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Polymorphism of Cytochrome P450 Aromatase Gene and its Association with Selected Reproductive Traits in Polish Holstein-Friesian Cattle

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Abstract: The aim of the study was to analyze the polymorphism of cytochrome P450 aromatase gene (*CYP19*) and the search for the association between the genotypes of two polymorphic sites (CYP19/Cyfr13I and CYP19/PvuII) in the P1.1 promoter region of the *CYP19* gene and the age at first calving and calving interval length in Polish Holstein-Friesian cattle. The study used a data set of 909 Polish Holstein-Friesian cows born in the years 1991-1998 and kept in five farms. The analysis of the *CYP19* gene polymorphism showed that homozygous AA genotype was most common in both CYP19/Cyfr13I and CYP19/PvuII loci while the least common, also in both loci was the BB genotype. The frequency of the A allele at both loci significantly exceeded the frequency of the B allele. The earliest age at first calving for both CYP19/Cyfr13I and CYP19/PvuII loci demonstrated BB homozygous cows and the latest was found for AA homozygotes. The longest first calving interval was characteristic of cows that were homozygous for the A allele at the CYP19/Cyfr13I locus whereas with respect to the CYP19/PvuII locus, heterozygotes had the longest first calving interval. The shortest first calving interval showed BB homozygotes at both investigated loci. Heterozygotes at the CYP19/Cyfr13I locus had the shortest second calving interval whereas regarding the CYP19/PvuII locus, the shortest second calving interval was found for AA homozygotes.

Key words: *CYP19* gene, cytochrome P450 aromatase, dairy cattle, reproductive traits, cows

INTRODUCTION

The key enzyme responsible for the biosynthesis of estrogens is cytochrome P450 aromatase, also known as estrogen synthetase. It is the enzyme complex consisting of two proteins: specific hemoglycoprotein, cytochrome P450 aromatase and a non-specific microsomal flavoprotein reductase (NADPH cytochrome P450 reductase) (Thompson and Siiteri, 1974). Aromatase is encoded by the *CYP19* gene, belonging to the CYP family that in cattle has been mapped to chromosome 10 and spans 125 kb of genomic sequence (Vanselow *et al*., 2000). Non-transcribed regulatory sequences (1.1, 1.2a, 1.2b, 1.3, 1.4 and 1.5) and the corresponding promoters span 89 kb while the 2-10 coding sequences comprise 36 kb. CYP19 is characterized by the presence of six tissue-specific promoter regions: P1.1, P1.2, P1.3, P1.4, P1.5 and P2 (Vanselow *et al*., 2004). The most characteristic sites of expression of cytochrome P450 aromatase gene are the gonads, placenta and brain. Aromatase proteins are identical, regardless of the type of tissue they are expressed in, even though the transcription products possess different 5'UTR regions, which are not translated (Vanselow *et al*., 1999b). Three promoters are involved in the expression of aromatase cytochrome P450 in the placenta: P1.1 which is the dominant placenta promoter (Kalbe *et al*., 2000), P1.2, which activity was also found in the ovaries and brain (Vanselow *et al*., 2000) and P1.3 (Furbass *et al*., 1997). The P1.4 promoter is responsible for the *CYP19* gene expression and biosynthesis of estrogens in the brain of cattle. This forms the basis for proper sexual behavior. Examining aromatase activity in certain areas of the rat hypothalamus, it was found that it plays an important role in the neurohormonal regulation of reproduction and dimorphism, including sexual behavior (Golovine *et al*., 2003). P2 promoter is the primary promoter active in bovine ovaries in follicular granulosa cells but lower activity of P1.1 and P1.5 promoters was also found. Furthermore, the P1.1 promoter is entirely responsible for the activity in the corpus luteum (Hamel *et al*., 2005; Lenz *et al*., 2004). The presence of UTR 1.1 transcripts

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was also detected in the ovarian tissues and the brain (Vanselow *et al*., 2001). The *CYP19* gene plays an important role in the mechanisms of reproduction. For this reason, the potential impact of the polymorphisms in this gene on traits related to reproduction and lactation of livestock is considered.

The aim of the study was to analyze the polymorphism of cytochrome P450 aromatase gene (*CYP19*) and the search for the association between the genotypes of two polymorphic sites in the P1.1 promoter region of the *CYP19* gene and the age at first calving and calving interval length in Polish Holstein-Friesian cattle.

