Association of LEP gene polymorphism with biochemical parameters of lipid metabolism and milk productivity of Holstein cattle

*Natali*a Safina[1,*], *Gazimzyan* Sharafutdinov[2], *Tahir* Akhmetov[3], *Rustam* Ravilov[3], and *Fanil* Vafin[4]

[1]Tatar Scientific Research Institute of Agriculture "Kazan Scientific Center of Russia Academy of Sciences", 420059 Kazan, Russian Federation
[2]Kazan State Agrarian University, 420015 Kazan, Russian Federation
[3]N.E. Bauman Kazan State Academy of Veterinary Medicine, 420029 Kazan, Russian Federation
[4]Federal State Budgetary Scientific Institution «Federal Center for toxicological, radiation, and biological safety», 420075 Kazan, Russian Federation

Abstract. The aim of the work was to study serum biochemical parameters and qualitative composition of milk of cows with various genotypes of the LEP gene. The research was conducted in 148 Holstein cow-heifers of Integrated Agricultural Production Centre "Stud farm named after Lenin" of the Atninsky district of the Republic of Tatarstan. Cattle genotyping was conducted by the AC-PCR method at the laboratory of the Department of Agrobiological Research of Tatar Research Institute of Agriculture of FRC KazanSC of RAS. The findings of allele and genotype calling of the LEP gene showed that the population under study is polymorphic and differs in genetic biodiversity. The study of serum biochemical parameters of experimental animals testified that the level of triglycerides, cholesterol and lipase is meaningfully lower in the blood of animals with the TT genotype. This indicates the rate of lipid metabolism in their body. During the analysis of milk productivity and parameters of the qualitative composition of milk, it was found that cow-heifers with the TT genotype of the LEP gene were superior to animals with other LEP gene genotypes in terms of milk yield for standard lactation (305 days), fat mass fraction, milk fat yield, milk fat and milk protein yield in total. Thus, it may be concluded that the TT genotype of the LEP gene has a positive effect on the economically important characters of cattle, which can be used in breeding in the future.

1 Introduction

Identification of the genes responsible for economic characters and further determination of the desired genotypes of the animals under study became possible with the introduction of the methods of molecular genetics into the selection of farm animals along with traditional methods. Genes regulating homeostasis and lipid metabolism, the

* Corresponding author: natysafina@gmail.com

polymorphic variants of which affect the qualitative composition of milk, in particular the content of fat and protein, are of special interest in dairy breeding.

Structurally leptin is a protein. It consists of 167 amino acids and has a molecular weight of 16 kDa. Leptin is mainly produced in adipose tissue and is secreted into blood circulation after the cleavage of a 21-amino acid signal peptide. The secretion occurs in response to changes in body fat percentage or energy status [1]. Its end product, the leptin protein, has an autocrine effect that inhibits insulin-stimulated glucose uptake and reduces adipogenesis of adipose tissue [2]. The bovine LEP gene is mapped on chromosome 4 and consists of 3 exons and 2 introns. About 60 SNP polymorphisms of the leptin gene are described in the literature. The LEP mutation (SNP T→C) leading to the replacement of cysteine with arginine (Arg73Cys) in the α-helix of the leptin polypeptide is identified at position 73 of the coding region [3, 4]. Leptin is interesting in breeding programs because it largely determines the milk productivity of livestock, the fat and protein content in milk, and also affects the duration of economic use and the productive longevity of cattle [5, 6].

Leptin also plays a major role in the control of body growth, adaptability, immune function, angiogenesis, renal function, haematopoiesis, reproduction, and not only acts as an endocrine signal in the brain and different peripheral tissues in which leptin receptors are expressed in fatal tissue, mammary gland, rumen, abomasum, duodenum and pituitary gland [7]. According to the intensity of metabolic processes occurring during lactation, and according to the biochemical serum parameters, it is possible to judge the amount of milk produced by milk cows, as well as its components and properties [8]. Leptin acts as a sensor of energy balance and metabolism. Its synthesis is conditional on the amount of fat in the animal's body. Produced directly into the blood circulatory system, leptin reflects the content of triacylglycerols in lipid depots [9, 10]. The functions of the endocrine system and signaling pathways within cells directly depend on the provision of the body with energy produced by blood lipid fractions, such as phospholipids, cholesterol, triglycerides and their derivatives [11].

The aim of the work was to study the biochemical parameters of serum lipid metabolism and the qualitative composition of milk in cows with various genotypes of the LEP gene.

