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EFFECT OF *CYP19* SNPS ON MILK PRODUCTION TRAITS OF JERSEY COWS

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Abstract. The aim of this study was to examine the relationship between genotype two SNPs located in the promoter region of *CYP19* gene and milk production traits. The study included 181 Jersey cows. The genotypes were identified by the PCR-RFLP method. The frequencies of the most common alleles were as follows: A – 0.98 (*CYP19/Pvu*II) and A – 0.94 (*CYP19/Cfr*13I). The results showed that there were no statistically significant associations between the individual genotypes of both SNPs and milk traits; however, the animals with heterozygous genotypes achieved the lowest milk yield and the highest protein and fat content in milk.

Key words: aromatase, daily milk yield, dairy cattle, milk production traits, *CYP19* gene

INTRODUCTION

Cytochrome P450 aromatase, also known as estrogen synthetase, is a complex enzyme with a molecular weight of about 58 kDa. It is composed of two proteins: specific hemoglycoprotein, the cytochrome P450 aromatase, and non-specific microsomal flavoprotein reductase [Conley and Hinshelwood 2001]. Aromatase belongs to the cytochrome P450 superfamily, in which about 460 different enzymes grouped in 74 families have been identified. It catalyzes the attachment of the oxygen molecule of to the organic molecule known as hydroxylase [Graham-Lorence et al. 1991].

Aromatase is responsible for converting androgen steroid precursors into estrogens, which has an indirect effect on mammary gland development and milk

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production. This gland is the site of the activity of cytochrome P450 aromatase, which is a product of the *CYP19* gene, and is essential for the conversion of androgens into estrogens [Miller et al. 1991].

The *CYP19* gene encodes cytochrome P450 aromatase, which plays an important role in the conversion of androgen hormons into estrogen hormones in all vertebrate species [Lin et al. 2007]. In cattle, the locus of the *CYP19* gene is located on chromosome 10, on the long arm of q2.6 [Fürbass et al. 1997]. The analysis of the bovine *CYP19* gene showed that the gene's locus has 125 kbp of genomic sequence. The sequence including untranslated exons (1.2a, 1.2b, 1.3, 1.4, 1.5) and the promoters respectively has 89 bp. Encoding exons 2–10 have 36 kbp [Vanselow et al. 2004].

The objective of this study is to identify two polymorphisms in the promoter region of the *CYP19* gene and to estimate their possible relationships to milk traits.

MATERIALS AND METHODS

The study included 181 Jersey cows kept in Wielkopolska Region of Poland. All the animals were kept in similar environmental conditions (one herd). They received standard feed and in the spring and summer were kept on pasture. They were milked twice a day using a mechanical milking machine. The herd performance was assessed using A4 – method, in accordance with the recommendations of the International Committee for Animal Recording (ICAR). The data related to SCC were collected from monthly test milkings. SCCs in the samples were determined by means of an instrumental method in compliance with the PN-EN ISO/IEC 17025 standard, using Combifoss equipment (including Fossmatic 5000 apparatus, Foss, Hillerod, Denmark). All the analyses were carried out in a certified milk analysis laboratory.

Full peripheral blood from the jugular vein of each cow was collected into vacuum tubes containing K3EDTA anticoagulant. The DNA isolation was performed using DNA isolation MasterPureTM (Epicentre®) following the isolation protocol supplied with the kit.

Two SNP type polymorphisms located in the P.1.1 promoter region of the *CYP19* gene (EMBL accession no. Z69241) were analyzed. Both SNPs are transitions – G1044A and G1902A. Amplifications of each of the tested fragments of the promoter region containing particular SNPs were carried out using primers following the work of Vanselow et al. [1999]. Amplification reactions were conducted in a final total volume of 20 µl, 0.2 mM of each dNTP, 10 pmol of each primer (forward and reverse), 50–100 ng of bovine genomic DNA, containing 1 unit of Taq DNA polymerase in a standard PCR buffer and sterile water.