MATERIALS AND METHODS

Data: The study used a data set of 909 Polish Holstein-Friesian cows, kept in five farms. Cows were born in the years 1991-1998. Animals were assigned to one of the four calving seasons, according to the month of calving:

- Spring season (S) included the months of 3-5
- Summer season (Su): 7-8
- Autumn season (A): 9-11
- Winter season (W): 12-2

Four genetic groups were distinguished based on the different share of Holstein-Friesian (HF) blood:

- Group 1: cows from 0-25% of HF blood
- Group 2: cows from 26-50% of HF blood
- Group 3: cows from 51-75% of HF blood
- Group 4: cows with >75% of HF blood

The data set also contained information on three genotypes (AA, AB, BB) within the two polymorphic sites in the P1.1 promoter region of the cytochrome P450 aromatase gene. Two polymorphic loci in the *CYP19* gene are referred to as CYP19/Cfr13I and CYP19/PvuII.

In addition, the data included information on the age at first calving of all cows and the length of two consecutive calving intervals for n_1 = 761 cows (1st calving interval) and n_2 = 470 cows (2nd calving interval), respectively.

Statistics: Statistical analysis of data included only those factors (year, calving season, the HF group, herd, CYP19/Cfr13I+CYP19/PvuII) within which the number of observations was >5. In addition, the analysis excluded records with missing data.

In order to investigate the significance of fixed effects on the reproduction traits analyzed, the General Linear Models procedure (GLM) has been applied and the following linear model was fitted to all reproduction traits examined:

$$Y_{ijklmno} = \mu + year_i + season_j + groupHF_k + herd_l + CYP19/Cfr13I_m + CYP19/PvuII_n + e_{ijklmno}$$

Where:

$Y_{ijklmno}$ = oth value of the trait
μ = Population mean
$year_i$ = ith effect of the year (i = 1991-1998)
$season_j$ = jth effect of the season (j = S, Su, A, W)
$groupHF_k$ = kth effect of the HF group (k = 1, 2, 3, 4)
$herd_l$ = lth effect of the herd (l = 1, 2, 3, 4, 5)
$CYP19/Cfr13I_m$ = mth effect of the CYP19/Cfr13I genotype (m = AA, AB, BB)
$CYP19/PvuII_n$ = nth effect of the CYP19/PvuII$_n$ genotype (n = AA, AB, BB)
e_{ijklmn} = Random error

Reproductive traits analyzed were statistically described using arithmetic means ($\bar{x}$) Standard Deviations (SD), Coefficient of Variations (CV) and the range of values (min-max). The significance of differences between the mean values calculated for the age at first calving and the length of the first and second calving interval was tested using the Duncan's test. SAS (2000) Statistical Software Package was used to perform all statistical analyzes.

RESULTS AND DISCUSSION

Table 1 presents the statistical characteristics of the studied reproductive traits. The range of data for all traits analyzed was very wide. Cows calved for the first time in August at the age of 23-44 months of age (722-1358 days), at the average age of nearly 30 months (905.1 days). The most recommended optimal age of first calving is 26 months due to the subsequent effectiveness of the use of cows. The length of calving interval for the studied cattle population ranged from 10 (315 days) to 29 months (883 days) for first calving interval and from 10-21 months (652 days) for second calving interval. The average length of calving interval was similar and amounted to almost 13 months which is a very good result. The lowest variation of the trait was found for the age at first calving where the Coefficient of Variation (CV) was 12.1% while the greatest variation was measured in the length of first calving interval (17.0%).

Table 2 presents the frequencies of genotypes and alleles in the cows tested. The AA genotype had the

Table 1: Statistical description of the studied traits

Traits	Range	Arithmetic mean	Standard deviation	Coefficient of variation (%)
Age at first calving (days)	722-1358	905.1	109.1	12.1
Length of first calving interval (days)	315-883	391.2	66.6	17.0
Length of second calving interval (days)	315-652	11383.9	58.9	15.3

Table 2: Allele and genotype frequency in the studied loci

		Genotype frequency			Allele frequency	
Traits	Polymorphism	AA	AB	BB	A	B
Age at first calving	**CYP19/Cfr13I**					
	Number of genotypes	664.000	220.000	25.000	0.851	0.149
	Genotype frequency	0.730	0.242	0.028		
	CYP19/PvuII					
	Number of genotypes	796.000	101.000	12.000	0.931	0.069
	Genotype frequency	0.876	0.111	0.013		
Length of 1st calving interval	**CYP19/Cfr13I**					
	Number of genotypes	553.000	189.000	19.000	0.851	0.149
	Genotype frequency	0.727	0.248	0.025		
	CYP19/PvuII					
	Number of genotypes	668.000	82.000	11.000	0.932	0.068
	Genotype frequency	0.878	0.108	0.014		
Length of 2nd calving interval	**CYP19/Cfr13I**					
	Number of genotypes	331.000	128.000	11.000	0.840	0.160
	Genotype frequency	0.704	0.272	0.023		
	CYP19/PvuII					
	Number of genotypes	410.000	54.000	6.000	0.930	0.070
	Genotype frequency	0.872	0.115	0.013		

highest frequency (73%) in the CYP19/Cfr13I locus and it slightly decreased in subsequent reproductive cycles (to 70.4%) in favor of the AB heterozygotes frequency. The least frequent was the BB genotype which frequency was 2.8% in the first production cycle and remained at similar levels in the consecutive production cycles. The frequency of alleles through all production cycles remained at a similar level, 85.1-84% for the A allele and 14.9-16% for the B allele.

