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# ASSOCIATIONS BETWEEN INTERLEUKIN-8 RECEPTOR (*CXCR1*) AND INTERLEUKIN-6 (*IL6*) POLYMORPHISM AND IMMUNITY TO MASTITIS IN THE BLACK-AND-WHITE VARIETY OF POLISH HOLSTEIN-FRIESIAN COWS

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**ABSTRACT**

The study involved 448 Black-and-White Holstein-Friesian cows. All animals came from one herd, they were kept in the same barn and in the same environmental conditions. Complete phenotypic data on the studied animals was collected. The aim of the research was to search for possible associations between SNPs in the *CXCR1* and *IL6* genes and *mastitis*. Identification of specific SNPs was possible due to the use of the PCR-ARMS method. Statistically significant associations were found between the analysed SNPs and resistance to *mastitis* and selected milk production traits.

**Key words:** animal breeding, *IL6* gene, *CXCR1* gene, dairy cattle, milk production traits, mastitis

## INTRODUCTION

The *mastitis*, or inflammation of the mammary gland, has for years been the main cause of economic losses in the dairy industry all over the world. Farmers and milk producers incur high costs due to treatment of sick animals, lower milk quality class, or premature culling of seriously ill cows [Aghamohammadi et al. 2018]. Aghamohammadi et al. [2018], who conducted research in Canadian dairy farms, estimated the cost of one *mastitis* case at CAD 662 per cow.

Bovine *mastitis* results from an immune response to microbial infections of the udder. The disease can also develop as a result of chemical, thermal or mechanical damage to the udder tissues leading to the formation of inflammation in the mammary gland in response to these lesions. The inflammation of the udder can take two main forms – clinical *mastitis* (CM) and subclinical *mastitis* (SCM). Clinical *mastitis* is characterized by pronounced changes in the appearance of the udder and milk. On the other hand, symptoms in subclinical *mastitis* are usually not noticeable and therefore subclinical *mastitis* is diagnosed on the basis of somatic cell count (SCC) in milk. Subclinical infections are much more frequent than CM [Amer et al. 2018].

About 70 years ago, scientists showed that the incidence of *mastitis* is associated with genetic factors. Later, the relationship between clinical *mastitis*, bacteriological status and SCC was demonstrated, and as a result dairy cattle genetic improvement programs were focused on preserving desirable animal traits [Weigel and Shook 2018]. It is known that *mastitis* develops in cows more sensitive to infections and depends to a large extent on the innate and acquired immunity of animals. Innate immunity plays a key role when the mammary gland is first exposed to pathogens that cause the disease to develop. Rapid activation of innate immunity mechanisms can lead to the reduction of damage to mammary tissues caused by pathogens. In response to inflammation, including *mastitis*, both elements of innate and acquired immunity must participate. In fact, without any joint action, each of these types of immunity is of little value [Sordillo 2018].

Inflammation of the mammary gland leads to an increase in SCC as a result of migration of neutrophils to the inflammatory focus. The increase in SCC results in an increased production of many chemotactic factors, such as interleukins [Sakemi et al. 2011]. Interleukins are cy-

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tokines that have several abilities, e.g. cell proliferation, migration and adhesion. They play an important role in differentiating and activating cells of the immune system. Some of them show pro- and anti-inflammatory effects [Brocker et al. 2010]. Pro-inflammatory mediators include interleukins 6 (Il-6) and 8 (IL-8), which can modulate immunity to infections [Seroussi et al. 2018, Chen et al. 2017]. Interleukin-6 shows a multidirectional effect, e.g. it stimulates the differentiation of B and T cells and activation of macrophages and NK cells, and stimulates the production of acute-phase proteins [Huang et al. 2014]. IL-6 can be used as an inflammatory marker in case of inflammation caused by bacteria. This type of interleukin is produced by macrophages and monocytes in response to other inflammatory cytokines [Naseem et al. 2016].

