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# Quantitative trait loci related to milk productivity in dairy cattle

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## Abstract

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Cattle breeding is an important sector of agriculture, providing raw milk and milk products to constantly growing interest and demands of the consumers. Milk productivity is genetically determined, but this trait is also strongly influenced by environment. The genetic potential could be fully revealed only under optimal growing conditions. Identification of the genetic endowments and productive traits of farm animals, as well as the good production, is essential for any producer. Modern methods of molecular biology allow the usage of DNA markers in scientific research to identify genetic polymorphisms in gene loci and associate them with phenotypic traits. With the development of modern research in animal husbandry, it becomes possible to study the patterns of organization and function of the genome and to understand the mechanisms for the realization of genetic information in normal and pathological conditions. This data analysis was successfully applied in the development of breeding programs in animal breeding practice, aiming faster genetic progress. This review aims to follow the studies related to the evaluation of candidate genes related to milk productivity in dairy cattle. Lactation in mammals is a process that directly depends on the hormonal balance of organisms. Genetic variation in some genes such as Prolactin, Lactoferrin and Leptin has a reliable influence on the quantitative and qualitative characteristics of milk.

Milk proteins are a large group of organic compounds important for the structure and normal function of the mammalian body. Specific to cow's milk are casein proteins, which come together in four forms (αS1-, αS2-, β- and κ-casein) and two whey proteins – α-lactalbumin and β-lactoglobulin. Genome-Wide Association Studies (GWAS), Transcriptomic Analysis, Whole-Genome Sequencing and PCR-RFLP are some of the modern methods for detecting genetic markers related to milk productivity. These approaches make it possible to find polymorphisms associated with desired productive traits.

*Keywords:* marker-assisted selection; DNA analysis; milk proteins; hormones

## Introduction

Milk productivity is an important trait in farm animals, as it is related to providing a major part of food products for people. In many countries, dairy farming has significant development potential and creates work positions in small rural and suburban areas. The local breeds raised in such farms and preserved as a genetic resource are a source of ecological raw materials for the production of traditional dairy products. Their range of distribution and the successful marketing of their products can have a significant social effect for the sustainable development of rural areas. Indigenous breeds, compared to commercial breeds, are kept in smaller herds. They often reduce intra-breed variability, increase the inbreeding coefficient, which is associated with adverse effects on the health status and productivity of the animals.

Modern breeds have high productivity, which can be modeled according to the change of economically important characteristics. They were created on the basis of complex crosses, and are a valuable source of polymorphic variation due to their high heterozygosity. An imbalance between quantitative and qualitative traits is observed in some of the specialized breeds (Zamorano-Algandar et al., 2023).

The milk productivity of animals is genetically determined, but it is also strongly influenced by environmental conditions. The full genetic potential of animals could be revealed only under ideal conditions of feeding and housing. This trait can be evaluated for any lactation period – from birth to weaning. Depending on the breed and individual qualities of the animals, this interval lasts from 150 days to 270 days and includes two sub-periods – lactation and milking (Nenov et al., 1991). Synthetic breeds are characterized by high milk yield, while local (autochthonous) breeds have a lower value of this trait.

A number of factors influence milk productivity: breed and individual characteristics of animals, age, lactation period, feeding and rearing, season, health status (Nenov et al., 1991).

Genes encoding the main hormones associated with lactation, as well as those responsible for the secretion of proteins in milk, are the main molecular markers that are studied as candidate genes for milk productivity in cattle. The development of molecular DNA-techniques determines their use as routine research methods in countries with a high standard of animal husbandry. PCR-RFLP is one of the most widely used methods for genotyping and establishing the polymorphic states of various genes, which was first documented in 1974 (Maheswaran, 2004). The main advantage of this method is the easy differentiation of the different genotypic variation due to cutting with specific restriction enzymes.

## *Candidate genes related to milk productivity*

Establishing the genetic variation of some loci in farm animals is essential for their breeding. A number of authors worldwide conducted studies related to the genetic variation of selected gene loci in different cattle breeds and its association with the milk productivity of the animals. Research has focused on both autochthonous and cultural breeds (Maletić et al. 2016; Maheswaran, 2004; Kaminski et. al., 2006; Kumar et al., 2019; Patel and Chauhan, 2017).

Prolactin is a major candidate gene related to milk productivity in cattle (Brym et al., 2005; Dybus et al., 2005; Mitra et al., 1995; Patel and Chauhan, 2017; Singh et al., 2015). It is a polypeptide hormone, synthesized and secreted by specialized cells of the anterior lobe of the pituitary gland. In mammals, it is essential for maintaining lactation and the quality characteristics of milk. In cattle, the gene encoding prolactin is located on chromosome 23, which is composed of five exons and four introns (Patel and Chauhan, 2017).

