Influence of complex genotypes of $GH$ and $PRL$ genes on milk productivity and milk quality of cows

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Abstract. The aim of the study is to analyze the obtained data on the effect of complex genotypes of the somatotropin ($GH$) and prolactin ($PRL$) genes on the milk productivity of cows, on the amount and mass fraction of fat and protein in milk, that is, on the quality indicators of dairy raw materials. The study was conducted on a sampling consisting of 158 cows at first calving of Holsteinized black-and-white breed of pedigree breeding unit, "Dusym" LLC of the Atinsky district of the Republic of Tatarstan. As a result of molecular genetic studies (PCR-RFLP technique), the livestock was divided into groups taking into account the complex genotype of the $GH / PRL$ hormone genes. The proposed evaluation of cows with different complex genotypes of the $GH$ and $PRL$ genes by animals selection and matching will further increase the amount of high-quality dairy raw materials.

1. Introduction

It is well known that the chemical and biological properties of prolactin, growth hormone and placental lactogen have a significant homology among themselves [1].

Growth hormone is involved in many important physiological processes, in particular in the regulation of growth, lacteal gland development and initiation of lactation [2, 3]. The $GH$ gene affects the growth, quality indicators of carcasses, milk productivity and reproductive qualities of cattle [4]. Along with cattle, the effect of the $GH$ gene polymorphism on growth rates in buffaloes has been proven. It has been reliably confirmed that the genotypes of the $GH$ gene are associated with earlier weaning of calves from breastfeeding, with a higher live weight [5]. Other studies of buffaloes have shown that the polymorphism of the $GH$ gene is associated with both the live weight of animals and their milk productivity [6]. Studies of the effect of genetic polymorphism in the $GH$ locus on the lipid metabolism of Modicana cows have confirmed positive effects on the level of total unsaturated and monounsaturated fatty acids, as well as on the quality of milk [7]. In general, genotypes of the $GH$ gene affect the milk yield of Holstein cows [8]. Evaluation of the correlation of polymorphic variants of the $GH$ gene with the milk productivity of Holstein cows showed a important significant ($P<0.05$-0.01) difference in the amount of milk fat and protein [9]. Similar studies by meta-analysis showed the presence of an association of $bGH/AluI$ gene polymorphism with milk yield for lactation of Holstein cows, while 4583 samples of cows from 20 samplings were analyzed [10]. Dispersion analysis proved the difference in bioenergetic parameters and lactotropic functions in Holstein cows carrying different alleles of the $GH$ gene [11].

Genotypes of the $PRL$ gene affect the live weight of calves at birth and weaning, the live weight of animals during insemination and calving, as well as the meat productivity of cows, as shown by
studies conducted on a sampling of Angus breed animals [12]. The obtained data on the differences in the distribution of allelic variants and genotypes of the PRL-RsaI locus among indigenous populations of cattle in India, indicating that there is a possibility for genetic selection based on milk productivity [13]. Studies of two samplings of different breeds and pedigrees have shown that certain genotypes of the \textit{PRL} gene significantly affect the milk yield, fat and protein content in the milk of cows of crossbred Holstein breed (Holstein x Simmental), while in cows of purebred Simmental breed such differences in milk productivity indicators were not revealed [14]. Along with this, studies conducted among cows of the Gir and Kankrej breeds also indicated the presence of differences in milk productivity (milk yield and fat mass fraction) depending on the \textit{PRL} genotype [15]. It is statistically proved that the genotypes of the \textit{PRL} gene significantly affect milk yield and the percentage of milk fat in Holstein cows [16]. The study of the genetic variability of the \textit{PRL} gene and its relation with the composition of milk in three breeds of cattle \textit{Bos indicus} (Rathi, Sahiwal and Kankrej) revealed the presence of a relation only with the mass fraction of protein in milk [17]. Along with cattle, the effect of the \textit{PRL} gene polymorphism on milk yield, milk yield peaks, mass fraction of protein in milk and total milk fat yield in buffaloes has been proven [18].

Probably, the markers of dairy and meat productivity of cattle are genotypes and alleles of the \textit{GH} and \textit{PRL} genes [19].

Uninterrupted supply of sufficient quantity of high-quality and safe dairy raw materials to processing enterprises and organizations is a guarantee of stable production of milk-based products, in particular fermented milk, cheese, canned, gerodietic, functional and others [20-26].

In this regard, the aim of our research was to study the milk productivity and quality composition of milk in crossbred (Black-and-white x Holstein) cows with different complex genotypes of somatotropin and prolactin.

2. Materials and methods

Studies were conducted in conditions of pedigree breeding unit "Dusym" LLC of the Atninsky district of the Republic of Tatarstan, on the homogeneous livestock of Black-and-white x Holstein heifers (n = 158).

DNA samples in the identification of animal genotypes by \textit{GH} and \textit{PRL} genes by PCR-RFLP technique were extracted from whole blood samples using a set of reagents "DNA-sorb B" (produced by the FBIS CRI of Epidemiology of Rospotrebnadzor, Russia).

The procedure for genotype identification by somatotropin (\textit{GH}) and prolactin (\textit{PRL}) genes was performed using appropriate PCR-RFLP techniques [27, 28].

