GENETIC MARKERS FOR HEALTHY MILK PRODUCTION

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summary

The development of DNA-based technologies, within the concept of selection marker gene, allows animals to evaluate the allelic variants of genes that are directly or indirectly related to production traits. Development and function of mammary is controlled mainly by growth hormone and prolactin, which are secreted by the anterior pituitary. The article presents the results of studies of the effect gene polymorphism pituitary transcription factor (POU1F1), kappa-casein gene (k-CSN) and prolactin gene (PRL) on milk production of cows of Frisian breed. Analysis of the genotypes was performed by PCR-RFLP (polymerase chain reaction–restriction fragment length polymorphism). It contains information describing how to conduct research and reagent kits for each of the genes studied. POU1F1 gene controls the transcription of genes PRL, TSH and GH, as well as the functioning of the breast. Mutations kappa-casein gene (k-CSN) affect the protein content in milk, cheese yield and cheese as well as on the coagulation properties of milk. All this makes the consideration of these three genes (POU1F1, k-CSN, PRL) as a promising candidate genes dairy cattle productivity. As a result of studies

have shown the presence of two populations of allelic variants of each gene and all three variants of genotypes. As a result of molecular genetic studies have investigated the genetic structure of Frisian breed and established three genotypes AA, AB and BB. The population was in Hardy-Weinberg equilibrium.

Animals genotype BB (POU1F1) characterized by the best milk yield per lactation (500.5 kg at (P <0,01) and 835.6 kg (P <0,001) compared with genotypes AB and BB, respectively), and genotype AB (POU1F1) was significantly associated with higher protein content. Genotype AB gene k-CSN provides higher performance milking (at 572.7 kg (P<0,05) compared with the AA genotype), whereas the effect of polymorphism of the gene for the protein and fat content in milk has not been established . PRL gene revealed that the animals of AA genotype have better milk yield per lactation. Superiority over similar genotype AG amounted to 388.2 kg and above analogy with the GG genotype at 564.1 kg (P<0,05).

Keywords: gene, polymorphism, productivity, POU1F1, k-CSN, PRLR, Frisian breed.

Introduction

In the current economic environment and foreign policy, it has increased substantially the relevance of the problem of increasing the productivity of farm animal genetic resources of domestic breeds[14]. One of the problems of domestic dairy cattle is the transition to the use of foreign tribal resources, in particular Frisian. Both genetic and environmental factors are known to influence production traits in cattle. Selection of animals with higher production or better reproductive performance is of great significance to breeders and consumers[2]. Current technologies enable scientists to improve on the accuracy and efficiency of traditional selection methods by applying genetic markers trough marker-assisted

selection. Therefore, genetic polymorphisms that are significantly associated with certain traits of interest are very useful [8]. Polymorphism detection in genes related to production traits and the identification of the allele which results in a phenotype of interest can allow for marker assisted selection (MAS) [12][17]. Genes affecting polygenic traits characterizing production performance are difficult to identify. In our conditions, such animals, giving 10 thousand Kg of milk per lactation, has a low reproductive capacity and propensity to various diseases, which significantly reduces the terms of economic use of high yielding cows and, consequently, the efficiency of production. Thus, it is advisable to increase the genetic potential of local breeds differing productive longevity of the use of modern breeding techniques. High efficiency for solving this problem having methods of DNA marker selection aimed at identifying genotypes of different genes markers of efficiency and selection of animals with desirable genotypes for further reproduction. Today, the interest of pituitary transcription factor genes (POU1F1), kappa-casein (k-CSN) and prolactin (PRL), as promising genetic markers productive qualities of cattle [7]. The aim of this work was to study the effect of gene polymorphism POU1F1, k-CSN and PRL on the performance of dairy efficiency of cows of Frisian breed.

Materials and methods

The subject of this research was the cows of Frisian breed of "ALHERA" region. Data on milk production obtained from the card directly on the farm. Genomic DNA of cows (n = 136) were isolated from 200µl of whole blood using a reagent kit Diatom DNA . Analysis was performed by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). Restriction of the amplified gene fragments POU1F1 carried out with the restriction enzyme HinfI, k-CSN- HindIII, PRL – RsaI . The size of restriction products were evaluated by electrophoresis on a 2% Agarose gel. Statistical analysis was performed by standard methods.