Singh et al. (2015) investigated intron 3 of the Prolactin gene and the region between exon IV and intron VI of the β-lactoglobulin gene. The selection of the animals was carried out on the basis of phenotypic indicators of milk yield. It was found a statistically significant influence of some of the genotypes on the productivity of the cows. In conclusion, it is indicated that these results are important for determining the direction of future studies. If a positive relationship between the polymorphism in the loci of the indicated genes and the observed indicators is confirmed, they will be able to be introduced as DNA markers in different breeding programs.

Similar are the conclusions made by Patel and Chauhan (2017), who, after analyzing the genetic variation in the Prolactin gene locus, in two dairy cattle breeds, observed significant and reliable differences in the productive indicators of animals with different genotypes.

Hatami et al. (2005) studied the polymorphism of the Prolactin (bPRL) gene and the Growth Hormone (bGH) gene in two cattle breeds. The aim of the presented research is to establish a relationship between the polymorphism of the indicated genes and the trait of milk productivity. In conclusion, it is stated that there is a significant influence of the genotype of the bPRL gene in the animals of both studied breeds on the fat content of the milk.

Lactoferrin was first isolated from cow‘s milk. It is one of the major serum transport proteins and belongs to the transferrin family. In mammals, this hormone is a major element of innate immunity. This is due to both its ability to bind to iron and to multiple additional mechanisms of action, bactericidal and protective properties against viruses, fungi and parasites (Kaminski et. al. 2006). Lactoferrin concentration in the milk is a useful indicator of lactation stage, mastitis infection and intramammary infection in dairy cows (Arnould et al, 2009). Animals have different resistance to mastitis, which changes over time. With the advancement of age, disease lesions become greater and the frequency of infection increases greatly (Arnould et al., 2009).

Arnould et al. (2009) observed a positive correlation between the content of lactoferrin in cow‘s milk and economic traits as fat and protein percentages, and somatic cell score. By the PCR method, all exons of the lactoferrin gene were amplified and then sequenced. No statistically significant effect of genetic variation in the gene locus and the specified productive characteristics was found. The small population size has been pointed out as the most likely reason for this. The need for further research is emphasized.

Lei et al. (2006) genotyped 80 cows for the lactoferrin locus. Animals were divided into two groups. Forty animals resistant to mastitis and forty animals susceptible to mastitis were selected from a population of 520 cows based on clinical appearance of the disease. Polymorphic variations in the lactoferrin locus were detected by PCR-SSCP analysis. For two of the base mutations (P1 and P4), significant differences in allele frequencies were observed between resistant and susceptible individuals to mastitis. Further study of these two markers and their influence on milk productivity indicators would allow for their application in proper animal selection and breeding.

Mastitis causes huge losses to dairy cattle worldwide. DNA analyzes and genetic markers are a possible approach for diagnosis of mastitis, concomitant infections and selection of animals with better resistance to the disease. Pham et al. (2022) investigated for an association between polymorphisms in the Lactoferrin (LTF) and Toll-like receptor 2 (TLR2) genes with mastitis score and milk component traits. The results indicated that the interaction between TLR2 genotypes and diseases has a significant impact on the fat and protein percentage of the produced milk. Therefore, TLR2 polymorphism could be used for the selection of cows with better milk compositions.

Using relevant methods of DNA analysis Kaminski et. al. (2006) determined the allelic frequencies of the Lactoferrin gene in Holstein-Friesian cows and investigated its association with the milk productivity traits. According to the authors, there is a reliable influence of the genotype of the animals on the indicated gene on the content of lactoferrin in milk.

Leptin is a peptide hormone responsible for energy intake and energy expenditure in the body. It participates in the regulation of feed intake, weight development, milk productivity, immune response and reproductive function of animals. It is secreted by the fat cells and transported through the blood to the receptors of the hypothalamus (Haruna et al., 2021a; 2021b).

Haruna et al. (2021a; 2021b), by single-strand conformational polymorphism (SSCP) analysis, found a total of five nucleotide substitutions c.239C/T (p.Ala80Val), c.396C/T (p.Gly132D), c.399T/C (p.Val133D), c.411T/C, (p.Ala137D) and c.495C/T (p.Pro165D) in exon 3 of the bovine leptin gene in New Zealand Holstein Friesian × Jersey dairy cows. The authors suggest that these variations could be explored as a means of decreasing the concentration of saturated fatty acids in milk.

