

ANALYSIS OF CYP19 GENE POLYMORPHISM AS FACTOR AFFECTING MILK PRODUCTION OF CATTLE

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ABSTRACT

The aim of this study was to determine the animal *CYP19/PvuII* genotypes, evaluate the genetic structure of population and estimate the effect of genotypes on long-life milk production traits in Slovak Simmental cattle. Blood samples were obtained from in total 150 Slovak Simmental cows originating from four different farms localized on eastern Slovakia. The polymorphism in *CYP19* gene was detected using PCR-RFLP method and *PvuII* restriction enzyme. In cows population was identified the presence of all three genotypes: AA (74.7%), AB (21.3%), and BB (4%). The A allele was more frequent (0.85 ± 0.02) than B (0.15 ± 0.02). In population was found deviations from HWE. The level of locus polymorphism in the analysed population was only low (PIC=0.212). The activity of *CYP19/PvuII* alleles were unbalanced ($N_e=1.33$) and the higher proportion of homozygous animals caused the decrease of observed heterozygosity ($H_e=0.21$). Based on F – statistic results the most of the genetic variation was distributed within all analysed individuals (F_{IT} 0.155). Evaluation of *CYP19/PvuII* genotype effect on milk production traits was carried out with linear models (GLM procedure). Based on the selected effect we were able to estimate the variability of analysed traits on in average 85%. The *CYP19/PvuII* genotype affected significantly the variability of milk and protein yield ($P<0.05$). The higher production of both traits was found in individuals with BB genotype. Statistically significant influence was detected also for sire ($P<0.05$), breeder ($P<0.0001$) and total number of completed lactations ($P<0.0001$).

Keywords:

INTRODUCTION

The reproduction and production traits are closely connected in cattle and therefore the genetic improvement of the reproduction traits is critical for the efficient course of lactogenesis and lactopoesis. The same physiological processes affect not only reproduction system but also lactation. Generally low numbers of genetic markers significantly influencing the reproduction traits were detected, because most of them are characterized by coefficients with small heritability. One of the promising possibilities is the analysis of different genetic variants of hormones and protein factors mediating their actions that have a key role in reproduction system (Kowalewska-Luczak, 2009; Szatkowska *et al.*, 2011).

In mammalian is reproduction regulated mainly by estrogens synthesized in the ovaries through the androgens aromatization. Except other functions these hormones stimulate lactogenesis in many species with well-developed mammary glands. In lactation the estrogens provide the stimulation of prolactin secretion and possibly other hormones from the pituitary gland (Kowalewska-Luczak, 2010). For the biosynthesis of estrogens from androgen precursors are crucial the enzymatic complex of two proteins, nonspecific microsomal flavoprotein reductase and specific haemoglycoprotein, which is known as cytochrome P450 aromatase. The converting of androgen steroid precursors into estrogens has also indirect effect on mammary gland development and milk production (Simpson *et al.*, 1994; Conley and Hinshelwood, 2001; Kowalewska-Luczak *et al.*, 2013).

The gene encoding cytochrome P450 aromatase (*CYP19*) was mapped on bovine chromosome 10 to band q2.6 (Vanselow *et al.*, 2001). The gene expression is regulated by tissue-specific alternative promoter regions which correspond to the 5'-UTR transcripts, but the coding region is identical for all tissues (Kalbe *et al.*, 2000). Then the increase of number of prolactin and growth hormone receptors influence the mammary cells. The *CYP19* gene is transcribed to six different promoter regions. The genomic sequence of *CYP19* locus (125kbp) includes untranslated regions (exons and promoter) and nine encoding exons that have 89 and 36 kbp, respectively (Vanselow *et al.*, 2008). In promoter regions of *CYP19* gene was found only several single nucleotide polymorphisms (SNPs) (Vanselow *et al.*, 2001) which were associated with milk production traits in cattle (Kowalewska-Luczak, 2010; Jędrzejczak *et al.*, 2011; Kowalewska-Luczak *et al.*, 2013). In this work was analysed the polymorphic site (A/G transition) in the

promotor 1.1 region that was recognized by restriction enzyme *PvuII* (Vanselow *et al.*, 1999).