Kit for DNA Extraction Diatom DNA Prep

The kit is intended to be used for easy and effective extraction of DNA from biological samples, based on selective sorption of DNA on the surface of silica-gel granules in the presence of a high concentration of chaotropic agent. The extracted DNA is used for further molecular biological reactions without additional purification. The kit is optimized for DNA extraction from the volume of 100µl (up to 20µg) from 100 samples.
The kit contains:
Lysing reagent – 60 ml;
10×Salt buffer for washing – 10 ml;
Glass beads, sorbing agent – 2 ml;
Reagent for elution, ExtraGene – 10 ml

Product specifications:

Extraction method	Sorption in a volume on the silica-gel granules
Purity of the extracted DNA	OD 260/280 – 1,6-2,0
Time consumption for DNA extraction	< 40 min
Yield of DNA	3-5 µg from 100 µl of blood

RFLP analysis—DNA sequence polymorphisms display different migration profiles from wild-type fragment patterns when DNA is digested with restriction fragments and separated by size using electrophoresis.

Results and Discussion

POU1F1 is a candidate gene for regulating the growth and development of cattle and other mammals, controls transcription of a gene of prolactin (PRL), thyrotropin (TSH) and growth hormone (GH). It is also required for effective functioning of breast cancer and increases milk production. According to the research of Drozdov E.V., mutation POU1F1, followed by disruption of the structure of its product we can have a significant impact on the expression of the genes controlled by them, and thus change the phenotypic expression of traits of milk production in cattle. [2] The gene POU1F1 in cattle localized on chromosome 1q21-22, located between TGLA57 and RM95 (A → G, AC_000158.1, 35008949..35024718), consists of six exons and five introns derived protein comprises 129 amino acids[12].

The researchers has been proven to influence kappa-casein gene (k-CSN) on milk productivity of cattle and technological properties of milk. According to Vladimir Kryukov in several populations has 6 different alleles of the gene. Genetic variants A and B alleles are most common. Many researchers have attributed to the presence of alleles in a higher protein content in milk, a higher yield of cheese and cheese, as well as the best coagulation properties of milk [3]. It is found that high-quality hard cheese can only be produced by a milk obtained from cows having genotype BB gene k-CSN. In many countries, to increase the frequency of the desired allele B gene k-CSN in programs of artificial insemination using semen of bulls with genotype BB and AB [10]. Locus k- CSN relates to synteny group U15 and stored in the chromosome 6. Variant A comprises amino acids threonine (Thr) and aspartate (Asp) at positions 136 and 148, respectively. Variant B Thr-136 is replaced by isoleucine (Iso), a Asp-148 to alanine (Ala). These differences are the result of point mutations in k-CSN [2].

Prolactin (PRL, lactogenic hormone mammotropin (luteotrophic hormone), growth hormone (GH), and chorionic somato mammotrophin (placental lactogen, PL) are a family of protein hormones with significant

sequence homology. Their molecules in various species account for 190-199 amino acid residues. Homology of amino acid sequences of human GH and PRL – 35%. All three hormones share common antigenic determinants and have a similar effect: have growth-stimulating and lactogenic activity [10].

PRL is involved in the initiation and maintenance of lactation in mammals and was originally identified as lactotropic hormone secreted by the pituitary gland. To date, we accumulated a lot of data about a variety of physiological functions of prolactin, including osmoregulation, reproduction, behavioral responses. Prolactin is synthesized in various tissues, including immune and endothelial cells, neurons and others. Moreover, part of prolactin in immune response regulation has led to the concept of a dual functional role of prolactin – as hormones and cytokines. Many biological functions, such as gene expression caseins are made through the prolactin receptor [8].

In cattle PRL gene is localized in the q21 region of chromosome 23 and is closely linked with the genes of class I and class II major histocompatibility complex [4]. The analysis studied the genetic structure of cows of breed Frisian genes POU1F1, k-CSN and PRL, set the desired impact and identified genotypes on these genes. In the studied sample of cows of Frisian breed gene POU1F1established the presence of the three genotypes AA, AB and BB at a frequency of 18.8; and 43.7%, respectively (Table 1). The highest frequency is determined for allele B(0.62) and genotype BB(43.7%).