IL-8 interacts with its two receptors, CXCR1 and CXCR2, to trigger neutrophilic activation, stimulate chemotaxis and increase phagocytosis. The CXCL8-CXCR1 / 2 signaling pathway is involved in the pathogenesis of many diseases. Both receptors are identical. They differ only in their ability to interact with other ELR+ chemokines (which feature the glutamate-leucine-arginine amino acid motif preceding conserved cysteines). CXCR1 weakly binds to other ELR+ chemokines, whereas CXCR2 binds to all other ELR+ chemokines [Ha et al. 2017, Galvão et al. 2011]. The interleukin-α receptor encoded by the CXCR1 gene has a high affinity for IL-8 [Pawlik et al. 2015].

In the light of the complex function of interleukin 8 and its receptor, and interleukin-6 in response to inflammation, we conducted studies to search for associations between polymorphic variants of genes encoding interleukin-8 receptor (*CXCR1*) and interleukin-6 (*IL6*), and *mastitis* and other milk production traits in the Black-and-White variety of Polish Holstein-Friesian cows.

## MATERIAL AND METHODS

The research material was peripheral blood collected from 448 Black-and-White Polish Holstein-Friesian cows. The animals were kept on one farm located in the north-west of Poland.

The cows were kept in one free-stall barn and were fed standardized feed rations according to the TMR (total mixed ration) system throughout the year. In addition, the animals received individually adjusted supplementary feed concentrates while milked and had constant access to water. The cows were milked twice a day in a herringbone milking parlour. The cattle were of different ages and in different stages of lactation. The health of the cows was monitored by an experienced veterinarian, who recorded all cases of *mastitis* and categorized them as *mastitis acuta* (MA) or *mastitis chronica* (MC). In addition, he recorded the duration of the disease, the number of MA and MC cases, the number of inflamed quarters, and the incidence of other diseases (inflammation of reproductive organs or hooves, respiratory infections – bronchitis and bronchopneumonia). The phenotypic data were collected based on samples taken during test-day milkings and included daily milk yield, SCC, and percentage content of fat, protein, lactose and dry matter in milk.

The collected peripheral blood samples were used to isolate genomic DNA, and the isolated DNA was analysed using the PCR-ARMS method (Table 1).

### Statistical analysis

The number of clinical cases and duration of the disease were determined for each subject. Also calculated were the mean number of clinical cases and the mean number of disease days per one intra-calving period (including full lactation and a dry period). Next, a multivariate analysis of variance (MANOVA) was performed to determine the significance of associations between *IL6* and *CXCR1* genotypes and the mean number of *mastitis acuta* cases per cow per year. The sources of variability included *IL-6* and *CXCR1* genotypes, the effect of the cow's birth year/season, the effect of the cow's calving year/season that preceded the occurrence of clinical *mastitis*, and the additive effect of the genetic background (sire). The applied model was as follows:

$$y_{ijklm} = \mu + G_i + P_j + R/SUR_k + R/SWY_l + \\ + b(HF_m) + e_{ijklm}$$

where:

$y_{ijklm}$ – number of *mastitis* cases per cow per lactation,
$\mu$ – mean number of *mastitis* cases in the herd per lactation,
$G_i$ – constant effect of the $i$-th genotype,
$P_j$ – random polygenic additive effect of the genetic background ($j$-th sire),
$R/SUR_k$ – constant effect of the cow's $k$-th birth year/season,
$R/SWY_l$ – constant effect of the cow's $l$-th calving year/season,
$b(HF_m)$ – regression on the percentage share of Holstein-Friesian genes,
$e_{ijklm}$ – random error effect.

