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# CANDIDATING GENETIC MARKERS RELATED TO MILK PRODUCTION CHARACTERISTICS AND TECHNOLOGICAL SUITABILITY OF MILK

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**Abstract.** Milk yield and composition are key factors for dairy industry, and both are determined by multiple genes. This study contains collective analysis of research on chosen candidate genetic markers associated with milk production traits and its technological suitability. The results of analyzed studies indicate that polymorphisms in the genes *GHR*, *IGF-1*, *CSN2*, *CSN3*, *FASN*, and *SCD1* affect the composition of milk, and thus its technological suitability. However, studies on *BLG* polymorphisms show variability in the results across researchers, highlighting the need for further analysis.

**Key words:** milk, polymorphism, technological suitability.

## INTRODUCTION

Milk yield and composition play a crucial role in the dairy industry (Čítek et al. 2019). The chemical composition of milk varies depending on the breed, season, lactation phase, feeding method, housing conditions, the region of origin, the age of the cows, and other factors (Król et al. 2011; Szewczuk 2016; Bondan et al. 2018; Olaniyan et al. 2023; Barłowska et al. 2024), which ultimately affect the quality of the finished product at later stages of production (Król et al. 2011; Manzocchi et al. 2021; Mituniewicz-Małek et al. 2022). The quality of the raw material supplied to the dairy is also of great importance in the processing process (Felipus et al. 2022), depending on such factors as: the scale and season of production (Barłowska et al. 2024), the method of obtaining milk, the conditions under which it is handled on the farm (Parente et al. 2020; Ismayilov et al. 2023; Kathiriya 2024), the collection system, and the size of the delivery (Henzl et al. 2018; Felipus et al. 2022). To produce high-quality fermented beverages and cheeses, milk must meet certain organoleptic parameters and be characterized by, among others, a good chemical composition and favorable bacterial microflora (Citek et al. 2021; Mitunkiewicz-Małek et al. 2022; Fedak et al. 2023). The technological suitability

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of milk is determined by the composition and proportions between individual components, especially dry fat-free mass, protein content, including casein (which constitutes about 80% of total protein), and mineral components (Litwińczuk 2015; Barłowska et al. 2024). The casein content in milk determines the speed of clot formation under the influence of rennet (Frederiksen et al. 2011) and its compactness (Król et al. 2011).

Among the various nutrients, protein is one of the most functionally versatile milk nutrients, and its content and functional properties can differ between mammalian species (Szewczuk and Kulig 2021). Functionally, milk proteins provide characteristic structure, solubility, water binding, viscosity, and heat stabilization properties to milk and dairy products (Pluta et al. 2022).

An important energy component of milk is fat, which gives it a pleasant taste, structure and consistency, which is important in processing, because by appropriately influencing the fat content we can obtain homogenized milk, skimmed milk, cream or butter (Parente et al. 2020). Fat is not a homogeneous substance, because it occurs in the form of dispersed fat globules dispersed in the aqueous phase of milk, creating an emulsion. The interior of the fat globules is filled with triacylglycerols (98% of all milk fat). The fat globule shell, in addition to glycoproteins, contains about 1.1% phospholipids, mono- and diacylglycerols, free fatty acids, sterols, carotenoids and fat-soluble vitamins (Jensen 2002; Miciński et al. 2012). According to Szewczuk and Kulig (2021), the greatest impact on the quality of fat is exerted by fatty acids produced in the rumen of ruminant animals, with the participation of microflora from acetate, β-hydroxybutyrate, triacylglycerols, lipoproteins and, in smaller amounts, from sterols, phospholipids and free fatty acids. Their properties depend on the length of the carbohydrate chain and the number of unsaturated bonds (Parente et al. 2020; Szewczuk and Kulig 2021). Its significant nutritional value and high digestibility (up to 99%) have made it play a major role in the human diet (Miciński et al. 2012; Szewczuk and Kulig 2021).

The microbiota present in milk is also of significant importance, as it is highly diverse in terms of both its quantity and species composition. It is influenced by factors such as the health status of the animal, the method of milk collection, storage, and transportation (Szewczuk and Kulig 2021; Felipus et al. 2022). The physiological microbiota consists of lactic acid bacteria (Gram-positive, non-spore-forming bacteria), which cause the natural acidification of milk (participating in the fermentation process) and affect the organoleptic characteristics of dairy products (Bintsis 2018). In raw milk, psychrotrophic bacteria (Gram-positive bacteria, such as *staphylococci* and *streptococci*, and Gram-negative bacteria, primarily of the *Pseudomonas genus*) are undesirable (Pukančíková et al. 2016). These bacteria produce lipolytic and proteolytic enzymes that can hydrolyze peptide bonds in proteins, hydrolyze milk fat into free fatty acids, thereby changing the physicochemical and functional properties of dairy products (Teh et al. 2011; Pukančíková et al. 2016).