The DNA amplification was performed using an initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 15 s, anneling at 55°C for 30 s and extension at 70°C for 2 min, ending with a final extension for 5 min at 72°C.

The resulting amplification products of both promoter region fragments of the *CYP19* – G1044A (288 bp) and G1902A (283 bp) were digested using *Pvu*II and *Cfr*13I, restriction enzymes respectively. The digesting was performed for at least 3 hours at 37°C. The resulting restriction fragments were separated on 2.5% agarose gels with ethidium bromide in the presence of pUC19/MspI standard.

The results were statistically analyzed. The relationship between different genotypes, different combinational genotypes and selected milk traits, which include daily milk yield (kg), the protein and fat content (%) in milk and the number of somatic cells in milk were analyzed. Statistical analysis of the relationships between the *CYP19* genetic variants and milk traits was conducted using STATISTICA® 8.0. A multifactorial, mixed, nested model from GLM (General Linear Model) package was used. The following formula was applied:

$$Y_{ijklm} = \mu + a_i + b_j + c_k + d_l + f_m(a_i) + e_{ijklm}$$

where:
Y_{ijklm} – the value of the observed trait of an individual,
μ – the average value of traits for the herd tested
a_i – the fixed effect of the corresponding genotype (i = 1, 2),
b_j – the effect of next lactation (j = 1, 2, 3, 4, 5)
c_k – the effect of season of lactation (k = 1, 2, 3, 4),
d_l – the effect of the next month lactation period (l = 1, 2, 3, ..., 14),
$f_m(a_i)$ – effect of cow, a factor nested within genotype/combination genotype *CYP19* (m = 181)
e_{ijklm} – random error.

RESULTS

The analysis of the fragments of PCR restriction products 288 base pairs long (digested with endonuclease *Pvu*II) showed the presence of two of the three possible genotypes: *AA* (288 bp) and *AB* (288 bp, 197 bp, 91 bp). While the restriction analysis of the fragment 283 base pairs long, using the *Cfr*13I restriction enzyme made it possible to identify two of the three possible genotypes: *AA* (fragments 235 bp and 48 bp long) and *AB* (283 bp, 235 bp and 48 bp).

Considering the occurrence of alleles and genotypes for the *CYP19/Pvu*II in the tested herd of Jersey cows revealed that homozygous genotype *AA* appeared with the highest frequency (0.98), heterozygous genotype *AB* was low (0.02), while the presence of genotypes BB was not demonstrated. The allele A occurred with a very high frequency (0.99), while the allele B occurred very rarely (0.01).

In the case of the *CYP19/Cfr*13I polymorphism, however, genotype *AA* occurred with the highest frequency (0.94), the genotype *AB* had much lower frequency (0.06), and the presence of BB genotype was not found. The allele A occurred at a frequency of 0.97, whereas that of allele B was 0.03.

The effect of polymorphisms in the promoter region of the P1.1 gene encoding cytochrome P-450 aromatase on traits such as daily milk yield, fat and protein content in milk, and the number of somatic cells in milk was studied. Table 1 shows the mean values and standard deviation for the traits investigated in the herd of dairy cattle observed at test milkings using the A4 method. The analysis involved two out of three possible genotypes for each polymorphism, since no animals with the homozygous genotype BB were identified.

