gene to milk yield for lactation was determined. The presence of polymorphism of the *POU 1 F 1* gene in Holstein cows and its relation to the signs of milk production. With the best indicators of the average daily milk yield and milk yield for 305 days of lactation, allele variant B of the *POU 1 F 1* gene is associated. Thus, studies conducted under different conditions show a significant effect of the polymorphism of the *POU 1 F 1* gene on the dairy productivity of cows and the possibility of using it as a genetic marker in the breeding and breeding work of dairy cattle. Diagnosis of allelic variants of the *POU 1 F 1* gene can be used in practical work to select heifers genetically predisposed to high average daily yield.

REFERENCES

1. Meuwissen T. Accelerating improvement of livestock with genomic selection / T. Meuwissen, B. Hayes, M. Goddard // Annu. Rev. Anim. Biosci. - 2013. - Vol. 1. P. 221-237.
2. Chung H. Molecular cloning of Pit-1 cDNA from porcine anterior pituitary and its involvement in pituitary stimulation by growth hormone releasing / H. Chung, T. Kato, K. Tomizawa, Y. Kato // Exp Clin Endocrinol Diabetes. - 1998. - Vol. 106.-P. 203-210.
3. Cohen L. Role of Pit-1 in the gene expression of growth hormone, prolactin and thyrotropin / L. Cohen, F. Wondisford, S. Radovick // Endocrinol Metab Clin North Am. - 1996. Vol. 25. - P. 523-540.
4. Carsai T. The Polymorphism of Pituitary Factor 1 (*POU1F1*) in Cattle / T. Carsai, V. Balteanu, A. Vlaic, V. Cosier // Anim Sci and Biotech. - 2012. Vol. 45 (1). - P. 142-146.
5. Leonova MA Genetic markers of cow milk Productivity of the red steppe breed / MA Leonova, LV Getmantseva, A.Yu. Kolosov, VN Pristupa // Science almanac of the Black Sea region countries. - 2015. Vol. eleven). - P. 29-33.
6. Heidari M. Effect of polymorphic variants of GH, Pit-1, and beta-LG genes on milk production of Holstein cows / M. Heidari, M. Azari, S. Hasani, A. Khanahmadi, S. Zerehdaran // Rus. J. Genet. - 2012. Vol. 48.-P. 503-507. doi: 10.1134 / S1022795412040060
7. Jia X. Polymorphism of *POU1F1* gene and PRL gene and their combined effects on milk performance traits in Chinese Holstein cattle / X. Jia, C. Wang, G. Yang, J. Huang, et al // Yi Chuan. - 2011. Vol. 33. P. 1359-1365