Key indicators of milk suitability for processing include heat stability, enzymatic coagulation, fat dispersion state, and fermentation capacity (Litwińczuk et al. 2015; Parente et al. 2020). Heat stability is an important criterion for its suitability for processing into products with a long shelf life, such as UHT milk and cream, condensed milk, and powdered milk. Additionally, factors such as the casein content, the protein-to-fat ratio, acidity, and rennet clotting time also determine the suitability of milk for processing (Barłowska et al. 2024). In cheese production, the most important factor determining the suitability of milk for cheese-making is the rennet clotting time, which is primarily influenced by the milk composition, including casein content and the proportion of its individual fractions (Litwińczuk et al. 2015).

Both milk yield and the nutrient levels in milk are polygenic quantitative traits. The specific number of genes affecting each trait is not yet known. Therefore, the search for genes responsible for shaping high levels of economic traits is the subject of extensive research, and identifying favorable genotypes within a herd allows for the selection of animals with the best traits (Duszyńska-Stolarska et al. 2015; Padzik and Szewczuk 2020; Szewczuk and Kulig 2021; Oster et al. 2023; Saleh et al. 2024).

The genes *IGF-1*, *GHR*, *CSN2*, *CSN3*, *BLG*, *FASN*, and *SCD1* play a crucial role in regulating processes related to milk production in animals. *IGF-1* and *GHR* are responsible for the development of mammary glands and the stimulation of lactation by influencing the proliferation of glandular cells and the synthesis of milk proteins. The genes *CSN2* and *CSN3* encode casein proteins, which are the main protein components of milk, affecting its structure and technological properties, such as cheese production. *BLG*, responsible for producing beta-lactoglobulin, is a key whey protein that plays a role in the digestibility of milk. *FASN* and *SCD1* regulate the synthesis of milk fat, ensuring an appropriate fatty acid profile, which influences the energy value and quality of the milk. In summary, these genes cooperate to optimize lactation efficiency, milk quality, and its nutritional and technological properties, which are of great importance in dairy animal breeding (Kulig et al. 2013; Deb et al. 2014; Szewczuk 2016; Neamt et al. 2017; Čítek et al. 2019; Cobanoglu et al. 2021; Liu et al. 2024).

The aim of this study is to present an overview of research on the relationship between polymorphisms in the *IGF-1*, *GHR*, *CSN2*, *CSN3*, *BLG*, *FASN*, and *SCD1* genes and their association with milk yield, composition, and technological suitability. The selected genes are involved in the biosynthesis of milk components. These studies have been conducted with a focus on the potential use of their results in programs aimed at improving functional traits.

## DETERMINATION OF CANDIDATE GENETIC MARKERS

The genetic improvement of livestock is based on enhancing individuals in successive generations, which is reflected in the intensity and effectiveness of selection within the herd. In addition to selecting animals based on phenotypes (Song et al. 2022), discoveries and advancements in molecular genetics have allowed for the improvement of breeding in terms of desirable genotypes, as well as faster progress in the overall productivity of breeding animals (Szewczuk and Kulig 2021). Consequently, there is a need to search for and identify genes whose variability may have a direct or indirect correlation with the productive traits of ruminants. Numerous genes encoding elements of the somatotropic axis are associated with growth, development, and the function of the mammary gland (including lactogenesis) and are hormonally regulated. This suggests a potential link to the milk production ability of cows, sheep, and goats (Parmentier et al. 1999; Plath-Gabler et al. 2001; Szewczuk et al. 2013; McCoard et al. 2016; Mirzaei-Alamouti et al. 2023; Ye et al. 2023).