Table 1. The mean value and standard deviation (SD) for milk performance traits in relation to genotype *CYP19*

Tabela 1.Wartości średnie i odchylenie standardowe (SD) dla cech użytkowości mlecznej w odniesieniu do genotypów *CYP19*

Genotype Genotyp	N/n	Milk, kg ± SD Mleko, kg ± SD	Fat, % ± SD Tłuszcz, % ± SD	Protein, % ± SD Białko, % ± SD	lnSCC ± SD
			CYP19/*Cfr*13I		
AA	170/2389	15.18 ± 4.72	5.82 ± 1.03	4.10 ± 0.56	5.182 ± 1.161
AB	11/173	15.01 ± 4.44	5.81 ± 1.11	4.22 ± 0.54	5.522 ± 1.161
Total Ogółem	181/3176	15.17 ± 4.70	5.82 ± 1.04	4.11 ± 0.55	5.205 ± 1.164
			CYP19/*Pvu*II		
AA	178/2520	15.18 ± 4.69	5.82 ± 1.03	4.10 ± 0.55	5.208 ± 1.164
AB	3/42	14.95 ± 5.43	6.21 ± 1.27	4.28 ± 0.56	5.021 ± 1.148
Total Ogółem	181/3176	15.17 ± 4.70	5.82 ± 1.04	4.11 ± 0.55	5.205 ± 1.164

N – the number of tested animals, n – number of observations.
N – liczba badanych osobników, n – liczba obserwacji.

Analyzing the results presented here, it was found that the animals with the heterozygous genotype were characterized by the lowest daily milk yield for both polymorphisms, whereas those with the homozygous genotype *AA* gave daily milk yield approximate to the average daily yield recorded for the herd.

The analysis the fat content in milk showed that the cows with the heterozygous *CYP19/Pvu*II genotype achieved the highest mean value of this trait, whereas the milk of the animals with the homozygous genotype *AA* was characterized by a content of fat at the level of the average value in the studied herd. In case *CYP19/Cfr*13I polymorphism has been shown that both *AA* homozygous and heterozygous animals achieved similar results, which were similar to the mean value of the tested milk traits. The protein content in milk was found to be the highest in the cows with the heterozygous genotype. The animals with the homozygous genotype *AA* revealed a lower value of this trait, and it was similar to the average protein content in milk of the Jersey cows in the herd studied.

Another analyzed trait was the level of somatic cells in milk. It was observed that the cows with the heterozygous genotype in the case of *CYP19/Pvu*II were characterized by the lowest number of somatic cells, whereas cows with the homozygous genotype *AA* were characterized by a smaller number of somatic cells, but similar to the average value in the herd studied. In the case of the *CYP19/Cfr*13I, it was shown that the animals with the heterozygous genotype were characterized by a greater number of somatic cells than those with the homozygous genotype *AA*, whose number of somatic cells was only slightly lower than the average value in the herd.

DISCUSSION

Milk production is strongly related to the secretion of estrogen. Estrogen produced by the ovaries affects the maturation of reproductive cells, is responsible for the level of hormones necessary for ovulation, and is essential for normal pregnancy and lactation. It is produced in the process of aromatization of androgens, whereby cytochrome P450 aromatase, encoded by the *CYP19* gene plays the key role [Amarenh and Simpson 1996, Lewis and Lee-Robichaud 1998]. The cascade of various processes during late pregnancy and hormonal control of the birth process contribute to the initiation of lactation. The hormonal regulation of lactogenesis involves progesterone, estrogen, prolactin, glucocorticoids and the growth hormone. The role of estrogen in lactogenesis is indirect – estrogen stimulates the secretion of prolactin and possibly other pituitary hormones [Akers 2000]. That is why, aromatase, an enzyme responsible for the conversion of androgen steroid precursors into estrogen may indirectly affect the development of the mammary gland, and milk production.

In this study, the occurrence of the *CYP19/Pvu*II and *CYP19/Cfr*13I genotype polymorphisms was slightly higher than those presented by other authors. The studies conducted on Jersey cattle and related to the *CYP19/Pvu*II showed

the presence of only one genotype – the *AA*, hence the frequency of this genotype equaled 1.0 and the frequency of A allele also equaled to 1.0 [Jędrzejczak et al. 2006]. In other studies the frequency of the A allele was lower than that obtained in this study. It was 0.88 for German Holstein cattle [Vanselow et al. 1999], 0.92 and 0.91 for Polish Holstein-Friesian strain Black and White respectively [Kowalewska-Łuczak 2009, Jędrzejczak et al. 2011]. In the case of *CYP19/Cfr*13I polymorphism for German Holstein cattle bred in [Vanselow et al. 1999], slightly lower frequency of the allele A (0.87) was determined, and for Polish Holstein-Friesian strain Black and White cattle [Kowalewska-Łuczak 2009] its frequency was also lower (0.86) than that in the Jersey cows in the herd studied.