In carrying out similar studies Drozdov EV et al. in the cattle herds of the Bryansk region found the prevalence of allele B and genotype BB POU1F1 gene in all studied populations [2], which is consistent with our research. The maximum prevalence of the genotype frequencies ob- served in animals BB black-motley breed: group of cows (81.0%), and the calves of black-motley breed of polymorphism in this gene was not observed. The highest frequency of A allele was identified in cows ayshirskoy breed. The

European and North American rocks observed in the prevalence of allele, in most cases is very significant [13]. It was also noted that cattle Iran is bucking the trend of the prevalence rate of A-allele. In groups of cows of black-motley breed in allele frequency in the range of 0.64 (Belarus) to 0.88 (Russia, Bryansk).

In the study of the distribution of frequencies of alleles and genotypes of the gene k-CSN in the study population were identified three possible genotypes AA, AB, BB. The lowest frequency was determined for the BB genotype, which occurred at a frequency of 6.3%. The frequency of A allele was 0.75, allele B – 0.25. The highest frequency in a population characterized by a homozygous genotype AA (56.3%). In studies of Goncharenko G. in the gene k-CSN for cows of red steppe breed established frequency of allele A 0.65, allele B–0.34, AA genotype – 40.8%, AB–48.9%, BB– 10.3 % [1]. Similar results were obtained Selionovoy MI (-0 Allele A, 62; B allele –0.38 genotypes AA–44% AB– 36% BB–20%) [9], LN Chizhova (Alleles A–0.74 and C–0.26; genotypes AA–58.5; AB–32.1 and BB–9.4%) [11].

Thus, our data are consistent with studies of several domestic authors in terms of the low incidence of homozygotes BB and significantly more prevalent allele, compared with allele B. On the other hand, our results, as well as data Selionovoy MI and Chizhova LN showed superiority over the frequency of homozygotes AA AB heterozygotes [9], while studies Goncharenko GM et al. found a slight superiority over the heterozygote AB homozygous AA [1]. Our studies of the genetic structure of Frisian breed cows according to PRL gene polymorphism showed the presence of genotypes AA, AG and GG with a frequency of 6.3; 31.3; 62.5%, respectively. The highest frequency was allele G (0,78) and the genotype GG (0,62). Studies Alfonso E. et al. PRL gene in the American-style Swedish cows in front showed the prevalence in the population allele (0.87). The frequencies of genotypes AA, AG and GG were 78.4; 17.2; and 4.4%, respectively [12]. Research results Tyulkina SV et al. [10] from

purebred and crossbred Holstein bulls of PRL gene locus showed that 53 out of 70 bulls (75.7%) had the AA genotype, 16 (22.9%) – AB and 1 (1 , 4%) – BB. The frequency of A allele was 0.87, and the allele B – 0.13. The frequency of allele A PRL gene in cattle herds of black-motley, Jersey, Kholmogory, Yaroslavl, Simmental breeds was 0,31-0,85. A higher frequency of allele A PRL gene was observed among cows of black-motley breed of 0.85, with the lowest frequency of allele A was the animals Jersey Cattle–0.31 [10].

Studies M. Ozdemir on different breeds of cattle in different directions productivity also showed different frequencies of genotypes and alleles of the gene PRL [16].

Table1: The distribution of the frequencies and alleles to the DNA-markers of the Frisian cows

DNA-marker	Allel frequency		Genotype frequency, %		
	A	B	AA	AB	BB
POU1F1/HinfI	0,38	0,62	18,8	37,5	43,7
k-CSN/HindIII	0,75	0,25	56,3	37,4	6,3
PRL/RsaI	A	G	AA	AG	GG
	0,22	0,78	6,3	31,3	62,4

The data (Table 2) showed the influence of gene polymorphisms POU1F1, k-CSN and PRL on productive qualities of cows. In the study of gene POU1F1, animals with BB genotype have better milk yield per lactation and surpasses analogues with genotypes AB in 500,5 (P <0,01) and AA to 835.6 kg (P <0,001). Fat content in the milk of cows of different genotypes was similar, but the concentration of the protein in milk was higher in cows with heterozygous genotype AB, which surpasses analogues AA genotype at 0,05% (P <0,01). Drozdov E.V. et al obtained opposite results where individuals of genotype AA gene POU1F1 nadoyu

at the average for a month was significantly superior to animals with other genotypes [2]. In the group of cows with Aagenotype individuals present with efficiency of more than 550kg of milk per month. Regarding indicators of body fat percentage in the milk there was no statistically significant differences between individuals with different genotypes.

