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CATHL2 gene polymorphism in relation to production traits in Holstein-Friesian cows

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The aim of this study was to identify SNPs mutations in the *CATHL2* gene and determine their potential association with dairy performance traits in Polish Black-and-White Holstein-Friesian (phf) cows. Genotypes of individuals were identified by PCR-RFLP. The frequencies of *CATHL2*/*Dde*I alleles were *C* – 0.693 and *T* – 0.307, and for *CATHL2*/*Hha*I polymorphisms, *G* – 0.763 and *C* – 0.237. The statistical analysis showed that cows with the *CC* (*CATHL2*/*Dde*I) and *CG* (*CATHL2*/*Hha*I) genotype produced higher milk yield than the other cattle genotypes. In the case of *CATHL2*/*DdeI* and *CATHL2*/*HhaI* polymorphisms, the highest somatic cell count was found in heterozygous *CT* and *CG* cows.

KEY WORDS: cathelicidins / *CATHL2/Dde*I / *CATHL2/Hha*I / Holstein-Friesian cows

The production traits of dairy cattle include daily milk yield (kg), milk protein and fat content (%), and somatic cell count. Daily milk yield and protein and fat content play a key role in the acquisition of milk with the desired properties. Somatic cells are important in terms of milk production and cow health. Somatic cells are present in the milk of healthy cows, but the count should not exceed 200,000/ml. Somatic cells are mainly cells of the mammary alveoli and the epithelial lining of the milk ducts. They are present in milk due to the natural exfoliation process resulting from wear and death during milk production. The standard somatic cell count also includes leukocytes that are found in milk and perform antimicrobial functions [13].

The somatic cell count may rise, which is indicative of infection or is associated with external factors such as contusion of the udder, improper milking or prolonged exposure to

cold temperatures. In the case of infection with pathogenic microbes, an increased somatic cell count is due to an influx of immune cells from the blood into the udder. The most common disease in dairy cattle is mastitis, which in a short time causes an increase in the number of somatic cells in milk, and depending on its form, in a reduction or complete cessation of milk production and possible damage to the udder [10].

Mastitis is inflammation of the udder caused by a variety of pathogenic microorganisms, such as Gram-positive and Gram-negative bacteria, viruses, mycoplasmas, moulds and fungi. The main pathogens causing mastitis are *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp. and *Candida* [7]. Defence mechanisms initiated during infection include exfoliation and renewal of the epithelial lining of the teat canal, which in turn cause a substantial increase in the somatic cell count in milk. In addition, substances that reduce tissue penetration by pathogens are activated. These include lysozyme, lactoperoxidase, lactoferrin, and cathelicidin [15]. Cathelicidin is a protein with numerous important functions, mainly antibacterial, antiviral and antifungal activity. However, it also has an important role in wound healing, angiogenesis and regulation of the immune system [4].

An important aspect of the study of dairy cattle performance is research on the effects of mutations in genes encoding proteins responsible for various processes that may affect lactation and milk quality.

The *CATHL2* gene has been mapped on cattle chromosome 22. This gene consists of four exons separated by three introns, and has a total of 1559 base pairs. *CATHL2* gene expression in healthy cattle has been demonstrated in tissues of organs such as the ovaries, small intestine, liver, spleen, lymph nodes, lung, and mammary gland [14].

The *CATHL2* gene encodes the Bac5 protein of the cathelicidin family. This protein is composed of three main parts: a signal peptide, a cathelin-like domain, and an active site domain with antimicrobial properties. The protein consists of 176 amino acids, of which the active site domain comprises 43 amino acids rich in proline and arginine. Due to the high content of amino acids in the variable region, the Bac5 protein performs functions supporting connective tissue regeneration [3].

In view of the role of cathelicidins as antimicrobial peptides, there is an important link between gene expression and the number of somatic cells. A high somatic cell count may indicate disease states that affect the amount of milk produced and its quality, including fat and protein parameters. Cathelicidin may have a positive effect by enhancing immunity, which in turn helps to maintain a high level of lactation and to limit deterioration of milk parameters.

The aim of the study was to analyse the frequency of SNP polymorphism genotypes and alleles in the *CATHL2* gene and to estimate the influence of the genotypes on selected performance parameters in a herd of cows.

Material and methods

The study was conducted on a herd of 150 Polish Holstein-Friesian Black-and-White dairy cows on a farm in the West Pomeranian voivodeship of Poland. The cows were kept in a free-stall barn and fed in a TMR (Total Mixed Ration) system. In spring and summer, the cattle grazed in the pasture. They were milked twice a day with a mechanical milking machine. Data regarding the cows' performance were obtained from breeding documentation kept by the Polish Federation of Cattle Breeders and Dairy Farmers. The somatic cell count (SCC), expressed in thousands per ml of milk, was log-transformed in Excel® (LnSCC) according to Ali and Shook [1] so that the trait met the conditions for normal distribution, as did daily milk yield (kg) and milk protein and fat content (%).