One such element that may influence milk yield in cows is insulin-like growth factor 1 (IGF-1), which is primarily synthesized in the liver in response to the secretion of growth hormone (GH) from the anterior pituitary, also known as somatotropin. Its secretion is controlled by two hypothalamic neurohormones: growth hormone-releasing hormone (GHRH), which stimulates its secretion, and somatostatin (SRIF), which inhibits it (Salvatori 2004; Szewczuk et al. 2009). The action of GH is mediated and regulated by numerous proteins that together form a complex system of interconnected interactions known as the somatotropic axis (Renaville et al. 2002). Once in the bloodstream, GH binds to growth hormone-binding proteins (GHBP) to form a specific complex that, due to its structure, increases its half-life (Baumann 1994). In target tissues, after dissociating from the complex, GH binds to a specific receptor (with the highest concentrations of these receptors found in hepatocytes, although they have also been detected in other organs and tissues, including the mammary gland of cows) on the surface of the target cell (GHR, growth hormone receptor) (Sinowatz et al. 2000; Plath-Gabler et al. 2001; Renaville et al. 2002; Abdolmohammadi and Zamani 2014; McCoard et al. 2016). The catalytic activity of the intracellular domain of the GHR (tyrosine kinase activity) triggers a signaling cascade that activates transcription factors for genes mediating GH signaling. The transmission of this signal is primarily mediated by insulin-like growth factor I (IGF-I) and its receptor, IGF-IR (Szewczuk et al. 2009).

The gene encoding IGF-1 in cattle has been mapped to the long arm of chromosome 5 (BTA5). Since *IGF-1* is located in the same region of chromosome 5 as more than 73 loci of other genes related to quantitative traits for milk and meat production (Reyna et al. 2010), it seems relevant to analyze potential associations with milk production and technological suitability of milk in cows.

The regulation of protein and fat synthesis, and indirectly lactose synthesis in the mammary gland, is hormonally controlled, particularly by prolactin (PRL), growth hormone (GH), thyroid hormones, and corticosteroids. Milk synthesis efficiency can be improved by leveraging knowledge about the transcriptional and post-transcriptional regulation of genes encoding proteins involved in the synthesis of these components in the mammary gland. According to Osorio et al. (2016), milk fat synthesis in cattle appears to be regulated by an interactive network between SREBP1, PPARγ, and LXRα, with a potential role for other transcription factors, such as Spot14, ChREBP, and Sp1. Milk protein synthesis is largely regulated by insulin, amino acids, and amino acid transporters through transcriptional and post-transcriptional pathways (the insulin-mTOR pathway plays a major role), while the transcriptional regulation of lactose synthesis is still poorly understood (Tian et al. 2022).

Transcription factors that control the expression of casein genes include the CCAAT/enhancer binding protein (C/EBP) beta, the glucocorticoid receptor, Signal Transducer and Activator of Transcription 5 (STAT5), and the Yin and Yang 1 protein, which is an inhibitor, binding to the octamer (Oct) sequence. Among these, STAT5 is the most important due to its role in controlling the expression of various casein gene variants and lactalbumin. The activated STAT5 signaling pathway binds to DNA fragments known as GAS (gamma interferon-activated sequence) and increases the expression of target genes (Wang et al. 2024).

Milk proteins in dairy milk consist of two groups: casein and whey proteins. The casein fraction represents approximately 80% of milk proteins and includes four types of caseins: αs1-casein (CSN1S1), αs2-casein (CSN1S2), β-casein (CSN2), and κ-casein (CSN3). Whey proteins comprise about 20% and are represented by α-lactalbumin (LALBA) and β-lactoglobulin (LGB), along with other proteins (Čítek et al. 2019). Milk proteins have a significant impact on the nutritional value and technological suitability of milk (Barłowska et al. 2011).

## INFLUENCE OF GENETIC POLYMORPHISMS ON PERFORMANCE TRAITS AND TECHNOLOGICAL SUITABILITY OF MILK

### *IGF-1*

The most widely studied IGF-1 gene polymorphism is the *IGF1/SnaB*I SNP being a *C/T* transition at position −472 in the 5 ‘non-coding region of *IGF-1* described for the first time in Angus cattle by Ge et al. (2001).

Research by Siadkowska et al. (2006) indicates no differences in daily milk yield between genotypes, however after converting milk yield to FCM (milk with fat correction) and VCM (milk with protein correction), heterozygotes were better than homozygotes. This was due to the higher content of fat and protein in the milk of cows with the *CT* genotype. Daily these cows gave 20 g more fat and 14.5 g more protein than cows with *TT* genotype. The *CT* genotype also turned out to be favorable for the fat and protein content of milk. The difference in protein and fat content between *AB* and *AA* cows was 0.07 and 0.14 percentage points, respectively ($P < 0.01$). The high fat and protein content of *AB* milk also resulted in a high total dry matter content, which was about 0.2 percentage points higher in *AB* than in other genotypes. There was no correlation between the cows' *IGF-1* genotype and milk lactose content.