Statistical analysis was also carried out to analyse the relationship between *IL-6* and *CXCR1* polymorphisms and SCC in milk. The sources of variability included factors such as: study year/season, parity, share of

**Table 1.** Primer sequences used in the PCR-ARMS reaction (according to Leyva-Baca et al. [2007], Blake [2009])

**Tabela 1.** Sekwencje starterów wykorzystywanych w PCR-ARMS (wg Leyva-Baca i wsp. [2007], Blake [2009])

| Analysed gene and polymorphism<br>Analizowany gen i polimorfizm | Primer sequences (5' – 3')<br>Sekwencje starterów (5' – 3') | Amplicon length, bp<br>Długość amplikonu, pz |
|---|---|---|
| *CXCR1*<br>*(IL8RA c.777G>C, rs208795699)* | Inner F: CGCACGCTATTTTCAGCCCAAATGGGGGAC | 175 (allele C) |
| | Inner R: AAAGATGACCCGCATGGCCCGGTGCATC | 262 (allele G) |
| | Outer F: AGGCATCTGGGCCCTGTCCGTGATCCTG | 379 (outer primers) |
| | Outer R: GCCAATGTCATTGCGGCGCTGACAGGTC | |
| *IL6*<br>*(rs43380663)* | Inner F: GGGCTCAGAGCAGAGGACCTCCCACC | 225 (allele C) |
| | Inner R: GCCACTGGCCTTGACTCGCCCAGCTA | 255 (allele T) |
| | Outer F: AGGCCCCCGAAGAACCCATTAAAATGCCT | 428 (outer primers) |
| | Outer R: TCCAGCAGGTCAGTGTTTGTGGCTGGAG | |

Holstein-Friesian genes, day of lactation, and cow effect as a random factor hierarchically nested in *IL-6* and *CXCR1* genotypes. Cow's birth year was not included as it coincided with parity in the subsequent years of the study. The study year was divided into seasons: winter (December – February), spring (March – May) summer (June – August) and autumn (September – November).

SCC in milk was transformed to a logarithmic scale (natural logarithm) to balance the distribution. To analyse the associations between lnSCC and milk production traits and the polymorphism of the *IL-6* and *CXCR1* genes, the following multifactor, mixed, hierarchically nested model was developed using the general linear model package:

$$y_{ijklm} = \mu + a_i + b_j + c_k + d_l + g_m + h_n(a_l) + e_{ijklm}$$

where:

$y_{ijklm}$ – mean values of SCC (lnSCC), daily milk yield, fat content and protein content,
$\mu$ – mean SCC in the herd (lnSCC),
$a_i$ – effect of *IL6* or *CXCR1* genotype,
$b_j$ – effect of parity,
$c_k$ – effect of study year/season,
$d_l$ – effect of lactation day,
$g_m$ – effect of the share of Holstein-Friesian genes,
$h_n(a_l)$ – cow effect (factor nested in *IL6* or *CXCR1* genotype),
$e_{ijklm}$ – error.

To test the significance of differences between the mean values of the number of clinical cases and SCC in milk in groups of cows with different *IL6* and *CXCR1* genotypes, Duncan's test was used. The calculations were made using the STATISTICA package.

## RESULTS

The frequencies and numbers of alleles and genotypes of the *CXCR1* gene in the studied herd of cows are presented in Table 2. An analysis of the data showed that the most frequent allele in the studied population was allele C (51.67410) and the most frequent genotype was genotype AC (71.65179), while genotype AA had the lowest frequency (12.500000). Table 3 shows the frequencies and numbers of genotypes and alleles for the *IL6* gene in the herd under study. It was found that the least frequent allele was allele T (33.59375). The least frequent genotype was genotype TT (6.02679) while the most frequent one was genotype CT (55.13393).

The results of an association analysis between the polymorphism in the *CXCR1* gene and *mastitis* and milk production traits (Table 4.) suggest that the most desirable value of SCC is characteristic of individuals with genotype AA, while cows with genotype AC have the worst SCC. The other traits under analysis were daily milk yield and percentage content of milk fat and protein. The highest values of these traits were characteristic of cows with genotype AA, whereas the lowest values were found in individuals with genotype CC in the case of protein and fat content and genotype AC in the case of daily milk yield.

