Identification of relationship of polymorphic variants of lactoferrin gene (LTF) in cows with milk production indicators depending on their lineage

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Abstract Dairy farming is striving to achieve positive results both in milk production increase and quality improvement. Unfortunately, the conventional breeding selection system does not consider the genotypic identity that reflects the level and direction of the processes occurring in the organism. The modern breeding selection is based on the selection by a set of features. In breeding, those animals that have more valuable characteristics are considered valuable. Animal husbandry can achieve great success only when analyzing the biology of the animal body and productivity. In order to increase the genetic potential of cows in Russia, it is necessary to take into account genetics and milk productivity. Analysis of milk productivity of cows with different LTF genotype in their pedigrees showed that the maximum milk yield was in cows of the Ayvengo line with the LTFAB genotype. These cows were distinguished from other cows by their high protein content, and these animals had the lactoferrin gene. And the animals of the Ayvengo line differed in the level of fat in milk. The comparative PCR-RFLP analysis of the blood DNA samples of the first-calf Holstein cows showed the benefit virtually by all milk production indicators (milk yield, milk protein and fat), including by somatic cell content in milk of the Ayvengo and Sovereign line cows with the LTFAB genotype. The Aydial line stock with the LTFAB genotype is worthy of notice regarding the selection activity.

1. Introduction
Milk is a liquid that is formed in the mammary gland in mammals. One of the main requirements for milk is its safety and exclusion of factors unfavorable for human life. Milk and dairy products differ not only in their nutritional value (vitamins, macromolecules, etc.), but also by its safety for human health [1,2]. The milk safety is provided by the bacterial count and somatic cell content that can indicate the existing inflammatory processes in the lacteal gland of cows – mastitis [3,4].
The mastitis in cows is studied on a sufficient level today. The International Dairy Federation informs that about 2% of cows suffer from the clinical form of mastitis in average, while more than 70% of cows have its latent (subclinical) form [5,6].

A great deal of today’s methods for latent mastitis diagnostics in cows and a variety of treatment methods do not guarantee complete recovery of the stock [7]. This disease is often recurrent that is due to the lack of proper animal management technology, veterinary services, feeding, compliance with veterinary hygiene standards, etc. This is why there arose a question of solving this problem by means of natural resistance of cows to mastitis [8]. Today, a gene encoding lactoferrin production can be a participant of such mechanism.

Lactoferrin is an iron-binding glycoprotein that belongs to the transferrin family, it has an antimicrobial effect both in vitro and in vivo, which is contained in milk and other eccrine secretions, such as saliva, tear drop, bile, urea, semen, vaginal fluids, nasal and bronchial secretions, as well as in blood plasma [9,10].

In breeding and breeding work, the ability of bulls to transmit resistance to various diseases, such as mastitis, to their offspring is of great importance. The main goal of this work is the molecular genetic analysis of cows for the detection of the lactoferrin gene using a polymerase chain reaction, as well as the study of the relationship between this gene and dairy productivity of cows.

2. Experimental
The experiment was conducted on 311 Holstein cows in the conditions of the agricultural production cooperative named after Lenin of Tatarstan. Milk sampling was conducted by control milking. We analyzed the quality of milk using the device "lactan 1-4", which determined the level of protein, fat, density and dry milk residue, as well as the device" Somatos-B", we determined the concentration of somatic cells. During the entire study period, all cows of the Lenin agricultural production cooperative were under the same conditions of maintenance, feeding, and veterinary care. To conduct molecular diagnostics, we isolated DNA from the blood of cows using reagents "DNA-Sorb-B" which is produced at the Central research Institute of epidemiology of the Federal service for supervision of human welfare. We obtained parts of the DNA using the programmable thermal cycler MyCycler T-100. The Taq DNA polymerase (5 U/ml) (MBI Fermentas) with the supplied buffer - 10× Taq buffer was used for PCR. A mixture of deoxynucleoside triphosphate (2.5 mM of each dNTP) (MBI Fermentas) was added to the reaction mixture in the final concentration of 0.25 mM. In our work we used the primers which were obtained using the method SibEnzyme (Novosibirsk):

- LTF-f: 5′-GCCTCATGACAACTCCCACAC-3′,
- LTF-r: 5′-CAGGTTGACACATCGGTTGAC-3′.