Similar results were obtained by Mehmannavaz et al. (2010) in a study of Iranian Holstein sires. Heterozygous bulls were characterized by higher efficiency and percentage of protein, but the differences between genotypes for these traits were not statistically significant ($P > 0.1$). Additionally, the substitution effect of the *T* allele was not statistically significant for milk, fat and protein yield and for fat and protein content.

Bonakdar et al. (2010) showed a relationship between polymorphism in milk production. The *TT* genotype was associated with the highest production, the *CC* the lowest, and the *CT* genotype was an intermediate value. There was a link between genotypes and the fat and protein content of milk. The *TT* and *CC* genotypes had a lower milk fat content than the *CT* group ($P < 0.1$). Similar results were obtained for milk protein efficiency, with the highest production for the *CT* type, and the lowest for the *TT* and *CC* groups ($P < 0.05$). The adjusted sum of fat and protein produced during the year was also the highest for the *CT* group ($P < 0.1$). Also, in the research of Polasik et al. (2014), the *CT* genotype was associated with a higher content of fat and protein in milk. Milk yield was also highest for the *CT* genotype, but the difference was small.

Different results in their research are presented by Mullen et al. (2011). These studies included ten polymorphisms (*IGF1i1*, *IGF1i2*, *IGF1i3*, *rs2901285*, *IGF1i5*, *IGF1i6*, *IGF1i7*, *IGF1i8*, *IGF1i9*, *IGF1i10*) of the *IGF-1* gene and none of these polymorphisms were associated with milk protein yield or milk protein content. The *rs29012855 G* allele was associated ($P < 0.05$) with reduced milk fat. Similarly, the *IGF1i6 G* allele and the *IGF1i3 G* allele were independently associated ($P < 0.05$) with reduced fat yield. In addition, *IGFi3* was the only protein performance related SNP with a *G* allele associated with decreased performance.

In the study by Szewczuk et al. (2012), no significant effect of *IGF1/SnaB*I polymorphism on milk yield, protein and fat yield, as well as protein and fat content in milk was found. However, cows with the *CT* genotype produced more milk per lactation (7679 kg) than cows with the other two genotypes (~7450 kg). Average fat and protein contents were at a similar level regardless of the genotype of the cows.

In the study conducted by Szewczuk et al. (2011) the influence of *IGF1/Tas*I polymorphism on milk characteristics in Polish Holstein-Friesian cows was analyzed. This polymorphism is the transversion of the *A* to *C* allele in the P1 promoter region of the *IGF-1* gene. There were statistically significant differences between individuals with different *IGF1/Tas*I genotypes in milk, fat and protein yield. In the 2nd and 3rd 305-day lactation cows with the *CC* genotype produced more milk (+515 kg and +463 kg, respectively) than the *AA* animals ($P \le 0.05$). Similar trends were observed for protein and fat. Cows with the homozygous *IGF-1 CC* genotype produced significantly more fat (+22 kg and +16 kg) and protein (both +14 kg) than the cows with the *AA* genotype.

Szewczuk et al. (2012) also investigated the relationship between the *IGF1R/i16/Taq*I polymorphism and the characteristics of milk production in Polish Holstein-Friesian cows. In the analyzed herd, a positive ($P \le 0.05$) effect of the *BB* genotype on the milk yield of lactation (+402 kg) in comparison with the *AA* genotype was found. Milk of cows with *AA* and

*AB* genotypes was characterized by a higher ($P \leq 0.05$) protein content (%) as compared to the milk of *BB* cows. There was no significant effect of *IGF1R/Taq*I polymorphism on fat and protein yield and milk fat content.

In studies conducted by Szewczuk (2016), on the influence of *IGF-1* gene polymorphisms on milk production traits in Montbeliarde cows, did not show differences in milk production traits among *IGF1/SnaB*I genotypes. In the first lactation, the *IGF1/Tas*I polymorphism was associated with differences in milk yield. The cows with the *CC* genotype homozygous had a higher milk, fat and protein yield than the cows with the *AA* ($P < 0.05$) and *AC* genotype ($P < 0.01$). No significant relationships were found in the second lactation, but similar trends were observed.