Table 5 presents the results of an analysis of associations between the studied genotypes and traits. Based on these data, it can be concluded that cows with genotype TT had the highest value of SCC, while animals with genotype CT had the lowest value. As for milk fat and protein content, TT individuals were found to produce milk with the highest percentage content of both fat and protein. Milk with the lowest percentage content of fat and protein was obtained from cows with genotype CC. The last milk production trait under analysis was daily milk yield. It was found that CC individuals produced more milk and TT cows produced least milk.

The above results show that the studied polymorphisms of the *CXCR1* and *IL6* genes have a pleiotropic effect

**Table 2.** Genotype and allele frequencies for the CXCR1 gene

**Tabela 2.** Frekwencje alleli i genotypów dla genu CXCR1

| Genotype/allele (*CXCR1*)<br>Genotyp/allel (CXCR1) | Number of cows<br>Liczba krów | Percentage<br>Procent |
|---|---|---|
| AC | 321 | 71.65179 |
| CC | 71 | 15.84821 |
| AA | 56 | 12.50000 |
| A | 215 | 48.32590 |
| C | 233 | 51.67410 |
| In total – Ogółem | 448 | 100 |

**Table 3.** Genotype and allele frequencies for the IL6 gene

**Tabela 3.** Frekwencje alleli i genotypów dla genu IL6

| Genotype/alleles (*IL6*)<br>Genotyp/ allel (IL-6) | Number of cows<br>Liczba krów | Percentage<br>Procent |
|---|---|---|
| CT | 247 | 55.13393 |
| CC | 174 | 38.83929 |
| TT | 27 | 6.02679 |
| T | 152 | 33.59375 |
| C | 296 | 66.40625 |
| In total – Ogółem | 448 | 100 |

**Table 4.** Mean values of daily milk yield, protein and fat content in milk and lnSCC in milk depending on the CXCR1 genotype

**Tabela 4.** Wartości średnie dobowej wydajności mlecznej, procentowej zawartości białka i tłuszczu oraz lnSCC w mleku z uwzględnieniem genotypów genu CXCR1

| Genotype<br>Genotyp | Daily milk yield, kg<br>Wydajność mleczna, kg | | Milk fat content, %<br>Zawartość tłuszczu, % | | Milk protein content, %<br>Zawartość białka, % | | LnSCC | |
|---|---|---|---|---|---|---|---|---|
| | Mean<br>Średnia | SD | Mean<br>Średnia | SD | Mean<br>Średnia | SD | Mean<br>Średnia | SD |
| AC | 32.119056$^{AB}$ | 10.563301 | 4.05277860$^{AB}$ | 0.81327398 | 3.61048291$^{AB}$ | 0.45160442 | 5.62234405$^{AB}$ | 1.39251971 |
| CC | 33.760202$^{A}$ | 11.407402 | 3.91634064$^{AC}$ | 0.84360608 | 3.44399663$^{AC}$ | 0.42898456 | 5.28134764$^{A}$ | 1.37555896 |
| AA | 34.128485$^{B}$ | 10.484644 | 4.20787879$^{BC}$ | 0.77631057 | 3.67345455$^{BC}$ | 0.45230400 | 5.18286977$^{B}$ | 1.24824207 |
| In total – Ogółem | 32.571701 | 10.724479 | 4.04455602 | 0.81799481 | 3.58892116 | 0.45285041 | 5.52628945 | 1.38677650 |

A,B,C – differences significant at $P \leq 0.01$ – różnice istotne przy $P \leq 0,01$.
a,b,c – differences significant at $P \leq 0.05$ – różnice istotne przy $P \leq 0,05$.