The first step in the analysis was isolation of DNA. Peripheral blood was drawn from the jugular vein of each cow into sterile tubes containing K_3EDTA as an anticoagulant. DNA was isolated using a MasterPure Kit (Epicenter Technologies) for DNA isolation according to the manufacturer's instructions.

Genotype analysis was performed by PCR-RFLP. SNPs 1730 *T>C* (rs109775410) and 1757 *G>C* (rs109848035) located in intron 3 of the *CATHL2* gene were analysed. The *CATHL2* gene fragment was amplified using PCR with appropriate specific primer sequences: forward – 5'-GGGCCTCGGTTTCATCTCTGTC-3' and reverse – 5'-AAGATCGGTGGGCGGATCGG-3'. The primer sequences were designed based on the *CATHL2* gene sequence (GenBank EU380692).

Amplification of the *CATHL2* gene fragment was optimized using initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 45 s, extension at 72°C for 30 s, and a final extension for 8 min at 72°C. After PCR, electrophoresis was carried out in a 1% agarose gel to evaluate the quality of the product.

The resulting amplification products were digested using restriction enzymes *Dde*I and *Hha*I for at least 3 hours at 37°C. The resulting PCR products and restriction fragments were separated on a 3% agarose gel with ethidium bromide in the presence of a pUC19/*Msp*I marker. The results were visualized and archived using an agarose gel documentation and analysis system.

Statistical analysis of the results was performed. The relationships between genotypes, combinations of genotypes and selected performance parameters, including daily milk yield (kg), milk protein and fat content (%), and somatic cell count were analysed. Statistical analysis of the relationships between *CATHL2/Dde*I and polymorphisms *CATHL2/Hha*I and milk performance traits was performed using Statistica 12 PL software [16]. Means ($\overline{x}$) and standard deviations (SD) were calculated, and one-way

analysis of variance was performed using the Duncan multiple range test. The significance of differences in the mean somatic cell count was assessed using the Kruskal–Wallis test.

Results and discussion

Analysis of the 285 bp PCR product following restriction enzyme digestion revealed three genotypes in the case of each enzyme used. The genotypes *TT* (285 bp), *TC* (285 bp, 202 bp and 83 bp) and *CC* (202 bp and 83 bp) were obtained for *Dde*I enzyme digestion, and genotypes *GG* (285 bp), *CG* (285 bp, 173 bp and 112 bp) and *CC* (173 bp and 112 bp) when the *Hha*I restriction enzyme was used.

Table 1 presents the results of the statistical analysis of the frequency of alleles and genotypes in the Polish Holstein-Friesian Black-and-White dairy cattle herd. For *CATHL2*/*Dde*I, the frequency of homozygote *TT* was the lowest, while the frequencies of the heterozygous *TC* genotype and the homozygous *CC* genotype were similar, with the recessive homozygotes occurring with the highest frequency. The frequency of the *T* allele was lower than that of the *C* allele. For *CATHL2/Hha*I polymorphism, the genotype frequency was highest for the homozygous *GG* genotype and the lowest for heterozygous *CG*. The *G* allele was the most frequent, while the frequency of the *C* allele was much lower.

Table 1
Genotype and allele frequencies in the cattle herd (n – number of cows)

Polymorphism	n	Genotype frequencies		Allele frequencies	
*CATHL2/Dde*I	16	*TT*	0.107	*T*	0.307
	60	*TC*	0.400	*C*	0.693
	74	*CC*	0.493		
*CATHL2/Hha*I	109	*GG*	0.727	*G*	0.763
	11	*CG*	0.073	*C*	0.237
	30	*CC*	0.200		

The next stage of the study was analysis of the relationships between individual polymorphisms genotypes and milk production traits. Statistical analysis was carried out for daily milk yield, percentage content of fat and protein in the milk, and somatic cell count. Table 2 presents the means and standard deviations for the production traits in relation to the genotypes.