### *GHR*

In the study by Komisarek et al. (2011) showed that the additive effect of *GH* and *GHR* genes on milk-related traits was significant only for the *GHR-F279Y* polymorphism. Replacement of the *T* allele encoding phenylalanine with variant A encoding tyrosine led to a reduction in milk, fat and protein yield. While the research of Viitala et al. (2006) indicate that *F279Y* had the greatest impact on the percentage of protein and fat. Moreover, the *TT (FF)* genotype was characterized by the lowest milk production compared to the *TA (FY)* and *AA (YY)* genotypes. The differences obtained in the results may be due to the small number of animals analyzed in this study and the exclusion of a very small group of *AA* genotypes from the association test.

The studies of Cobanoglu et al. (2021) on the influence of the *GHR/Alu*I polymorphism on milk production traits in Jersey and Holstein-Friesian cows in the Black Sea and Marmara regions indicate the effect of genotype on the fat and protein content in milk. It was observed that Holstein cows with the *AA* genotype had a significantly higher daily milk yield (24.64 kg / d) than animals with the *GG* genotype (19.40 kg). Similarly, cows with the *AA* genotype showed significantly higher milk yield in the 305-day lactation (8472.4 kg) than the animals with the *GG* genotype (7032 kg). In contrast, Holstein cows with the *GG* genotype achieved a higher percentage of milk protein (3.46%) than animals with the *AA* and *AG* genotypes 3.78% and 3.87%, respectively. However, the differences between the other features analyzed in the study (percentage of fat, protein and fat yield) were not significant. However, animals with the *AA* genotype showed higher values ($P < 0.05$) of the analyzed traits related to performance than the cows with the *AG* and *GG* genotypes. Overall, the *GHR/Alu*I relationship with FC and PC turned out to be significant in Jersey with $P < 0.05$. On the other hand, the daily milk yield at P <(0.05), the annual milk yield and the amount of protein at $P < 0.01$ were significantly associated with the *GHR/Alu*I polymorphism in Holstein cows. In particular, it is worth noting that animals with *AA* and *AG* genotypes were distinguished by higher daily and annual milk yield, but less protein than animals with the *GG* genotype.

### *CSN2*

The research by Heck et al. (2009) showed that β-casein genotypes were associated with protein yield ($P < 0.01$) and differences in the relative concentrations of all 6 major milk proteins (all $P < 0.01$). Cows with the A1 allele were characterized by lower protein efficiency than cows with the A2 allele, which was due to the reduced milk production. Variant A1 was also associated with higher relative concentrations of αS1-casein and κ-casein and lower relative concentrations of αS2-casein and β-casein compared to variant A2. Variant B β-casein was associated with higher relative concentrations of κ-casein compared to variants A1 and A2. Cows heterozygous for β-casein (A1A2, A1B or A2B) had a higher relative concentration of β-CN and a lower relative concentration of αS1-CN compared to homozygous animals (A1A1 or A2A2). Similar results were obtained by Bonfatti et al. (2010) additionally showing that the influence of the B allele of the *CSN2* gene on the content of whey proteins was insignificant.

Research on milk protein haplotypes by Čítek et al. (2019) showed that, compared to the *CSN2* A2 bearing haplotypes, the haplotypes containing the *CSN2* A1 allele were characterized by a lower content of β- and αS2-casein and a higher content of αS1- and κ-casein. The main effect of the *CSN2* A1 allele was a noticeable decrease in γ-casein content. The results of this study indicate that the A2 allele of *CSN2* can have a positive effect on the expression of β-casein compared to the most common alleles, without altering the content of other protein fractions.

Analysis of *CSN2* association in the study by Čítek et al. (2019) showed a statistically significant difference between the *AB* and *BB* genotypes in the protein content of milk ($P < 0.05$). The trend clearly showed a positive effect of the *A* allele on milk protein content, but the differences between *AA* and *AB* genotypes and between *AA* and *BB* genotypes were not significant, possibly also due to the very low number of *AA* homozygotes.

### *CSN3*

Heck et al. (2009) showed that the κ-casein genotypes were associated with the percentage of protein ($P < 0.001$) and the differences in the relative contents of all 6 major milk proteins (all $P < 0.001$). Variant B κ-casein was associated with a higher percentage of protein, and variant E with a lower percentage of protein compared to variant A. Variant B κ-casein was associated with a lower relative concentration of α-lactoglobulin and αS1-casein and a higher relative concentration of αS2-casein compared to variant A. Variant B of κ-casein was associated with a higher relative concentration of κ-casein, and variant A with a lower relative concentration of κ-casein compared to variant E.