**Table 5.** Mean values of daily milk yield, protein and fat content in milk and lnSCC in milk depending on the IL6 genotype

**Tabela 5.** Wartości średnie dobowej wydajności mlecznej, procentowej zawartości białka i tłuszczu oraz lnSCC w mleku z uwzględnieniem genotypów genu IL6

| Genotype<br>Genotyp | Daily milk yield, kg<br>Wydajność mleczna, kg | | Milk fat content, %<br>Zawartość tłuszczu, % | | Milk protein content, %<br>Zawartość białka, % | | LnSCC | |
|---|---|---|---|---|---|---|---|---|
| | Mean<br>Średnia | SD | Mean<br>Średnia | SD | Mean<br>Średnia | SD | Mean<br>Średnia | SD |
| CT | 32.593534$^{A}$ | 10.795204 | 4.07554481$^{Aa}$ | 0.85991177 | 3.60203157$^{A}$ | 0.46637152 | 5.61587904$^{A}$ | 1.40748281 |
| CC | 32.732840$^{B}$ | 10.733083 | 3.95928668$^{Ba}$ | 0.73965723 | 3.55820323$^{B}$ | 0.43043092 | 5.45484629$^{B}$ | 1.36551284 |
| TT | 30.860606$^{AB}$ | 9.661324 | 4.44363636$^{AB}$ | 0.83827792 | 3.70951515$^{AB}$ | 0.46138684 | 5.10332331$^{AB}$ | 1.20897756 |
| Total – Ogółem | 32.571701 | 10.724479 | 4.04455602 | 0.81799481 | 3.58892116 | 0.45285041 | 5.52628945 | 1.38677650 |

A,B,C – differences significant at $P \leq 0.01$ – różnice istotne przy $P \leq 0,01$.
a,b,c – differences significant at $P \leq 0.05$ – różnice istotne przy $P \leq 0,05$.

on milk production traits, that is they affect SCC, daily milk yield, and percentage content of fat and protein.

Table 6 presents the mean duration of *mastitis chronica* and *acuta* in relation to the *CXCR1* genotype. The longest duration of *mastitis chronica* was recorded among individuals with genotype CC (4.250) and the shortest in cows with genotype GC (2.728). In the other type of udder inflammation – acute *mastitis* – the longest duration of the disease was observed in animals with genotype GG (6.957) and the shortest in individuals with genotype CC (2.988). Table 7 contains data on the mean number of cases per cow in one inter-calving period depending on the *CXCR1* genotype. It was found that individuals with CC and GG genotypes suffered from *mastitis chronica* and *acuta* most frequently (1.150 and 1.809, respectively). In contrast, the lowest incidence of *mastitis chronica* and *acuta* was observed in animals with GG and CC genotypes (0.660 and 0.988, respectively).

The other tables (Tables 8 and 9) show the mean duration of *mastitis chronica* and *acuta* depending on the genotype of the *IL6* gene and the mean number of cases per cow in one inter-calving period depending on the *IL6* genotype. The longest duration of both *mastitis chronica* and *acuta* was recorded in TT subjects (5.250 and 7.750, respectively), and the shortest in CC and CT individuals (2.483 and 6.239, respectively). Moreover, the highest incidence of *mastitis chronica* was observed in cows with genotype TT (1.750) and the lowest incidence was reported in animals with genotype CC (0.703). In the case of *mastitis acuta*, the highest incidence was observed for genotype TT (2.500) and the lowest for genotype CT (1.731).

To sum up the data on clinical *mastitis* cases, it was found that cows with GG and TT genotypes were characterized by the longest duration of *mastitis* (10.119 and 13.000, respectively), while the shortest duration of the disease was observed in CC and CC animals (7.238 and 8.959, respectively). In addition, the number of *mastitis* case was highest in animals with genotypes GC and TT whereas in cows with genotypes CC and CT the incidence of the disease was lowest (2.138 and 2.558). The above data may suggest an association between the duration of the disease and its incidence.

## DISCUSSION

Many studies indicate that the *CXCR1* gene is highly polymorphic and may be used in animal genetic selection. To confirm the potential, Pighetti et al. [Pighetti et al. 2012] analysed 36 SNPs and reconstructed the haplotypes. In addition, one of the most frequently studied SNPs of this gene – CXCR1 +777 – is associated with altered neutrophil function [Pighetti et al. 2012].