Dar et al. (2021) reported the discovery of new SNPs in exon 3 region of leptin gene, in indicine and taurine crossbred cows (Karan Fries). SNP at position rs29004509 (C > T) had positive correlation ($r = 0.376$; $P < 0.05$) with milk yield. In conclusion, it was stated that statistically significant effects of genetic variation in the locus of the studied gene and with milk yield traits were established. According to this fact, the leptin gene can be considered as a potential genetic marker to select elite cows for genetic improvement.

Applying the methods of DNA analysis (PCR-RFLP) Komisarek and Dorynek (2005, 2006) investigated the polymorphism in the Leptin gene in 213 bulls of the Holstein Friesian breed and the polymorphism in the leptin gene and the leptin receptor gene in 219 cows of the Jersey breed. In cattle, the leptin receptor gene (LEPR) is located on the chromosome 3 (Komisarek and Dorynek 2006). The leptin gene and the leptin receptor gene (LEPR) are accepted as candidate genes affecting productive qualities. According to the authors, the LEP and LEPR polymorphisms may have a certain relationship to feed intake, carcass composition, milk yield and calving period. The analysis of the results did not show a statistically significant effect of the LEPR polymorphism on the quantitative and qualitative characteristics of the milk produced during the lactation period. The authors observe a statistically proven influence regarding the content of fat and protein in milk. Animals with the TT genotype were characterized by the lowest levels of both traits. In conclusion, the researchers indicated that there was a possible effect of the leptin receptor gene polymorphism on the composition of the milk of Jersey cows, and mainly on the fat content. The authors believed that the relationship should be confirmed in future studies involving a larger number of animals, which would increase the reliability of the statistical tests and the reliability of the results.

Leptin has been shown to be involved in the protective mechanisms of the mammary glands in cows. Its secretion level increases significantly in response to viral or bacterial infections (Jemmali et al., 2017). Leptin receptors are expressed in various cells and tissues, including those of the immune system. This is related to the performance of phagocyte cells (Jemmali et al., 2017). In this regard, this gene is the subject of studies associated to the animal‘s resistance to mastitis. Jemmali et al. (2017) analyzed the polymorphism of the gene responsible for the biosynthesis of leptin by PCR-RFLP technique. The study included 160 animals from dairy Holstein breed cows situated in four Tunisian governorates: Bizerte, Nabeul, Kairouan, Kasserine. A substitution mutation at position 1180 (C:T) was found, which changes the expression of the gene. The results showed that two alleles A and B and three genotypes AA, AB, BB with a dominance of the allele A were observed in the studied population. Animals carrying the BB genotype could contribute to a reduction of somatic cells in cattle. Based on this observation, they are more resistant to mastitis; this peptide can be considered as a candidate gene for udder health.

Milk protein genes, such as casein and lactoglobulin, are also the subject of many studies (Felenczak et al., 2006; Maletić et al., 2016; Miluchová et al., 2009; Sodhi et al., 2018).

Milk proteins are a large group of organic compounds important for the structure and proper function of the mammalian body (Maletić et al., 2016). Specific to cow's milk are the proteins of casein, which comes together in four forms (αS1-, αS2-, β- and κ-casein), and two whey proteins – α-lactoglobulin and β-lactoglobulin (Hristov et al., 2008).

Casein is the most abundant protein in cow's milk, making up about 80% of the total proteins and is the main component of cheese. K-casein is one of the four fractions of this protein and is determined by a gene located on chromosome 6 in cattle (Felenczak et al., 2006; Maletić et al., 2016).

β-lactoglobulin is one of the whey proteins that makes up about 14–53% of the total whey protein. It is found in sheep, cow, pig and dog's milk. Due to intra- and interspecies genetic variation, it exists in several forms. The gene encoding β-lactoglobulin is located on chromosome 11 (Maletić et al., 2016). Genetic variation at the κ-casein, β-casein and β-lactoglobulin gene loci was analyzed by Antonopoulos et al. (2021), Hill et al. (1997), Kumar et al. (2019), Lunden et al. (1997), Maletić et al., (2016), Miluchová et al. (2014), Rachagani et al. (2006), Sodhi et al. (2018).

Sulimova et al. (2007) conducted a similar study by determining the allelic polymorphism of the κ-casein gene (CSN3) in five cattle breeds raised in Russia and defined it as a genetic marker. The authors emphasized that the genetic characterization based on the polymorphism of the indicated gene was important for the selection practice in dairy cattle breeding. Nevertheless, additional studies were needed in order to use it to determine phylogenetic relationships between populations,

Maletić et al. (2016) detected polymorphism at the κ-casein (κ-CN) and β-lactoglobulin (β-Lg) loci in the indigenous Busha breed and the cultivated Holstein-Friesian cattle breed by PCR-RFLP analysis. Specific primers and restriction endonucleases were used for amplification of κ-CN and β-Lg genes. The percentage distribution of genotypes in both breeds was similar. The percentage of animals with the heterozygous genotype AB was higher in both studied breeds. The homozygous genotype BB was with the lowest frequency in both breeds as well. Busha cows had a higher presence of the A allele for both genes. According to the authors, the results in this work could be used as a basis for future studies in this direction.