Research by Bartonova et al. (2012) indicate that the relationship between the *CSN3* gene and daily milk yield was significant. The association analysis performed with the *CSN3* gene showed that there was a highly significant difference between the genotypes *AA* and *AE*, *AB* and *AE*, and *AE* and *BB*, and a significant difference was observed between the genotypes AE and EB with regard to the daily milk yield. No relationship was found in the studies between the *CSN3* gene and the efficiency of fat and protein in milk.

Also, the research by Deb et al. (2014) showed that subjects with the *AB* genotype had significantly ($P < 0.05$) higher total milk yield and productivity after 300 days than subjects with the *AA* genotype. There was also no significant difference between the two identified genotypes in terms of percentages of fat, protein and lactose.

Similarly, the highest milk production was recorded for the *AA* genotype at the *CSN3* locus (5887.76 ± 115.7 kg) in the studies by Neamt et al. (2017). This production was significantly higher ($P \leq 0.003$) than that observed for the *BB* genotype (5619 ± 86.34 kg). There were no significant differences between *AA* and *AB* genotypes in the study (5839.37 ± 117.8 kg). Milk production related to the *AB* genotype turned out to be significantly higher ($P \leq 0.007$) compared to the production related to the *BB* genotype (5619 ± 86.34 kg). The *AA* genotype favored a higher milk fat content (4.19 ± 0.05%) compared to the *AB* genotype (4.08 ± 0.06%, $P \leq 0.04$) and *BB* (4.01 ± 0.02%, $P \leq 0.008$). In the assessment for *AA* and *AB* genotypes, the percentage of milk protein had significantly lower values compared to the *BB* genotype (3.27 ± 0.03%, $P \leq 0.012$, 3.29 ± 0.03%, $P \leq 0.036$, 3.4, respectively ± 0.02%).

### *BLG*

Heck et al. (2009) indicates that the *BLG* genotype was associated with differences in the relative concentration of all 6 milk proteins (all P < 0.001) but not with any feature of milk production (all P > 0.23). Compared to variant B, variant A was strongly associated with a higher relative concentration of β-lactoglobulin and a lower relative concentration of the other 5 major milk proteins. Cows with *BB*, *BA* and *AA* genotypes had mean relative β-lactoglobulin concentrations of 6.58, 8.15 and 9.52%, respectively.

In the studies of Karimi et al. (2009), no significant relationship was found between different *BLG* genotypes and milk yield characteristics of the analyzed cows. Milk from the cows with the *BLG BB* genotype had a higher fat content, while the milk yield and the protein content of the cows with the *BLG AB* genotype were higher, but the difference was not statistically significant.

However, the research by Neamt et al. (2017) indicate the influence of the *BLG* gene on milk productivity. The highest milk production was recorded for genotype *AB* (5906.54 ± 166.76 kg), significantly different from that produced by *AA* genotypes (5809 ± 117.58 kg, P ≤ 0.033) and BB (5812 ± 115.11 kg, P ≤ 0.038). The mean protein content in milk of 3.34 ± 0.05% was related to the *AB* genotype, while the value of 3.31 ± 0.04% (P > 0.57) was related to *AA*, and 3.33 ± 0.01% (P > 0.081) with *BB* genotype. This underlines that the *BLG* genotype has no significant effect on the protein content of the milk. In this study, the total milk protein yield was significantly lower in the homozygous *AA* genotypes (192.27 ± 5.43 kg, P ≤ 0.042) and *BB* (193.53 ± 3.94 kg, P ≤ 0.026) compared to the *AB* heterozygous genotype (197.27 ± 6.19 kg). There were no significant differences (P > 0.34) between the homozygous *AA* genotypes (192.27 ± 5.43 kg) and *BB* (193.53 ± 3.94 kg) for the total milk protein yield.

### *FASN*

Certain polymorphisms in the *FASN* gene have been associated with higher milk yield and fatty acid composition in dairy cattle. In particular, the g.21272422 C>T polymorphism has been shown to affect the milk yield and protein yield, with individuals carrying the *CC* genotype showing higher production levels compared to those with the *CT* genotype (Liu et al. 2024).

Roy et al. (2006) analyzed the effect of two SNPs of the *FASN* gene on milk fat content. The *G>C* substitution in exon 1 and the *A>G* substitution in exon 34. The study showed that the *C* allele in exon 1 is significantly associated with high milk fat content compared to high and low groups of Holstein Friesian cows. The *C* allele in exon 1 eliminates the putative binding site of the Sp1 transcription factor. This site may be important as it is conserved in the human *FASN* exon 1. Different secondary structures are predicted for the two 5'-UTR variants, suggesting that this site may be involved in modulating mRNA stability. The *G* allele at exon 34 (which produces alanine) was associated with an increased content of milk fat.