Galvão et al. [Galvão et al. 2011] conducted a study to evaluate the relationship between SNP polymorphism at position +735 in the IL-8 receptor gene (*CXCR1*) and the incidence of *mastitis* and milk production traits. The results of the study confirmed that the incidence of clinical *mastitis* is associated with the studied polymorphism in the *CXCR1* gene. The incidence of the disease was highest in cows with genotype GG (the highest incidence) and slightly lower in CC and heterozygous animals. Milk yield was also related to the polymorphism under study. The best results for this trait were observed in heterozygous individuals and the lowest – in animals with genotype GG [Galvão et al. 2011].

A study by Chen et al. 2011 [Chen et al. 2011] showed that the analyzed SNPs are related to *mastitis*. It was shown that -1830AA, -1768TT and -344TT in the *CXCR1* gene significantly correlated with the lowest SCS for each SNPs. Moreover, 10 different haplotypes were determined in the study based on all 4 SNPs (CXCR1c.-1830A>G, CXCR1c.-1768T>A, CXCR1c. 344T>C, CXCR1c.783C>A). Correlation studies between these 10 haplotypes and SCS showed that only one haplotype (Haplo2-ATTA) is statistically significantly related to the level of SCS [Chen et al. 2011].

In their study, Polish scientists analysed six SNPs in the *CXCR1* gene (c. +291C>T, c. +365T>C, c. +816C>A, c. +819G>A, +1093C>T, and + 1373C>A) and their possible association with *mastitis*. It was shown that only 1 SNP (c. +365T>C) out of the six was significantly associated with *mastitis* in Polish cows. Animals with genotype CC were affected by udder inflammation most frequently [Pokorska et al. 2016].

Beecher et al. [Beecher et al. 2010] conducted studies to assess the relationship between polymorphisms in the TLR2 and *CXCR1* genes and udder health indicators in dairy cattle (health indictor traits). The research included 246 lactating dairy cows from 5 different breeds from one farm and 848 Holstein-Friesian bulls. It was shown that TLR4-2021 is statistically significantly related to the percentage content of protein and fat in milk, while in the case of bulls no such association was observed. An analysis of CXCR1-777 indicated the existence of a statistically significant association between the studied polymorphism and milk fat yield among bulls. Association studies between CXCR1-777 and SCS did not show significant differences in cows but showed tendencies to associate [Beecher et al. 2010].

Pawlik et al. [Pawlik et al. 2015] analysed the relationship between two SNPs of the *CXCR1* gene and susceptibility to *mastitis*. 554 Polish Holstein cows were genotyped and 140 of them were examined microbiologically. The authors assessed the differences in test-day SCC and *Staphylococcus aureus* among cows having different genotypes and haplotypes. CXCR1+472 was fo-

**Table 6.** Duration of *mastitis chronica* and *mastitis acuta* depending on the CXCR1genotype

**Tabela 6.** Liczba dni zachorowań na *mastitis chronica* i *acuta* w zależności od genotypu genu CXCR1

| Genotype<br>Genotyp | N[1] | Duration of MC[2], days<br>Długość trwania MC[2], dni | | Duration of MA[3], days<br>Długość trwania MA[3], dni | | Duration of MC[2]+MA[3], days<br>Długość trwania MC[2]+MA[3], dni | |
|---|---|---|---|---|---|---|---|
| | | Mean<br>Średnia | SD | Mean<br>Średnia | SD | Mean<br>Średnia | SD |
| GC | 321 | 2.728 | 8.611 | 6.199 | 17.161 | 8.927 | 21.997 |
| CC | 71 | 4.250 | 18.558 | 2.988 | 10.021 | 7.238 | 27.675 |
| GG | 56 | 3.234 | 11.150 | 6.957 | 17.705 | 10.191 | 28.123 |
| Total – Ogółem | 448 | 3.006 | 10.927 | 5.777 | 16.346 | 8.782 | 23.498 |

[1] number of all genotypes, [2] *mastitis chronica*, [3] *mastitis acuta*.
[1] liczba wszystkich genotypów, [2] *mastitis chronica*, [3] *mastitis acuta*.