Studies of the genetic polymorphism in exon 7 of the β-casein gene in Greek domestic cattle breeds and in the Holstein-Friesian cattle breed also showed a higher frequency of the A allele in indigenous breeds (Antonopoulos et al., 2021).

These results were also confirmed by Miluchová et al. (2009). In the population of Slovak Pinzgau cattle, a higher frequency of genotype A1A2 and of allele A1 was observed at the β-casein locus, compared to those of the other alleles and genotypes.

Badola et al. (2004) investigated the genetic diversity of the β-lactoglobulin in eight meat Indian cattle breeds. They found a higher level of allele A gene compared to three cultured breeds. A low effect of genetic variation in the gene locus and on fat percentage in cattle was observed. On the other hand, Badola et al. (2003) reported significant effect of β-lactoglobulin genotype on the milk yield from the first lactation.

A study conducted with Sahiwal, Holstein Friesian, Jersey and crossbred cattle breeds revealed both alleles (A and B) and the three possible genotypes (AA, BB, AB) in all of the breeds. The AA genotype produced significantly higher milk yield in Sahiwal cattle whereas the BB genotype resulted in higher milk yield in Holstein Friesian cattle. In the other cattle breeds the genotypic effect was non-significant. It was concluded that future studies of this gene could clarify its role for milk productivity in cows and it could be included in breeding programs as a genetic marker.

The possible relationship between the polymorphism of other genes with milk productivity and technological qualities of milk in cows has been addressed in numerous scientific publications (Czarnik et al., 2005, 2006; Jia et al., 2023; Kaupe et al., 2007; Komisarek and Dorynek, 2006; Ma et al., 2021; Zamorano-Algandar et al., 2023).

Kaupe et al. (2007) investigated the association between two gene polymorphisms (CYP11B1 and DGAT1) and milk yield, reproductive and some productive traits in Holstein-Friesian cattle bred in Germany. The two genes are located on chromosome 14, which, according to the authors, has been the subject of research with the aim of establishing major genes – markers of certain quantitative traits. CYP11B1 is a gene encoding an enzyme that catalyzes the metabolism of corticosteroid hormones in cattle. (Kaupe et al.,2007) indicated that steroid hormones and cortisol are the main regulators of the fatty acid composition in the plasma and tissues of animals. DGAT1 is a gene identified as a candidate marker related to milk productivity in cattle (Kaupe et al.,2007).

In a review article, Ma et al. (2021) described the genetic markers associated with milk production traits in dairy cattle recorded from 2010 to 2021. The information was collected from refereed journals in databases such as PubMed, ScienceDirect, Web of Science, SpringerLink, Scopus, and Google Scholar. The authors made a detailed review of four methods Genome-Wide Association Studies (GWAS), Transcriptomic Analysis, Whole-Genome Sequencing and DNA polymorphisms, and their association with milk production traits in dairy cattle for screening of genetic markers for milk production in dairy cattle. In conclusion, it was stated that the presented analysis could be helpful to select the potential genetic markers for the genetic improvement of milk production traits in dairy cattle.

Genome-Wide Association Study (GWAS) is an increasingly used method for the discovery of markers for quantitative traits. This approach allows to find SNPs associated with desired productive indicators. Such approach was used by Zamorano-Algandar et al. (2023) in Holstein Dairy Cows. Due to its high milk yield, this breed is one of the most widespread worldwide. When exposed to high temperatures, during hot seasons, animals reduce feed intake and milk production. The body‘s response to heat stress is individual and varies within high limits. It can be assumed that it is genetically determined and it is possible to find DNA markers related to the adaptation process. Such markers could genes that regulate metabolic functions necessary to meet energy needs and minimal heat production. The authors reported the detection of six single nucleotide polymorphisms (SNPs) that could be used for milk production in heat-stressed Holstein cows. Only three of these SNPs, in the genes TLR4, GRM8, and SMAD3, were further validated as markers for milk production and thermotolerance traits (i.e., rectal temperature and respiratory rate) in two independent Holstein cow populations.