The aim of this study was to determine the animal genotypes, evaluate the genetic structure of population based on polymorphism in *CYP19* gene and estimate the effect of *CYP19* genotype on long-life milk production traits in Slovak Simmental cattle.

MATERIAL AND METHODS

Biological samples were obtained from in total 150 Slovak Simmental cows originating from four different farms localized on eastern Slovakia. Genomic DNA of each individual was extracted from blood samples according to Miller *et al.* (1988) and analysed for assessment of concentration by spectrophotometer measuring of the optical density at wave of length of 260 nm. Genotyping of animals was carried out by PCR-RFLP method according to Vanselow *et al.* (1999). The *PvuII* enzyme was used for digestion of amplifying PCR products (405bp) and identification of restriction fragments that characterized the present of each allele. Results from PCR reaction and subsequent digestion of PCR products were visualized by use of horizontal electrophoresis and 2% agarose gels in 0.5 x TBE (120 V for 40 min).

Alleles and genotypes frequency of SNP *CYP19/PvuII* was calculated by direct counting and the deviations from Hardy – Weinberg equilibrium (HWE) were tested by Chi-square (χ^2) test. Population genetic parameters, including observed and expected homozygosity (H_o), heterozygosity (H_e) and effective allele numbers (N_e) were determined according to Yeh *et al.* (2000). Polymorphic information content (PIC) was evaluated according to Botstein *et al.* (1980). Moreover the Wrights' fixation indices (F_{IT} , F_{IS} and F_{ST}) were calculated according to Belkhir *et al.* (1996 – 2004).

All statistical analyses for determination of associations between *CYP19* genotypes and long-life milk production traits in Holstein cows were prepared using SAS Enterprise Guide 4.2 software (SAS Institute Inc., 2009). The significance of differences based on *CYP19* genotype effect on analysed traits was evaluated by following linear models:

$$Y_{ijklmno}^{1,2,3} = G_i + BT_j + S_k + B_l + AFC_m + CL_n + II_p + e_{ijklmno}$$

where: $Y_{ijklmno}^{1,2,3}$ – milk, protein and fat yield, G_i – effect of *CYP19/PvuII* genotype, BT_j – effect of breed type, S_k – effect of sire, B_l – effect of breeder, AFC_m – effect of age at first calving, CL_n – effect of total number of completed

lactation, CI_0 – effect of average calving interval, II_p - effect of average insemination interval, $e_{ijklmnop}$ – random error.

RESULTS AND DISCUSSION

All three genotypes of SNP *CYP19/PvuII* were detected in analysed population of Slovak Simmental cows. Table 1 shows observed frequency of alleles and genotypes. The A allele was in population more frequent than B allele. The highest frequency was observed for animals with AA homozygous genotype, and the lowest for heterozygous AB genotype. In the population was found deviation from Hardy-Weinberg equilibrium which was due by the difference between observed and expected genotype frequencies. The higher proportion of homozygous animals (about 74%) was transferred to the low number of observed heterozygosity (Tab. 1). Only low level of polymorphic information content for

analysed loci was found. The effectiveness of loci allele impact has been expressed in population by effective allele number that demonstrated unbalanced activity of alleles (Tab.1). Based on F – statistic results the most of the genetic variation was distributed within individuals in whole population (F_{IT} 0.155) and then among individuals within subpopulations (F_{IS} 0.122). Only very low proportion was explained by differences among the groups (F_{ST} 0.037). Subpopulations were created and evaluated based on animals originating from different farms. The positive value of F_{IS} indicated also the sufficient proportion of heterozygous animals in analysed population. The similar distribution of *CYP19/PvuII* alleles was reported in populations of Polish black and white cows with the different proportion of Holstein breed, Jersey cows (Jędrzejczak et al., 2011) and Holstein cows (Kowalewska-Luczak, 2010; Szatkowska et al., 2011).