**Table 7.** Number of cases per cow in one inter-calving period depending on the CXCR1 genotype

**Tabela 7.** Liczba zachorowań przypadająca na jedną krowę w jednym okresie międzywycieleniowym w zależności od genotypu genu CXCR1

| Genotype<br>Genotyp | N[1] | Number of cases of MC[2]<br>Liczba przypadków MC[2] | | Number of cases of MA[3]<br>Liczba przypadków MA[3] | | Number of cases of MC[2]+MA[3]<br>Liczba przypadków MC[2]+MA[3] | |
|---|---|---|---|---|---|---|---|
| | | Mean<br>Średnia | SD | Mean<br>Średnia | SD | Mean<br>Średnia | SD |
| GC | 321 | 0.874 | 2.577 | 1.776 | 4.341 | 2.650 | 5.763 |
| CC | 71 | 1.150 | 4.267 | 0.988 | 2.457 | 2.138 | 6.290 |
| GG | 56 | 0.660 | 1.773 | 1.809 | 3.843 | 2.468 | 5.364 |
| Total – Ogółem | 448 | 0.897 | 2.841 | 1.658 | 4.070 | 2.555 | 5.804 |

[1] number of all genotypes, [2] *mastitis chronica*, [3] *mastitis acuta*.
[1] liczba wszystkich genotypów, [2] *mastitis chronica*, [3] *mastitis acuta*.

**Table 8.** Duration of *mastitis chronica* and *mastitis acuta* depending on the IL6 genotype

**Tabela 8.** Liczba dni zachorowań na *mastitis chronica* i *acuta* w zależności od genotypu genu IL6

| Genotype<br>Genotyp | N[1] | Duration of MC[2], days<br>Długość trwania MC[2], dni | | Duration of MA[3], days<br>Długość trwania MA[3], dni | | Duration of MC[2]+MA[3], days<br>Długość trwania MC[2]+MA[3], dni | |
|---|---|---|---|---|---|---|---|
| | | Mean<br>Średnia | SD | Mean<br>Średnia | SD | Mean<br>Średnia | SD |
| CT | 247 | 3.036 | 12.210 | 6.239 | 19.163 | 9.274 | 26.507 |
| CC | 174 | 2.483 | 8.339 | 6.477 | 17.203 | 8.959 | 21.769 |
| TT | 27 | 5.250 | 11.841 | 7.750 | 15.000 | 13.000 | 19.893 |
| Total – Ogółem | 448 | 2.881 | 10.624 | 6.408 | 18.114 | 9.288 | 24.202 |

[1] number of all genotypes, [2] *mastitis chronica*, [3] *mastitis acuta*.
[1] liczba wszystkich genotypów, [2] *mastitis chronica*, [3] *mastitis acuta*.

**Table 9.** Number of cases per cow in one inter-calving period depending on the IL6 genotype

**Tabela 9.** Liczba zachorowań przypadająca na jedną krowę w jednym okresie międzywycieleniowym w zależności od genotypu genu IL6

| Genotype<br>Genotyp | N[1] | Number of cases of MC[2]<br>Liczba przypadków MC[2] | | Number of cases of MA[3]<br>Liczba przypadków MA[3] | | Number of cases of MC[2]+MA[3]<br>Liczba przypadków MC[2]+MA[3] | |
|---|---|---|---|---|---|---|---|
| | | Mean<br>Średnia | SD | Mean<br>Średnia | SD | Mean<br>Średnia | SD |
| CT | 247 | 0.827 | 2.809 | 1.731 | 4.351 | 2.558 | 6.021 |
| CC | 174 | 0.703 | 2.000 | 1.942 | 5.083 | 2.645 | 5.788 |
| TT | 27 | 1.750 | 3.568 | 2.500 | 3.225 | 4.250 | 5.745 |
| Total – Ogółem | 448 | 0.810 | 2.519 | 1.857 | 4.648 | 2.668 | 5.901 |