Jia et al. (2023) studied the SEC13 gene, responsible for the expression of a protein that was the core component of the cytoplasmic COPII complex, which mediates material transport from the endoplasmic reticulum to the Golgi complex. It also participated in and involved in metabolic pathways of milk synthesis such as citric acid cycle, fatty acid, starch and sucrose metabolisms. Variation of SEC13 expression was found in different stages of the lactation period in cows. According to the authors, this gene was suitable for a candidate gene influencing milk production characteristics. In this research, by sequencing the whole coding and partial non-coding regions of SEC13, four single nucleotide polymorphisms (SNPs) were found. In conclusion, it was indicated that the observed polymorphism in the SEC13 gene locus has a significant effect on milk yield and composition in a Chinese population of Holstein cows. The identified SNPs can be used as candidate genetic markers.

### *Studies in Bulgaria*

In Bulgaria, DNA analyses in cattle were mainly aimed at establishing genetic diversity in some breeds, through microsatellite markers (Bozhilova-Sakova et al., 2023; Teneva et al., 2005; Teneva et al., 2007; Viryanski et al., 2019; Viryanski et al., 2022).

Hristov et al., (2014b; 2015) performed a mitochondrial DNA analysis of two autochthonous breeds Bulgarian Gray cattle and Shorthorn Rhodopean cattle breed for revealing the genetic profile and structure of the populations. A detailed comparative analysis with other Balkan cattle populations was also carried out. The data showed an mtDNA profile with multiple haplotypes with no phylogenetic relationships between them. Hristov et al., (2012; 2014a) established the genetic profiles of the αS1-casein and κ-casein genes in the mentioned two Bulgarian breeds. The aim was to compare them with other European populations and to determine their position in terms of biodiversity. According to the authors, it can be concluded that the Bulgarian breeds had specific genotypic profiles close to those of other cattle in Southeast Europe.

Using the PCR-RFLP method, Yordanova et al. (2014d) detected differences in allelic and genotypic frequencies at the αs1-casein (CSN1S1) locus between two widespread cattle breeds (Bulgarian Brown Cattle and Bulgarian Black and White Cattle) and two endangered cattle breeds (Rhodopean Short-Horned Cattle and Iskar Cattle). Significant difference was found in the frequency of heterozygous genotype – a higher frequency was announced in the endangered cattle breeds.

The same breeds were also a subject of a research of Angelova et al. (2014). The authors found that the K-casein (CSN3) gene in all studied breeds was represented by the most frequent genotypes – AA, AB and BB. Bulgarian Brown Cattle and Rhodopean Short-Horned Cattle revealed the following genotypes – AA, AB, BB, AH, BH, and Bulgarian Black and White Cattle showed the presence of genotypes AA, AB, BB, BH, HH. It was indicated that the main purpose of the research was related to the protection of the genetic resources of cattle breeds in Bulgaria.

In an extensive study, a team from the Agricultural institute, Stara Zagora, Agricultural Academy, Sofia, Bulgaria investigated the genetic polymorphism in four gene loci: αs1-casein (CSN1S1), β-casein (CSN2), K-casein (CSN3) and β-lactoglobulin (LGB) in Bulgarian Brown Cattle and Bulgarian Black and White Cattle (Angelova et al., 2021a; Angelova et al., 2021b; Angelova et al., 2021c; Angelova et al., 2021d; Yordanova et al., 2021a; Yordanova et al., 2021b; Yordanova et al., 2021c). A statistically significant effect was established between the observed genotypes on some of the factors affecting the milk productivity in cows.

These results were also confirmed by research of scientists from the Bulgarian Academy of Sciences and Sofia University "St. Kliment Ohridski". The genetic variation in the loci of three genes – αS1-casein, κ-casein and β-lactoglobulin in four local breeds – Bulgarian Rhodopean cattle, Bulgarian black pied cattle, Bulgarian Gray cattle, Rhodop Shorthorn cattle breed was analyzed (Hristov et al., 2011a; 2011b; 2014c; Neov et al., 2013; Teofanova et al., 2011; Zlatarev et al., 2008). The authors found a correlation between the genetic variants of the genes and the qualitative and quantitative characteristics of the milk. According to their work, it could be concluded that these genes could be applied as genetic markers for the selection of animals with desirable milk qualities and genetic improvement of these local breeds as part of the national genetic pool.

## Conclusion

In this review, a number of scientific references have been studied. They reported a statistically significant effect of a large group of genetic markers on the expression of phenotypic traits such as milk yield and milk composition in cattle. The collected information allows to be concluded that the application of the developed methods for the analysis of the allelic variation of genes related to animal productivity can be used for the so-called marker-assisted selection (MAS) in animal breeding.

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