In the study there was a significant influence of gene polymorphism k-CSN on milk production of cows. The presence of AB genotype is associated with a better milk yield per lactation. Thus, the milk yield per lactation, cows with genotype AB exceeds analogues with genotype AA in 572.7 kg (P <0,05). At the same time, the relationship of genotypes with a mass fraction of fat is not included in our study . Cows with BB genotype were excluded from the analysis, because of sample (n = 1) does not provide reliable results, nonetheless ; genotype AB animals carrying the desired allele intended to increase the mass proportion of protein is 0.09%. In the future when dealing with the breed you need to increase the proportion of animals with a desired genotype BB in the population, in order to increase milk production of cows of Frisian breed.

Table2:Milk productivity of cows of different genotypes POU1F1, k-CSN and PRL

Genotype of gene	Milk yield for 305 days of lactation, kg	Mj, %	MPS, %
POU1F1/HinfI			
AA	4959,6 ± 112,8	3,88±0,04	3,08±0,02
AB	5294,7±104,6**	3,92±0,01	3,13±0,003**
BB	5795,2±137,8***	3,88±0,03	3,12±0,007
k-CSN/HindIII			
AA	5259,1±187,2	3,89±0,02	3,09±0,01
AB	5831,8±175,2*	3,89±0,01	3,18±0,06
BB	-	-	
PRL/RsaI			
AA	5815,5±201,0*	3,90±0,07	3,15±0,02
AG	5427,3±227,0	3,95±0,01	3,12±0,01
GG	5251,4±196,5	3,88±0,03	3,10±0,06

 *P<0,05, **P<0,01, ***P<0,001

In studies of Selionovoy M.I., cows of Frisian breed with genotype AB gene k-CSN udoyu superior to their counterparts with genotype AA at 154.1 kg. In these studies, the highest productivity was observed in genotype BB that we have not studied. As in our case, the effect of the gene k-CSN with indicators of fat content in milk is not established [9]. At the same time, the results of research and other Goncharenko. Significant effect of the gene k-CSN on the performance and yield of milk fat from cows of Frisian breed were found [1]. Thus, the structure of the allelic gene may contribute significantly to polygenic traits of milk production, at least in the Frisian breed . This B-allele associated with increased

productivity, can be seen as relatively rare. In the analyzed group of cows is absent and there is a high probability of extinction in this sector in the coming generations. PRL gene revealed that the animals AA genotype have better milk yield per lactation and surpasses analogues genotype AG for 388.2kg and 564.1 in the genotype GGkg (P<0,05).   Effect of PRL gene genotypes on the amount of fat and protein in the milk of the study population, has not been established, but there is a positive trend in animal genotype AA, which are relatively unique GG genotype had better scores on the protein mass fraction of 0.05%. Research results Alfonso E. et al. [12] gene PRL showed that animals with AA genotype had a higher milk production during lactation than animals with genotype AG and GG (p <0.05), consistent with our results. Studies of S.N. Dong for a large population of local Holstein cows also showed that cows with AA genotype of the gene PRL was higher milk protein content than cows with genotypes AG and GG [14].

Conclusion

The pituitary-specific positive transcription factor (*POU1F1*) gene has been the subject of many recent studies because of its important roles in growth and development of mammals.

As a result of studies, it found the effect of genotypes of genes POU1F1, k-CSN and PRL in the milk production of cows of Friesian breed. For cows of Friesian breed desirable genotypes established POU1F1 / BB, k-CSN / AB and PRL / AA consolidation that in the study population will increase the milk yield per lactation to 835,6 (P <0,001), 572,7 (P <0, 05) and 564.1 kg (P<0,05), respectively. No significant differences in the mass fraction of fat in milk has been established. The largest mass fraction of protein in milk was observed in animal genotype AB / POU1F1, which surpasses analogues on the 0,05% (P <0,01). These results confirm the possibility of using these DNA-

markers as additional criteria for the selection of animals for increasing milk productivity. The animals breeding selection assisted with genetic markers can increase the production traits or optimize reproduction performance in dairy cattle. The results from many associations analysis of this effect shows potential positive effect of B allele occurrence on growth and negatively on milk production performance. Preferably average values for milk, growth and reproduction parameters.

These studies are part of a comprehensive study of domestic cattle breeds, which aims to study the genetic basis of the variability of milk production in cattle by examining the polymorphism of a number of key genes involved in the development of breast cancer and her lactation capacity.

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