2 Materials and methods

In the course of the experiment conducted in 2018-2019 on the basis of the Department of Agrobiological Research of Tatar Research Institute of Agriculture of FRC KazanSC of RAS and on the basis of Integrated Agricultural Production Centre “Stud farm named after Lenin” of the Atninsky district of the Republic of Tatarstan, biological samples were taken. The whole blood of 148 Holstein cow-heifers was drawn from the tail vein into preservative tubes containing EDTA anticoagulant (APEXLAB, China). Purified DNA was isolated from the obtained samples using the “AmpliPrime DNA-sorb-B” kit (NextBio, Russia). A set of primers was used to identify genotypes by allele-specific polymerase chain reaction (AC-PCR) [12, 13].

The reaction mixture, with a total volume of 20 µl, including the specified primers with a use rate of 0.25-1.00 µm, also included 2 µl of dNTPs, 0.2 µl of the Taq DNA polymerase with 2 µl of the Taq buffer supplied with it, 2 µl of purified DNA and deionized H2O (Sibenzyme, Russia).

The reaction mixture was amplified on “T-100 Thermal Cycler” and “My Cycler” thermocycling devices (Bio-Rad, USA) under specifically developed temperature and time conditions optimized for the primers used (Table 1).

Table 1. Temperature and time conditions for AC-PCR

preliminary denaturation	denaturation	annealing	elongation	cycles	final elongation
94 °C 5 min	94 °C 10 sec	60 °C 10 sec	72 °C 10 sec	40	72 °C 5 min

Visualization of reaction products after electrophoretic separation in agarose gel was carried out in a UV transilluminator, followed by fixation and documentation in the Gel&Doc system (Bio-Rad, USA). The detection of the LEP gene polymorphism under study for CC, TC and TT genotypes was carried out by the detectable mutation (SNP T→C Arg73Cys) of the DNA sequences.

The frequency of occurrence of allelic variants and genotypes was calculated according to the guidelines [14]. The significance of the variability between the expected and observed distribution of genotypes of the PON1 gene was tested by Pearson's chi-squared test (χ^2) and for compliance with the Hardy-Weinberg law of genetic equilibrium in the population.

Data on milk yield were obtained from the established electronic file "SELEX. Dairy cattle w.7.1.0.0." (AWS Plinor, Russia). The content of the mass fraction of fat and protein was determined on "Clever-2M" device in accordance with the shop instructions (Biomer, Russia).

Biochemical analysis of blood serum samples in terms of lipid metabolism was carried out according to practical standards ("VETTEST" LLC, Russia). The findings obtained in the course of scientific research are processed by the biometric method. The degree of confidence was determined by the Student's t-test for independent samples.

3 Findings and discussion

As a consequence of DNA identification of Holstein cattle, C and T alleles, and CC, TC, TT genotypes were identified, suggesting the genetic biodiversity of the livestock under study (table 2). Genotype distribution analysis showed that most animals are heterozygous TC genotype carriers – 59.5%. The CC genotype frequency is 27.0%. The smallest number (13.5%) are representatives of the homozygous TT genotype.

Table 2. Distribution of alleles and genotypes of the LEP gene.

Frequency	Genotype frequency						Allele frequency		χ^2
	CC		TC		TT				
	n	%	n	%	n	%	C	T	
Observed	40	27.0	88	59.5	20	13.5	0.568	0.432	6.61
Expected	48	32.2	73	49.1	27	18.7			

The examination of the genetic equilibrium between the observed and expected distribution of genotypes in accordance with the Hardy-Weinberg law testified that the value of $\chi 2=6.61$ is below the critical value ($p<0.01$). There is a shift towards an increase in the homozygosity of the LEP gene.

Based on the research findings of the bovine LEP gene polymorphism of some authors, it was established that animals of the heterozygous TC genotype predominate in livestock populations [15-17]. However, according to most foreign researchers, the homozygous CC genotype is dominant in livestock populations [18-20]. There are also data on the predominance of the TT genotype among various herds, breeds and populations of cattle [21].

Data for biochemical research of blood serum of cows with various genotypes of the LEP gene (figure 1) indicate significant differences in terms of lipid metabolism.