Table 2

Mean values ($\bar{x}$) and standard deviations (SD) for milk yield traits in relation to *CATHL2*/*Dde*I and *CATHL2*/*Hha*I genotypes in the cattle herd (n – number of cows)

Genotype	n	Milk yield (kg)	Fat content (%)	Protein content (%)	LnSCC*
TT	16	16.27 ±2.10	3.95 ±0.54	3.28 ±0.24	3.26 ±1.06
TC	60	16.37 ±2.37	3.79 ±0.52	3.27 ±0.21	3.42 ±1.23
CC	74	16.41 ±2.37	3.87 ±0.49	3.24 ±0.19	3.40 ±1.35
GG	109	16.40 ±2.36	3.86 ±0.51	3.25 ±0.20	3.34 ±1.24
CG	11	16.75 ±2.96	3.81 ±0.60	3.31 ±0.20	3.97 ±1.51
CC	30	16.17 ±2.01	3.81 ±0.49	3.25 ±0.22	3.37 ±1.27

*LnSCC – natural logarithm of somatic cell count

For *CATHL2/Dde*I polymorphism, the highest milk yield was noted in animals with the *CC* genotype, and the values for both fat and protein content were the highest for animals with the *TT* genotype.

In the case of *CATHL2/Hha*I polymorphism, the highest milk yield and content of milk protein were found for heterozygous cows, and milk fat content was the highest for animals with the *GG* genotype. Regarding the somatic cell count, the highest counts in the case of both *CATHL2/Dde*I and *CATHL2/Hha*I were found in cows with the heterozygous *CT* and *CG* genotypes, respectively. For *CATHL2/Dde*I polymorphism, the lowest somatic cell count was found for animals with the *TT* genotype. For *CATHL2/Hha*I polymorphism, the milk of cows with *GG* and *CC* genotypes had similar somatic cell counts, which were lower than for animals of the heterozygous genotype.

Milk production is closely linked to the physiological state of the cow. In the case of udder disorders, it is important that the animal's defence mechanisms quickly eliminate the inflammatory factor. Elements representing the first line of defence include proteins of the cathelicidin family, which exhibit bactericidal, antiviral and antifungal properties. Research on polymorphisms within the *CATHL2* gene may provide information on an increased or decreased role of cathelicidins in dairy cows [17].

Due to the role of Bac5 in the bactericidal process, it is important to determine the incidence of polymorphisms within the *CATHL2* gene and their association with dairy performance [5]. The *CATHL2* gene has been shown to be expressed in healthy bovine tissues, including the mammary gland. This indicates that the Bac5 protein functions as an antimicrobial peptide that controls the physiological state of the udder. Whelehan et al. [17] have also studied the expression of genes encoding cathelicidins in the case of high

somatic cell counts in Polish Holstein-Friesian Black-and-White cows. They showed that high somatic cell counts do not have a clear effect on expression of these genes. At the same time, it cannot be overlooked that the genes are also expressed in healthy tissues, so an infection causing an increase in somatic cell count need not be associated with increased expression of the genes.

This should not diminish the importance and role of cathelicidins in defence mechanisms activated during udder disease and as a preventive mechanism, due to the presence of cathelicidins in healthy tissues. Liaox et al. [8] have demonstrated that the occurrence of polymorphisms within genes encoding cathelicidins can affect performance traits, and the results can be used in selection programmes.

Mei et al. [9] have also reported an association between polymorphism in the cathelicidin genes and production traits.

Many studies have been conducted to detect polymorphisms of other genes that could be used as markers of production traits in dairy cattle. Pawar et al. [11] have demonstrated an association between bovine growth hormone (*GH*) polymorphism and higher milk yield in dairy cattle.

Polymorphism in the *STAT5* gene, also known as mammary gland factor (MGF), has been investigated by Brym et al. [2], who found relationships between polymorphism and production traits.

There has been a great deal of research on the associations between genotypes of both defensins (also classified as antimicrobial proteins – AMPs) and cathelicidins and dairy production traits. Ryniewicz et al. [12] have investigated the relationship between defensin gene polymorphism and performance parameters. Krzyżewski et al. [6] have also shown associations between the bovine β4-defensin gene and milk traits, which can be used as a marker in dairy cow selection.

Polymorphism within the *CATHL2* gene indicates that for *CATHL2/Dde*I, the best parameters for protein and fat content and the highest milk yield were observed for homozygotes. The case of *CATHL2/Hha*I is different. Here, the highest milk yield was recorded for heterozygotes, but the protein and fat parameters were the highest for homozygotes.

It is important to note the somatic cell count, which was highest in the milk of heterozygotes. This may indicate that in the case of homozygous genotypes the *CATHL2* gene encoding the Bac5 protein is expressed to a small but greater extent.

Research on polymorphisms in genes encoding cathelicidins can provide information on the effect of these mutations on dairy cow performance. Knowledge of how *CATHL2* polymorphism is associated with milk performance traits and somatic cell count may be useful for improving dairy cattle selection and breeding programmes.

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