A similar relationship between milk yield and the *CYP19/Pvu*II polymorphism was observed by Jędrzejczak et al. [2011] and Kowalewska-Łuczak [2010] – the cows with genotype *AA* were characterized by the highest yield of milk, but the data was obtained from the full lactations. However, other milk traits (the fat and protein yield and fat and protein content in milk) presented by these authors, indicate that cows with the homozygous genotype *AA* achieved the highest mean values of the traits analyzed. On the other hand, the animals with homozygous BB genotype were characterized by the lowest mean values of the milk traits studied.

The SNP *CYP19/Cfr*13I studies of Polish Holstein-Friesian strain Black and White cattle, based on complete lactation [Kowalewska-Łuczak 2010], showed that the highest mean values of most of the analyzed milk traits (milk, the protein and fat yield, and protein content) were achieved by cows with homozygous genotype BB. While the animals with heterozygous genotype achieved the lowest value of the trait studied for protein content of the milk and fat yield, and animals with homozygous *AA* genotype were characterized by the lowest yield of milk and protein and high fat content in the milk.

There has been some continuing trends in both of the two polymorphisms as far as milk traits in the Jersey cattle herd studied. In both of the examined polymorphisms – the *CYP19/Cfr*13I and *CYP19/Pvu*II – the animals with heterozygous genotypes achieved the lowest milk yield and the highest protein and fat content in milk. Due to the *AB*sence of homozygous BB genotype in the herd of studied cows, it was difficult to assess whether it would impact the traits analyzed.

Given the fact that in this study only two of the three possible genotypes for both polymorphisms analyzed were identified, a further analysis of both polymorphic locations in the promoter region of the gene encoding cytochrome P-450 aromatase designed to estimate the relationship between all the possible genotypes and milk production traits seems necessary.

CONCLUSION

In summary, the results of the statistical analysis of the *CYP19/Pvu*II and *CYP19/Cfr*13I genotypes do not indicate whether that the *CYP19* polymorphisms can be used in selection for milk production traits. However, further studies are needed to verify the associations between the *CYP19* genotypes and milk production traits. In addition, the results obtained in our study should be confirmed in larger numbers of cows of different breeds representing all possible genotypes.

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WPŁYW SNP W GENIE *CYP19* NA CECHY UŻYTKOWOŚCI MLECZNEJ KRÓW JERSEY

Streszczenie. Celem pracy było zbadanie zależności między poszczególnymi genotypami dwóch SNPs zlokalizowanych w regionie promotorowym genu *CYP19* a cechami użytkowości mlecznej. Badaniami objęto 181 krów rasy jersey. Genotypy poszczególnych osobników oznaczano za pomocą metody PCR-RFLP. Frekwencje alleli występujących z najwyższą częstością były następujące: 0,98 (*CYP19/Pvu*II) i 0,94 (*CYP19/Cfr*13I). Statystyczna analiza wyników badań nie wykazała istotnych zależności między poszczególnymi genotypami w odniesieniu do obydwu analizowanych SNP i cech użytkowości mlecznej. Jednak w przypadku zwierząt o heterozygotycznych genotypach wykazano, że osiągały niższą wydajność mleka oraz cechowały się wyższą zawartością białka i tłuszczu w mleku.

Słowa kluczowe: aromataza, bydło mleczne, cechy użytkowości mlecznej, dzienny udój mleka, gen *CYP19*

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