Similar results were obtained by Kowalewska-Luczak (2009) who examined a population of 1083 cows of Polish Holstein-Friesian breed and found that the frequency of A and B alleles at CYP19/Cfr13I locus was 86 and 14%, respectively while the frequency of the most frequent AA genotype was equal to 74 and rarest BB to 3%. The frequency of alleles at the CYP19/Cfr13I locus was also similar in the case of 44 individuals of the German Holstein cattle to the frequency obtained in the current study and amounted to 87% for the A allele and 13% for the B allele (Vanselow *et al*., 1999a).

The most common genotype at the CYP19/PvuII locus was an AA homozygote which frequency in all production cycles ranged from 87.2-87.8%. The BB homozygote was the least frequently occurring CYP19/PvuII genotype in all the production cycles and its frequency was in the range from 1.3-1.4%. Allele frequency in all production cycles remained at the same level. The frequency of the A allele was 93-93.2% and the B allele 6.8-7%.

Other studies have reported similar frequencies of genotypes and alleles. The study by Jedrzejczak *et al.* (2006) carried out on 266 Black and White cows, found frequencies of AA homozygotes at 89.8% while the BB homozygotes at 0.4%. The incidence of the A allele in this population was 0.947 and the B allele-0.053. Another study of the same group on 171 Jersey cows, found that the frequency of the A allele was 100%. The frequency of the AA genotype in a population of 472 Black and White cows was 85% while the rarest BB genotype was 0.4%. The frequency of A and B alleles amounted to 92.3% and 7.7%, respectively (Jedrzejczak *et al*., 2011; Szatkowska *et al*., 2011). The study of Komisarek and Dorynek (2002) analyzed the polymorphism in the CYP19/PvuII locus in 39 Holstein-Friesian bulls and did not detect the presence of BB genotypes while the frequency of AA homozygotes was 85%. In turn, the frequency of A and B alleles of the bulls was 92 and 8%, respectively. Kowalewska-Luczak (2009) found similar frequencies of alleles in the CYP19/PvuII locus that amounted to 91% for the A allele and 9% for the B allele. Analysis of the gene frequency in the German Holstein cattle (Vanselow *et al*., 1999a) showed a slightly lower frequency of the A allele (88%) and higher of the B allele (12%) when compared to the results obtained in the present study.

Figure 1 shows the average age at first calving in relation to the CYP19/Cfr13I and CYP19/PvuII genotypes.

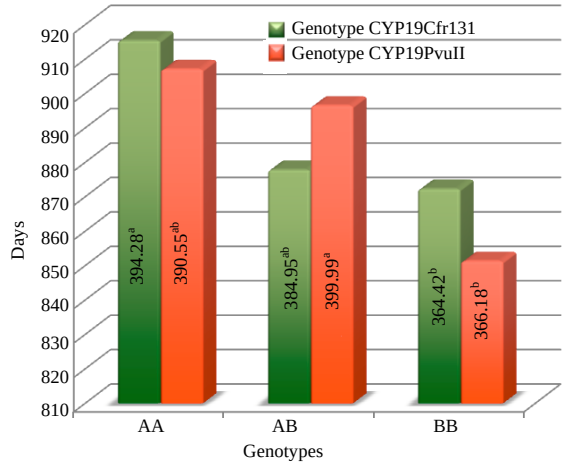

Fig. 1: Average age at first calving in relation to the CYP19/Cfr13I and CYP19/PvuII genotypes. Means marked with the same capital letter differ significantly at $p \leq 0.01$ and means marked with the same small letter differ significantly at $p \leq 0.05$

The lowest age at first calving, 28 months (872.32 days) had cows with the BB genotype at the CYP19/Cfr13I locus while the highest amounting to 30 months (915.43 days) had cows with the AA genotype. These cows calved for the first time at the significantly later age ($p \leq 0.01$) compared to the cows with homozygous BB and heterozygous AB genotypes.

In the case of CYP19/PvuII locus, cows that were AA homozygotes were characterized by the highest age at first calving (29 months to 907.07 days) which was significantly higher ($p \leq 0.01$) than the age of cows with the BB genotype (27 months to 851.33 days). Age at first calving of BB homozygotes was significantly lower ($p \leq 0.05$) than the age of heterozygous AB cows.

Average length of first calving interval in relation to the genotypes at CYP19/Cfr13I and CYP19/PvuII loci is shown in Fig. 2.