Alim et al. (2014) showed the effect of six SNPs (*g.8948C>T*, *g.13965C>T*, *g.14439T>C*, *g.16907T>C*, *g.17924A>G* and *g.18663T>C*) in the *FASN* gene on fat content in milk (P < 0.05 to < 0.01), and one of them (*g.8948C>T*) also had a significant effect on the amount of fat and protein in milk (P < 0.05).

In the study by Ciecierska et al. (2013) on the *g.17924A>G* polymorphism, individuals with the *AA* genotype were characterized by the highest milk and protein efficiency. Cows of this genotype in the first lactation produced 36 kg more fat and 27 kg more protein than animals with the *AG* genotype (p ≤ 0.05), but in the subsequent lactations the differences in the amount of fat and protein were not statistically significant.

### *SCD1*

The research by Kulig et al. (2013) indicate relationships between *g.10329C>T* genotypes and the breeding value for protein content in Polish Holstein-Friesian cows. A statistically significant difference was observed between *TT* and *CC* cows. The cows with the *TT* genotype were characterized by a higher content of fat and protein, but the *CT* individuals were characterized by a higher breeding value in terms of milk, fat and protein yield.

Studies on the *SCD1-A293V* polymorphism by Soltani-Ghombavani et al. (2016) showed the effect of the *SCD1-A293V* genotype on the annual milk yield. The individuals with the *AA* genotype had a lower annual milk yield compared to the individuals with the *AV* genotype ($P < 0.01$). These studies also revealed the effect of the *SCD1-A293V* polymorphism on the percentage of protein. Cows homozygous for the *V* allele showed higher milk protein content compared to *AA* and *AV* genotypes ($P < 0.01$) and replacing the *A* allele with the *V* allele increased the protein content by 0.02%. The same polymorphism (*SCD1-A293V*) in relation to the characteristics of milk production was also analyzed by Kęsek-Woźniak et al. (2020). There were differences in the amount of fatty acids in milk. The milk of cows with the *VA* genotype had the highest content of C8:0 and C10:0 acids in comparison to the homozygotes. Additionally, homozygous *AA* individuals had the lowest content of C12:0 and C13:0 acids. Moreover, the group of 18-carbon fatty acids was found to be related to *A293V* SNP. The saturated form, C18:0, was found in the milk of first phase *VV* cows in abundance, but significant differences ($P < 0.05$) were only observed between *VV* and *AA* genotypes.

Further studies confirm that *SCD1* plays an important role in regulating fat metabolism, which influences the content of unsaturated fatty acids in milk. In dairy cattle, higher *SCD1* activity leads to higher oleic acid (C18:1) content, which is beneficial for milk fat quality. Changes in the expression of this gene can thus lead to a better fat profile in milk and its higher nutritional value (Wang et al. 2024).

## CONCLUSIONS

The results of the research on the relationship between the genetic variants *IGF1/SnaB*I and *IGF1/Tas*I with the milk performance traits are consistent. Most studies confirmed the beneficial effect of the *CT* genotype of the *IGF1/SnaB*I polymorphism on the content of fat and protein in milk and milk yield. In the case of the *IGF1/Tas*I polymorphism, the *CC* genotype was the most favorable in terms of milk production, protein and fat content. In contrast, the *BB* genotype was the most favorable regarding protein and fat content in the *IGF1R/i16/Taq*I polymorphism (Ge et al. 2001; Bonakdar et al. 2010; Szewczuk et al. 2012; Szewczuk 2016).

The research shows the negative influence of the *TT* genotype of the *GHR-F279Y* polymorphism on milk production. On the other hand, the *GG* genotype of the *GHR/Alu*I polymorphism had a positive effect on protein content, and the *AA* genotype on milk yield. There was no effect of *GHR* polymorphisms on the percentage of fat and protein in milk (Komisarek et al. 2011; Cobanoglu et al. 2021).

In case of *CSN2*, the A2 allele influenced the increased content of β- and αs1-casein in milk, and the A1 allele increased the content of αs1- and κ-casein. Additionally, individuals with the *AA* genotype were characterized by a higher protein content in milk (Heck et al. 2009).

Studies on the κ-casein polymorphism showed that individuals with the *AA* genotype had higher milk yield, as well as a higher fat content in milk. The *BB* genotype, on the other hand, was associated with a higher protein content in milk (Čítek et al. 2019).