Table 1 Distribution of *CYP19/PvuII* alleles and genotypes in analysed population

Genotype frequency			Allele frequency		χ^2 test	H_0	H_e	N_e	PIC
<i>CYP19/PvuII</i> ^{AA}	<i>CYP19/PvuII</i> ^{AB}	<i>CYP19/PvuII</i> ^{BB}	<i>CYP19/PvuII</i> ^A	<i>CYP19/PvuII</i> ^B					
0.747	0.213	0.040	0.853±0.020	0.147±0.020	0.196	0.787	0.213	1.334	0.212

$P < 0.05$

The average values of evaluated long-life milk production traits are described in table 2. The data of milk production traits were obtained in maximum from the six and in the minimum two completed lactations. In the higher proportion of cow was found only 3 completed lactations (45%). Different effects were included in GLM procedure to estimate the relationship between *CYP19/PvuII* marker and analysed traits. Except *CYP19* genotypes, they were selected as fixed effects for linear model the breed type, sire and farmer, age at first calving, numbers of completed lactations, average calving and insemination interval. The average values of analysed traits in relation to the specific *CYP19/PvuII* genotype and significance of selected factors shows table 3. Based on used fixed effect we were able to determine the variations of traits on in average 85%. The *CYP19/PvuII* genotype affected significantly the variability of milk and protein yield ($P < 0.05$). The higher production of both traits was found in individuals with

BB genotype. Statistically significant influence was detected also for sire ($P < 0.05$), breeder ($P < 0.0001$) and total number of completed lactations ($P < 0.0001$). Another factors affected the production on milk, protein and fat only non-significant.

Table 2 Basic statistical measurements of analysed long-life milk production traits in cows

Trait	n	mean	SD	min	max
Milk yield (kg)	150	20786.59	7725.68	6131.00	43683.00
Protein yield (kg)	150	709.647	255.29	224.00	1403.00
Fat yield (kg)	150	840.37	306.68	250.00	1970.00

Table 3 The association between *CYP19/PvuII* genotypes and milk production traits

Trait	<i>CYP19/PvuII</i> ^{AA}			<i>CYP19/PvuII</i> ^{AB}			<i>CYP19/PvuII</i> ^{BB}			Factors
	n	mean	SD	n	mean	SD	n	mean	SD	
Milk yield (kg)		21117.30*	7895.99		19349.10*	7300.75		21690.50*	6678.03	Breed type Sire*
Protein yield (kg)	114	719.13*	261.17	30	663.90*	235.39	6	758.17*	248.46	Breeder*** Age at first calving Number of lactation ***
Fat yield (kg)		850.88	315.09		790.10	371.00		892.17	291.26	Average calving interval Average insem. interval

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$

In previous studies the *PvuII* polymorphism in *CYP19* gene was analysed in associations with milk production (Kowalewska-Luczak, 2009; Kowalewska-Luczak, 2010; Jędrzejczak et al., 2011) and also with reproduction and functional traits (Szatkowska et al., 2011, Othman et al., 2014). However, only several authors reported the statistically significant relationship between *CYP19* gene polymorphisms and milk performance. The associations were found mainly for reproduction traits. In population of Holstein cows was found the positive effect of heterozygous *CYP19/PvuII* genotype on lengths of days open and calving interval. But the variability of reproduction strongly depended on the order of lactation (Szatkowska et al., 2011). In addition Zaborski and Grzesiak (2011) observed by use of the linear models the effect of *CYP19* on calving difficulty. The effect of bovine *CYP19* gene polymorphic variants was also described in activity of ovaries (Othman et al., 2014). All these findings suggest the great potential of this bovine gene for genetic improvement of economically important traits. The animals selection based on the genetic markers (mainly SNPs) in connection with traditional selection can increase the effectiveness of animals breeding. The *CYP19* gene is strictly related to hormonal metabolism and physiology of reproduction and therefore can be selected as genetic marker to prediction of the milk production. But all these assumptions should be evaluated first in populations of breeds with different genetic background which contain higher number of individuals.

CONCLUSION

In present study was found the relationship between polymorphism localized in promotor region of bovine *CYP19* gene and milk performance in Holstein cows. The *CYP19/PvuII* genotype influenced significant the values observed for milk, protein and fat yield in long-life milk production. The study results suggested a potential of *CYP19* gene for improvement of milk production that resulted from its physiology function in organisms. Due to interaction with estrogens the *CYP19* gene affect indirect the mammary gland development and also milk production. The animals selection in dairy cattle assisted with genetic markers

can increase the production traits or optimize reproduction performances that are the most important economical parameters for cattle breeders.

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