A description of the sequence of oligonucleotide primers (SibEnzyme Company, Russia), the size of amplicons of the studied genes, as well as their restriction fragments are described in Table 1.

| Gene | Primer structure | Amplicon size, bp / restrictase | Genotype and restriction fragments, bp |
|------|------------------|--------------------------------|-------------------------------------|
| \textit{GH} | GCTGCTCCTGAGGGCCCTTC CATGACCCTCAAGTGCTTCCG | 211 / AluI | GH/VV 211, GH/NL 211/159/52, GH/LL 159/52 |
| \textit{PRL} | CGAGTCCCTATGAGCCTGGTTCTT GCCTTCCAGAAGTGGTTTGC | 156 / RsaI | PRL/AA 156, PRL/AB 156/82/74, PRL/BB 82/74 |

The value of milk yield of cows was determined by the calculated method, using the indicators of weekly control milkings, both for full and incomplete lactation (for 305 days, 240 days, and more, respectively). Indicators of milk composition was determined on the device – analyzer "LAKTAN 1-4".

Standard indicators of statistical processing of research results were carried out by the method using variational analysis. The reliability of these studies was confirmed by the Student's criterion.
3. Results and discussions

In Black-and-white x Holstein cows, the frequency of the somatotropin and prolactin genes alleles occurrence was at the level of: $GH/L – 0.90$ and $GH/V – 0.10; PRL/A – 0.90$ and $PRL/B – 0.10$. The occurrence of individual genotypes of these genes was respectively: $GH/LL – 80.4\%$, $GH/VL – 19.6\%$; $PRL/AA – 79.7\%$, $PRL/AB – 20.3\%$. At the same time, such genotypes of $GH/VN$ and $PRL/BB$ genes of the somatotropin and prolactin were not found in this population, which ultimately affected the occurrence of complex $GH/PRL$ genotypes. In the studied sampling of animals, 4 complex genotypes of the somatotropin and prolactin genes were identified. The occurrence of complex genotypes was in the following order: $63.9\%$ (genotype $LL/AA$) > $16.5\%$ (genotype $LL/AB$) > $15.8\%$ (genotype $VL/AA$) > $3.8\%$ (genotype $VL/AB$).

We also conducted a study of indicators of milk productivity and milk quality (milk yield for lactation, content of mass fraction and amount of fat in milk, content of mass fraction and amount of protein in milk) in crossbred Black-and-white cows with different complex genotypes of hormone genes: somatotropin and prolactin (Table 2).

Table 2. The milk productivity of cows with different complex genotypes of the genes of somatotropin and prolactin.

| Genotype | n  | Milk yield, kg | Fat | Protein |
|----------|----|----------------|-----|---------|
|          |    |                | %   | kg      |        |
| $LL/AA$  | 101| 4263±71.9*     | 3.84±0.02 | 163.7±2.75* | 3.22±0.01 | 137.3±2.31 |
| $LL/AB$  | 26 | 3928±69.1      | 3.87±0.03* | 152.0±3.09  | 3.21±0.02 | 126.1±2.45  |
| $VL/AA$  | 25 | 4100±173.0     | 3.77±0.04  | 154.6±6.72  | 3.21±0.02 | 131.6±5.59  |
| $VL/AB$  | 6  | 3827±167.5     | 3.88±0.08  | 148.5±7.14  | 3.18±0.02 | 121.7±6.12  |

* - P<0.05

The milk producing ability of cows with different complex genotypes by the genes of the somatotropin and prolactin hormones was from 3827 kg (with the genotype $VL/AB$) to 4263 kg (with the genotype $LL/AA$). Cows of the first calving with complex heterozygous genotype $VL/AB$ were inferior in abundance to their peers of complex genotypes $LL/AB$, $VL/AA$ by 101-273 kg and $LL/AA$ by 436 kg (P<0.05) of milk.

The indicator of the fat mass fraction content in milk was in the range from 3.77 % (genotype $VL/AA$) to 3.88 % (genotype $VL/AB$). The first heifers of the complex $VL/AA$ genotype were 0.07-0.11% inferior to the groups of analogues of other genotypes in terms of the fat mass fraction content in milk. Provided that, the intergroup difference of animals of complex genotypes $LL/AB$ and $VL/AA$ was significant and amounted to 0.10 % (P<0.05). The amount of fat in milk, the first heifers with the complex $LL/AB$ genotype had a lower indicator than their counterparts with other complex genotypes by 3.5-15.2 kg of milk fat. At the same time, the intergroup difference between animals of homozygous and heterozygous complex genotypes $LL/AA$ and $VL/AB$ was significant and amounted 15.2 kg (P<0.05). The highest amount of milk fat obtained is typical for cows with a complex homozygous genotype $VL/AA – 163.7$ kg.

The indicator of the protein mass fraction content in milk was in the range from 3.18 % (genotype $VL/AB$) to 3.22 % (genotype $LL/AA$). Cows of the first calving of the complex $VL/AB$ genotype were slightly inferior to the groups of herd mates of other genotypes by 0.03-0.04 % in terms of the total protein mass fraction in milk. The amount of total protein in milk, cows of the heterozygous complex genotype $VL/AB$ had a significantly lower indicator than their counterparts with other complex genotypes by 4.4-15.6 kg of milk protein. At the same time, the intergroup difference between animals of homozygous and heterozygous complex genotypes $LL/AA$ and $VL/AB$ was significant and amounted to 15.6 kg (P<0.05).

4. Conclusion

In such way, studies have shown that using PCR analysis of the genetic characteristics of cows by the genes of somatotropin and prolactin, it is possible to give a forecast of quantitative and qualitative
indicators of milk productivity, which in the end is the key to ensuring the production of more high-quality dairy products. Thus, the highest amount of high-quality milk was obtained from first heifers with the homozygous complex genotype \( LL / AA \) by the genes of the somatotropin and prolactin hormones in comparison with analogues of other genotypes. Apparently, the presence of the "desirable" alleles \( PRL/A \) and \( GH/L \) in the genome had a greater impact on the milk productivity of first heifers and the quality of their milk.

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