Some authors of the studies did not find a relation between the presence of the polymorphic form of the *BLG* gene and the features of milk productivity, however, studies linking the polymorphisms of this gene with milk productivity indicate individuals with the *AB* genotype as having the highest milk yield. There are no significant statistical differences in protein and fat content between individuals of different genotypes (Neamt et al. 2017).

The *FASN g.21272422 C>T* polymorphism has been associated with the characteristics of milk productivity. Individuals with the *CC* genotype show the highest milk and protein yield than *CT*. Additionally, the *C* allele in exon 1 was significantly associated with high milk fat content (Liu et al. 2024).

Studies on the *g.10329C>T* polymorphism indicate that individuals with the *TT* genotype were characterized by a higher content of fat and protein, while heterozygous individuals are characterized by a higher milk, fat and protein yield (Kulig et al. 2013).

The *SCD1-A293V* polymorphism was associated with milk yield, with the *AV* genotype being the most preferred. On the other hand, the *VV* genotype was characterized by the highest protein content in milk. In addition, this polymorphism affects the content of fatty acids in milk. The milk of individuals with the *VA* genotype contained more C8: 0 and C10: 0 acids (Soltani-Ghombavani et al. 2016).

Both growth hormone (GH) and insulin-like growth factor 1 (IGF-I) play a key role in the development and growth of animals, and their interactions significantly influence milk production and its composition. GH stimulates the hepatic synthesis of IGF-I, which in turn affects GH secretion and modulates the expression of its receptor. IGF-I also has a significant impact on reproduction, lactation, and feed intake through its interaction with the somatotropic axis (Nicolini et al. 2013; Brown-Borg 2022). Studies have shown that both genes, *IGF-I* and *GH*, play a fundamental role in milk production and composition in dairy cattle, and changes in these genes can affect milk yield and quality (Pereira et al. 2005; Valencia et al. 2022). Understanding the mechanisms by which these genes function and interact with each other allows for the optimization of milk production and the improvement of dairy product quality (Silveira et al. 2019; Ahmadzadeh et al. 2019).

Research on polymorphisms in the *IGF-I* and *GH* genes is important for the dairy industry, as both genes are associated with productive and reproductive traits in animals. There is evidence suggesting that specific variants of these genes may have positive or negative associations with milk production, reproductive performance, and growth rate in dairy cattle and other species (Saleh et al. 2022). Identifying these polymorphisms can help breeders select herds with favorable gene variants, contributing to the rapid improvement of efficiency and, therefore, the profitability of milk production (Saleh et al. 2023). Moreover, studying the genetic basis of these traits can support the development of targeted management strategies that maximize dairy herd performance (Nugroho et al. 2022). Therefore, research on polymorphisms in the *IGF-I* and *GH* genes is crucial for the advancement of the modern dairy industry.

The *SCD1* gene is one of the key genes influencing milk quality, particularly fat content and fatty acid profile. Polymorphisms in this gene can have varying effects depending on the genotype, with benefits in increasing milk protein and fat content, especially in the *AV* genotype. Studies highlight the importance of *SCD1* in regulating milk fat quality, which is relevant for genetic selection in animal breeding, aiming to achieve better milk quality and more efficient milk production (Wang et al. 2024).

The identification and understanding of polymorphisms in these genes enable breeders to select individuals with advantageous genetic profiles, accelerating the improvement of dairy cattle productivity and profitability. By incorporating genetic knowledge into breeding strategies, it is possible to optimize milk production and quality, ensuring the continued advancement of the dairy industry.

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## KANDYDUJĄCE MARKERY GENETYCZNE ZWIĄZANE Z CHARAKTERYSTYKĄ PRODUKCJI MLEKA ORAZ PRZYDATNOŚCIĄ TECHNOLOGICZNĄ MLEKA

**Streszczenie.** Wydajność i skład mleka są kluczowymi parametrami dla przemysłu mleczarskiego, determinowanymi przez wiele genów. Praca zawiera zbiorczą analizę badań wybranych kandydujących markerów genetycznych związanych z cechami produkcji mleka i jego przydatnością technologiczną. Wyniki analizowanych badań wskazują, że polimorfizmy genów *GHR*, *IGF-1*, *CSN2*, *CSN3*, *FASN* i *SCD1* wpływają na skład mleka, a tym samym na jego przydatność technologiczną. Natomiast badania nad polimorfizmami *BLG* cechuje zróżnicowanie wyników, co wskazuje na potrzebę dalszych analiz.

**Słowa kluczowe:** mleko, polimorfizm, przydatność technologiczna.