The temperature and time profiles for PCR analysis were optimized in the preliminary laboratory experiments that ensured the optimal amplification of gene sites.

Studies of samples for PCR hydrolysis were performed using endonucleases restriction EcoRI by SibEnzyme (Novosibirsk) in the calculation of 10 units of enzyme per sample.

During the entire period of research and production experience in the laboratory, such indicators as cow milk yield, the level of protein and fat in milk, the total output of fat and protein in animals for the entire study period, namely 305 days, were taken into account. Data processing was performed on a computer in accordance with the methodological recommendations of E. K. Merkureva (1977).

3. Results and considerations
Studies have shown that the analysis of data from PCR DNA samples in 311 cows and further processing of the results showed that the LTF gene was detected in 68% of animals (chieftain line) 75% (Ayvengo line) and, respectively, the population with the LTFAB gene was within 24% and 42%, respectively. (figure 1).
Figure 1. Percentage distribution of frequency of lactoferrin genotypes regarding lineage.

For cows belonging to the Sovereign line, the pattern of the genetic test results was so that in this group, the population with the heterozygous $LTF^{AB}$ (57.1%), the polymorphic variant, prevailed over the homozygotic genotypes for allele A (42.9%). It should be noted that there were no animals with the desirable $LTF^{BB}$ genotype in this population.

Analysis of the frequency of distribution of variants of the lactoferrin gene, the majority were animals with the $LTF^A$ allele: Ayvengo – 0.879 and Aydial – 0.875, Rockman and Chief – 0.864 and 0.863, Chieftain – 0.844 and Sovereign – 0.714. The maximum frequency of the desirable $LTF^B$ allele was stated among the population belonging to the Sovereign line – 0.286 (table 1). Testing for variability between the observed and expected distribution using the chi-square method showed that all values $\chi^2$ in the study livestock were below the critical level ($\chi^2 = 5.99$; $P \leq 0.05$). All the study livestock showed the increased homozygosis, this is especially notable in the Chief line where the expected distribution is marked by a surge towards the homozygotic genotype BB. Irrespective of this, the genetic equilibrium in the population is not disturbed in any line according to the Hardy-Weinberg's law.

Table 1. Observed and expected distribution of frequency of lactoferrin genotypes regarding lineage of cows.

| Line   | Number of animals | Frequency of genotypes | Frequency of alleles |
|--------|-------------------|------------------------|---------------------|
|        | heads             | $LTF^{AA}$          | $LTF^{AB}$          | $LTF^{BB}$          | $A$  | $B$  | $\chi^2$ |
|        |                   | Observed frequency, heads | Expected frequency, heads | Observed frequency, heads | Expected frequency, heads | Observed frequency, heads | Expected frequency, heads |       |       |       |
| Ayvengo| 33                | 10.6                  | 25                    | 25                    | 8                    | 7                    | 0                    | 1     | 0.879 | 0.121 | 0.63  |
| Aydial | 24                | 7.7                   | 18                    | 18                    | 6                    | 5                    | 0                    | 1     | 0.875 | 0.125 | 0.49  |
| Rockman| 51                | 16.4                  | 37                    | 38                    | 14                   | 12                   | 0                    | 1     | 0.863 | 0.137 | 1.29  |
| Sovereign| 7                | 2.3                   | 3                     | 4                     | 4                    | 3                    | 0                    | 1     | 0.714 | 0.286 | 1.12  |
| Chief  | 180               | 57.9                  | 131                   | 134                   | 49                   | 42                   | 0                    | 4     | 0.864 | 0.136 | 4.47  |
| Chieftain| 16               | 5.1                   | 11                    | 11                    | 5                    | 4                    | 0                    | 1     | 0.844 | 0.156 | 0.55  |
| TOTAL  | 311               | 100                   | -                     | -                     | -                    | -                    | -                    | -     | -     | -     |
The milk production analysis of the first-calf cows with different LTF genotypes regarding their lineage showed that the Ayvengo line stock with the heterozygous \( LTF^{AB} \) genotype had the highest milk yield. Their milk yield made 7,172 kg of milk for the first lactation (table 2).