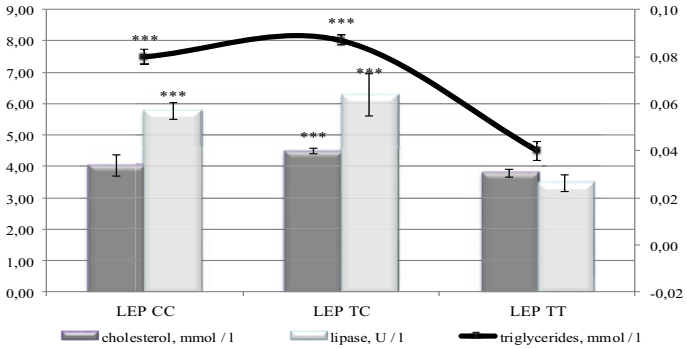

Fig. 1. Blood biochemical parameters of Holstein cattle with various genotypes of the LEP gene (*** - $p < 0.001$, the difference between the highest and the given indicator)

It is notorious that the content of cholesterol and triglycerides in the blood characterizes the rate of fat metabolism in the body. Our research found that the highest cholesterol content was observed in cows with the TC genotype and amounted to 4.48 mmol/l, and the lowest cholesterol content was observed in cow-heifers with the TT genotype – 3.79 mmol/l. The significant difference in this indicator between the groups was 0.69 mmol / l (15.4%; $p<0.001$).

The triglyceride content in the blood of cow-heifers with the CC genotype was twice as high as of cows with the TT genotype – by 0.040 mmol/l (50%; $p<0.001$), and the difference between TC-individuals and individuals with the TT genotype was 0.047 mmol/l (54%; $p<0.001$).

Individuals with the TT genotype were also characterized by low lipase levels in the blood. The statistically significant difference between them and animals with CC and TC genotypes was 2.30 and 2.82 U/L (or 39.9 and 44.8 %; $p<0.001$).

The research of parameters of milk productivity and the content of components in milk (table 3) suggest that following the results of the first standard lactation (305 days) cows with the TT genotype of the LEP gene boasted in terms of milk yield, content of fat mass fraction, milk fat yield, milk fat and milk protein yield in total. However, most findings of the research of other authors indicate a higher milk productivity of cows with the CC genotype [17, 20, 22].

Table 3. Association of LEP gene polymorphism and traits of milk productivity of Holstein cows.

Traits	Genotype		
	CC (n=40)	TC (n=88)	TT (n=20)
Milk yield, kg	6876.4±177.8	6676,9±129,3	6958,1±163,7
Fat, %	3.74±0.03	3.63±0.04	3.84±0.05**
Protein, %	3.32±0.04	3.32±0.03	3.31±0.04
Milk fat yield, kg	257.2±5.3	221.7±3.9	267.2±6.5**
Milk protein yield, kg	228.3±7.1	213.4±4.8	230.3±6.5
Total milk fat + protein yield, kg	485.5±12.4	464.1±10.4	497.5±13.0*

* - $p < 0.05$; ** - $p < 0.01$, the difference between the highest and the given indicator

The high mass fraction of fat, exceeding by 0.21% ($p<0.01$), was in the milk of cows with the TT genotype than of heterozygous TC animals. This is indicative of previously published data from other cattle researchers [17, 20, 22]. In terms of milk fat yield, this group also had an advantage – 267.2 kg. This is more than 10 kg (3.7%) of the fat mass in the milk of individuals with the CC genotype of the LEP gene, and 45.5 kg (17.0%; $p<0.01$) more than the indicator of individuals with the TC genotype. Our findings are consistent with other researches [20].

No significant differences in the content of the protein mass fraction and milk protein yield were found among groups of animals with various genotypes of the LEP gene. Although there is some evidence that cows with the TT genotype have protein and milking capacity, compared to cows of other genotypes [17, 20, 22].

The sum of milk fat and protein has the maximum yield in the group with the TT genotype-497.5 kg. The difference was 33.4 kg (6.7 %; $p<0.05$) compared to cow-heifers with the TC genotype, and 12.0 kg (2.4%) – with the CC genotype.

4 Conclusion

During the allele and genotype calling by the AC-PCR method, it was found that the leptin gene polymorphism (LEP) is observed in the population of interest of Holstein cattle. The distribution analysis of SNP alleles and genotypes (Arg73Cys) of the LEP gene revealed that most animals are carriers of the heterozygous TC genotype – 59.5%, the proportion with the CC genotype occurs with a frequency of 27.0%, the smallest number (13.5%) are representatives of the homozygous TT genotype.

Analysis of biochemical parameters of serum lipid metabolism showed that animals with the TT genotype are characterized by intensive metabolism and more successful secretion of lipid fractions from blood to milk. Their parameters for milk yield and qualitative composition of milk exceeded the results of lactation activity of cows with other genotypes of the LEP gene.

Thus, the determination of genotypes together with data on the content of the level of lipid metabolism parameters in the blood, and the use of this information will make it possible to predict milk yields, produce milk with excellent technological characteristics and successfully conduct selective and stock breeding at agricultural enterprises. In this connection, the increase in the genetic potential of cattle for economically important household characters is expected.

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