[1] number of all genotypes, [2] *mastitis chronica*, [3] *mastitis acuta*.
[1] liczba wszystkich genotypów, [2] *mastitis chronica*, [3] *mastitis acuta*.

und to be significantly associated with SCC in test milk. However, no statistically significant relationship was found between CXCR1+735 and SCC. In addition, a statistical analysis did not show any statistically significant relationship between *CXCR1* and *mastitis* caused by *Staphylococcus aureus* [Pawlik and wps. 2015].

Zhou et al. [Zhou et al. 2013] found 4 SNPs *CXCR1* in their studies and analysed these polymorphisms for their effect on milk traits. The research was carried out on 648 Holstein, Luxi Yellow and Bohai Black cows. There was a statistically significant association between c.337A> G and c.365C> T and somatic cell scores [Zhou et al. 2013].

In their study, Hagiwara et al. [Hagiwara et al. 2001] analyzed the level of interleukin 6 in blood serum and in milk from cows with *mastitis* and healthy ones. Higher concentrations of IL-6 were observed in *mastitis* cows on the first day of the disease. Higher concentrations of the same interleukin were also recorded in the whey of sick animals [Hagiwara et al. 2001]. To date, only a few studies have been conducted to evaluate the effect of the *IL6* gene on *mastitis* in cows. Fonseca et al. [Fonseca et al. 2011] examined the level of expression of various genes, including *IL6*, associated with the response to inflammation in two groups of animals – healthy and sick. The obtained results were compared between sick and healthy cows and no statistically significant differences were found between *IL6* mRNA levels in the two groups of cows [Fonseca et al. 2011]. In another study by the same author, the level of *IL6* gene mRNA was also analyzed in healthy and diseased cows. There were no statistically significant differences in the expression of the gene encoding interleukin 6 [Fonseca et al. 2009].

## CONCLUSION

The study has shown that there is an association between the analyzed SNPs in *CXCR1* and *IL6* genes and resistance to *mastitis*. However, further research must be carried out in different conditions, i.e. on a larger herd or other dairy cattle breeds, to assess the usefulness of the obtained results for the dairy cattle selection programmes.

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## POSZUKIWANIE ASOCJACJI POMIĘDZY POLIMORFIZMEM GENU RECEPTORA INTERLEUKINY-8 (*CXCR1*) ORAZ INTERLEUKINY-6 (*IL6*) A ODPORNOŚCIĄ NA MASTITIS WŚRÓD KRÓW RASY POLSKIEJ HOLSZTYŃSKO-FRYZYJSKIEJ ODMIANY CZARNO-BIAŁEJ

### STRESZCZENIE

Badaniem objęto 448 krów rasy holsztyńsko-fryzyjskiej odmiany czarno-białej. Wszystkie zwierzęta pochodziły z jednego stada, były trzymane w tej samej oborze i hodowane w tych samych warunkach środowiskowych. Zebrano kompletne dane fenotypowe dotyczące badanych zwierząt. Celem badania było poszukiwanie asocjacji między SNPs w genach *CXCR1* i *IL6* a *mastitis*. Identyfikacja określonych SNP była możliwa dzięki zastosowaniu metody PCR-ARMS. Stwierdzono istnienie statystycznie istotnych powiązań pomiędzy analizowanymi SNP a odpornością na *mastitis* i wybranymi cechami użytkowości mlecznej.

**Słowa kluczowe:** hodowla zwierząt, gen *IL6*, gen *CXCR1*, bydło mleczne, cechy produkcji mlecznej, mastitis