The longest first calving interval in the case of CYP19/Cfr13I locus exhibited AA homozygotes in which this period lasted almost 13 months (394.28 days) and was significantly longer ($p \leq 0.05$) than the shortest first calving interval of almost 12 months (364.42 days) occurring in homozygous BB cows.

With respect to the CYP19/PvuII locus, the longest first calving interval lasting 13 months (399.99 days) was characteristic of the heterozygous AB cows and was significantly longer ($p \leq 0.05$) than the shortest 12 months first calving interval (366.18 days) found in BB homozygotes.

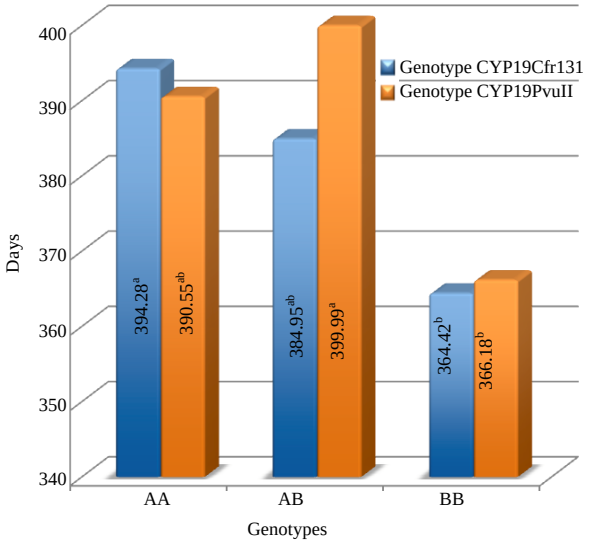

Fig. 2: Average length of first calving interval in relation to the genotypes at CYP19/Cfr13I and CYP19/PvuII. Means marked with the same small letter differ significantly at $p \leq 0.05$

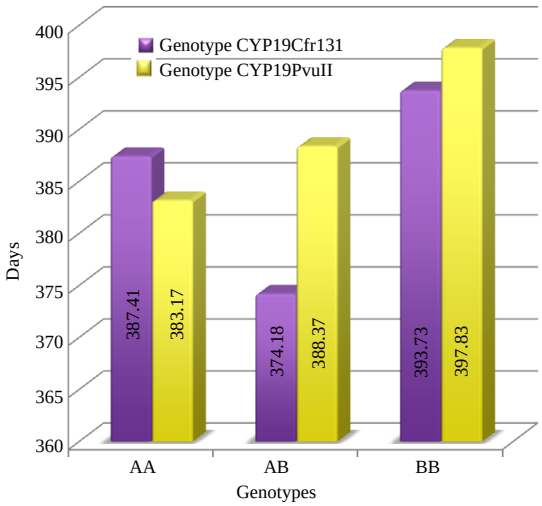

Fig. 3: Average length of second calving interval in relation to the genotypes at CYP19/Cfr13I and CYP19/PvuII

Figure 3 shows the average second calving interval, in relation to the CYP19/Cfr13I and CYP19/PvuII genotypes. There was no significant difference between the average lengths of second calving interval for both genotypes.

AB heterozygotes at CYP19/Cfr13I locus demonstrated the shortest second calving interval amounting to 12 months (374,18 days). The shortest second calving interval in the case of CYP19/PvuII locus,

also amounting to 12 months (383.17 days) had cows with the AA genotype. BB homozygotes at both CYP19/Cfr13I and CYP19/PvuII loci were characterized by the longest second calving interval of about 13 months (393.73 and 397.83 days, respectively).

Szatkowska *et al.* (2011) investigated the association of the polymorphism at CYP19/PvuII locus with length of the calving interval of 472 Polish Holstein-Fresian cows and found that the longest first and second calving interval exhibited AA homozygous cows while the shortest BB homozygotes. However, in the case of the third calving interval, cows that had homozygous BB genotype had the longest and AB heterozygotes the shortest calving interval.

CONCLUSION

Based on the experiments conducted we can draw the following conclusions:

- Homozygous AA genotype was most common in both CYP19/Cyfr13I and CYP19/PvuII loci while the least common also in both loci was the BB genotype. The frequency of the A allele at both loci significantly exceeded the frequency of the B allele
- The earliest age at first calving for both CYP19/Cyfr13I and CYP19/PvuII loci demonstrated BB homozygous cows and the latest was found for AA homozygotes
- The longest first calving interval was characteristic of cows that were homozygous for the A allele at the CYP19/Cyfr13I locus whereas with respect to the CYP19/PvuII locus, heterozygotes had the longest first calving interval. The shortest first calving interval showed BB homozygotes at both investigated loci
- The longest second calving interval (for both loci) was found for BB homozygous cows. Heterozygotes at the CYP19/Cyfr13I locus had the shortest second calving interval whereas regarding the CYP19/PvuII locus, the shortest second calving interval was found for AA homozygotes

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