**Table 2**: Relationship of polymorphic variants of LTF gene with milk production indicators in cows regarding their lineage.

| Line   | Genotype | Milk yield, kg | Protein, % | Protein release, kg | Fat, % | Fat release, kg | Somatic cell content, ths./ml |
|--------|----------|----------------|------------|--------------------|--------|----------------|-----------------------------|
| Ayvengo| \( LTF^{AA} \) | 6,315±222 | 2.99±0.05  | 188.8±7.3          | 4.02±0.06 | 253.9±10.6 | 209.4±16.8b                |
|        | \( LTF^{AB} \) | 7,172±255 | 2.91±0.07  | 208.7±12.7         | 3.84±0.14 | 275.4±14.6 | 202.7±18.3b                |
| Aydial  | \( LTF^{AA} \) | 6,225±218 | 3.04±0.04  | 189.2±7.3          | 3.88±0.08 | 241.5±11.9 | 267.8±16.3a                |
|        | \( LTF^{AB} \) | 5,997±261b | 3.04±0.12  | 182.3±19.1         | 4.24±0.22 | 254.3±15.7 | 269.3±21.5                |
| Rockman | \( LTF^{AA} \) | 6,625±216 | 2.89±0.04b | 191.5±6.8          | 3.72±0.07b | 246.5±8.4  | 245.4±15.1a                |
|        | \( LTF^{AB} \) | 6,593±369 | 2.96±0.06  | 195.2±11.2         | 3.68±0.10a | 242.6±15.1 | 228.3±19.3                |
| Sovereign | \( LTF^{AA} \) | 5,889±124b | 3.02±0.06  | 177.8±6.9          | 4.00±0.02 | 235.6±4.5  | 399.7±58.9                |
|        | \( LTF^{AB} \) | 6,597±386 | 3.05±0.17  | 201.2±19.8         | 4.04±0.10 | 266.5±14.8 | 257.0±59.5                |
| Chief   | \( LTF^{AA} \) | 6,051±99a  | 2.88±0.03b | 174.3±3.3a         | 3.86±0.04a | 233.6±4.4  | 293.5±16.9                |
|        | \( LTF^{AB} \) | 6,333±158b | 2.89±0.05  | 183.0±5.0          | 3.88±0.07 | 245.7±6.8a | 277.5±20.1                |
| Chieftain | \( LTF^{AA} \) | 6,473±202 | 2.70±0.12a | 174.8±11.3         | 3.86±0.19 | 249.9±5.3  | 266.5±24.8a               |
|        | \( LTF^{AB} \) | 5,990±359a | 2.88±0.06  | 172.5±14.2         | 3.97±0.13 | 237.8±18.3 | 269.0±26.1                |

\( a \)- \( P < 0.05; \) \( b \)- \( P < 0.01.\)

The Ayvengo, Sovereign and Chief line cows with the heterozygous \( LTF^{AB} \) gene showed the largest milk yield among the study population of the livestock. Their milk yield made 6,333-7,172 kg in average. With regard to the herdmates with the \( LTF^{AA} \) gene according to the similar father lines, the difference by the milk yield made 282-857 kg of milk, respectively. At this, the difference in the milk yield of the Ayvengo cows of two groups of genotypes \( LTF^{AB} \) and \( LTF^{AA} \) was the largest and made 857
kg of milk. It should be noted that the milk yield in the cows with the LTF$^{AB}$ gene with regard to the Aydial and Chieftain line herdmates with the LTF$^{AA}$ gene was reliably lower by 228 kg (P<0.01) and 483 kg (P<0.05), respectively. However, the Rockman and Chieftain line population with the homozygotic LTF$^{AA}$ gene showed the reliably high milk yield with regard to the population with the heterozygous genotype, by 32 kg and 483 kg, respectively.

The Aydial and Sovereign line cows distinguished by higher protein content, and the both groups had the identified genes of the lactoferrin. At this, the protein content was reliably lower in the Rockman and Chief line population with the LTF$^{AA}$ gene made 0.07% (P<0.01) and 0.01% (P<0.01), respectively. The cows with the LTF$^{AA}$ gene of Ayvengo (4.02%), Sovereign lines (4.00%), and their herdmates with the LTF$^{AB}$ gene of Aydial (4.24%), Sovereign (4.04%) and Chieftain line (3.97%) stood out by fat content in the milk. And the reliable difference between two study groups of cows with the LTF$^{AB}$ and LTF$^{AB}$ genes was in the Chief line group and made 0.02% (P<0.05).

The largest protein release was characteristic for the population with the heterozygous LTF$^{AB}$ gene of Ayvengo (208.7 kg), Rockman (195.2 kg) and Sovereign lines (201.2 kg). The difference with the herdmates with the LTF$^{AA}$ gene made 19.9 kg, 3.7 kg and 23.4 kg, respectively. The group of the Chief line cows with the LTF$^{AA}$ gene had the reliably (P<0.05) low (by 8.7 kg) protein release with regard to the herdmates with the heterozygous LTF$^{AB}$ gene.

The groups of Ayvengo, Aydial and Sovereign line animals with the LTF$^{AB}$ gene were distinguished by the largest milk fat release and made 275.4 kg, 254.3 kg and 266.5 kg, respectively. The difference with the herdmates with the LTF$^{AA}$ gene made 21.5 kg, 12.8 kg and 30.9 kg, respectively. The Chief line cows with the heterozygous LTF$^{AB}$ gene had the reliably high milk fat release by 12.1 kg (P<0.05) with regard to the population with the homozygotic LTF$^{AA}$ gene.

The somatic cell content in milk of the cows with different genes of the lactoferrin gene and depending on lineage was within 209.4 ths./ml (LTF$^{AA}$ genotype of Ayvengo line) and 399.7 ths./ml (LTF$^{AA}$ gene of Sovereign line). At this, the Rockman line population with the LTF$^{AB}$ gene had the reliably lower somatic cell content in 1 ml of milk as compared with the herdmates with the LTF$^{AA}$ gene, that is 17.1 ths. Generally for all the lines, the animals with the LTF$^{AB}$ gene stood out in the somatic cell content with regard to the analogues of the LTF$^{AA}$ gene; the difference made 6.7-17.4 ths./ml.

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4. Conclusion
Therefore, according to the comparative analysis results of the first-calf Holstein cows, the Ayvengo and Sovereign line animals with the LTF$^{AB}$ gene showed the benefit virtually by all milk production indicators (milk, protein, fat), including by somatic cell content in milk. The Aydial line stock with the LTF$^{AB}$ gene is worthy of notice regarding the selection activity for increase of the protein and fat content in milk.

References
[1] Ganiev A S et al. 2018 Research Journal of Pharmaceutical, Biological and Chemical Sciences 9(6) 1504-9
[2] Zinnatov F F et al. 2020 Int. J. Res. Pharm. Sci 11(2) 1428-32
[3] Hairullin D D et al. 2020 Int. J. Res. Pharm. Sci 11(2) 2241-3
[4] Yakupov T R et al. 2020 Int. J. Res. Pharm. Sci 11(1) 290-3
[5] Yakupov T R et al. 2019 Research Journal of Pharmaceutical, Biological and Chemical Sciences 10(2) 1331-7
[6] Smolentsev S Yu et al. 2018 Research Journal of Pharmaceutical, Biological and Chemical Sciences 9(2) 948-50
[7] Khristoforovich P K et al. 2016 Research Journal of Pharmaceutical, Biological and Chemical
Sciences 7(4) 2214-21
[8] Matveeva E L et al. 2015 Research Journal of Pharmaceutical, Biological and Chemical Sciences 6(4) 207-9
[9] Valiullin L R et al. 2017 Bali Medical Journal 6(2) 88-91
[10] Semenov E I et al. 2018 Indian Veterinary Journal 95(6) 16-9