



Genetic polymorphism of Pit-1 gene associated with milk production traits in Holstein cattle

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ABSTRACT

The aim of this study was to evaluate the effect of Pit-1 gene polymorphism on long-life milk production traits in the selected population of Holstein cattle. Biological material was obtained from hair roots of 89 Holstein cows originating from the farm with high milk production in Slovak Republic. We use the PCR – RFLP method to detect Pit-1 gene polymorphism (restriction enzyme HinfI). In cows population we identified the presence of all three genotypes: AA genotype (260 bp) of 5 cows, genotype AB (260 bp, 190 bp, 70 bp) of 23 cows and BB genotype (190 bp, 70 bp) of 61 cows. The highest frequency had homozygous BB genotype (69%). The frequency of occurrence of allele B (0.81 ± 0.03) was higher than the frequency of allele A (0.19 ± 0.03). The level of locus polymorphism in the analyzed population was median ($PIC=0.2564$). We observed unbalanced activity of alleles ($N_e=1.43$) and a higher ratio of homozygous individuals, which caused decrease of expected heterozygosity ($H_e=0.31$). Evaluation of Pit-1/HinfI 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 analyzed traits on 79%. The Pit-1/HinfI genotype affected the variability of milk, protein and fat yield in long-life production only non-significant ($P>0.05$).

(Keywords: cattle, milk traits, Pit-1, polymorphism)

INTRODUCTION

Studies conducted in recent past have successfully determined the genotypic profiles at many economically important candidate gene loci like κ -casein, β -lactoglobulin, growth hormone, butyrophyllin, STAT-5a, prolactin, pituitary specific transcription factor etc. in several cattle breeds (Mukesh *et al.*, 2008).

Pituitary transcription factor (Pit-1) has been shown to be a positive regulatory factor of growth hormone (GH), thyrotrophin β -subunit (TSH- β), and prolactin (PRL) in the mammalian pituitary (Doosti *et al.*, 2011). Genetic and biochemical analysis indicated the main role of Pit-1 in the cell stimulation of growth hormone during gene transcription (Aytekin and Boztepe, 2013). The inhibition of Pit-1 synthesis leads to a marked decrease in expression of PRL and GH to a dramatic decrease in proliferation of cell lines producing PRL and GH (Beigi *et al.*, 2010; Heidari *et al.*, 2012; Selvaggi and Dario, 2011). Expression of Pit-1 gene is superior to growth hormone and prolactin genes (somatotrophic and lactotrophic cells) and also to expression of hormone specific activators of these cells (Scully *et al.*, 2000). In mammals, Pit-1 has three different splicing variants, the major type, Pit-1 α , and two other splicing variants, Pit-1 β and Pit-1T (Theil *et al.*, 1989). All of the splicing variants are biologically active. Pit-1 variants

act differentially on the promoters of target genes. Pit-1 α strongly activates Pit-1 α and Pit-1 β promoters (Tanaka *et al.*, 1999). Pit-1 β has a 26-amino acid insert in the transactivation domain because of alternative splicing of the Pit-1 gene transcript at the end of intron one. Consequently it lost the ability to activate the PRL and Pit-1 promoters and preferentially activates the GH gene promoter. Pit-1T contains a 14-amino acid insert in the transactivation domain because of an alternate 3' splice acceptor site and was found to be expressed in thyrotroph-derived cells and stimulates only TSH- β expression (Ferry *et al.*, 2005; Zhao, 2002). Bovine Pit-1 as 129 amino acid protein is member of DNA-binding POU family of homeo-domain transcription factor. This has been sublocalized to the centromeric region of bovine chromosome 1, located midway between TGLA57 and RM95 (Moody *et al.*, 1995).

In Pit-1 gene have been until now indentified various mutations, which caused genetic disorder in regulation of growth hormone and prolactin. Because they are necessary for development of mammary gland and consequently milk production gene encoding Pit-1 have a great potential as genetic marker for evaluation of milk production traits. The aim of this study was to evaluate the effect of Pit-1 gene polymorphism on long-life milk production traits in the selected population of Holstein cows.

MATERIAL AND METHODS

In this study were analyzed a total of 89 biological samples obtained from selected Holstein cows originating from the farm with best milk production in Slovak Republic. Genomic DNA for molecular – genetic analysis was extracted from hair roots according to Gábor (2009) and concentration was estimated by spectrophotometer measuring of the optical density at wave length of 260 nm. Following identification of Pit-1 polymorphism/genotypes was performed by PCR-RFLP method with using primers (FOR: 5' -ACT CGC TAT TAC ACA ATA GGA GAG CCT- 3', REV: 5'-TCC TGC CAA CTC CTC ACC TCC C - 3') according Ozdemir (2012). A genotyping of Pit-1 allelic variants was carried out by digestion of 260 bp PCR products and *HinfI* restriction enzyme. Results from PCR amplifications and digestion of PCR products were analyzed by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (130 V for 40 min) (Broody and Kern, 2004) stained with GelRed (Biotium) prior to visualization under UV light.

Frequency of Pit-1 alleles and genotypes for the entire population were estimated by direct counting and the differences of the observed and expected frequencies of genotypes were tested using Chi-square (χ^2) analysis. Genetic indices of populations, including observed and expected gene heterozygosity (H_e), homozygosity (H_o) and effective allele numbers (N_e) were performed by Popgene32 software version 1.3 (Yeh *et al.*, 2000). Moreover, polymorphism information content (PIC) was calculated according to Botstein *et al.* (1980).

Associations of the Pit-1 genotypes with milk production traits were determined by analyses of quantitative traits. Statistical analysis was performed using SAS Enterprise Guide 4.2 software (SAS Institute Inc., 2009) and significance of differences based on genotypes effect of production and reproduction traits were tested by following general linear models:

$$Y_{ijklmn}^{1,2,3} = G_i + BT_j + S_k + A_l + L_m + DL_n + e_{ijklmn}$$

where: $Y_{ijklmn}^{1,2,3}$ – milk, protein and fat yield, G_i – effect of Pit-1/*HinfI* genotype, BT_j – effect of breed type, S_k – effect of sire, A_l – effect of age at first calving, L_m – effect of number of lactation, DL_n – effect of days of lactation, e_{ijklmn} – random error.

RESULTS AND DISCUSSION

The Pit-1 gene located on bovine chromosome 1 consists of 6 exons. In the 6 exon of bovine Pit-1 gene the restriction fragment length polymorphism using restriction enzyme *HinfI* was detected (Moody et al., 1995). Table 1 summarised alleles and genotypes frequencies of Pit-1 gene polymorphism (called *HinfI*) in the analyzed populations of Holstein cows. The highest frequencies were observed for homozygous BB genotype, and lowest for AA genotype. Analyzed population was in Hardy-Weinberg equilibrium ($P > 0.05$). High frequency of BB genotype was reflected also in observed frequency of B allele, which was predominant. Basic genetic indices of population are presented in Table 1. Number of heterozygous animals (26%) was transferred to the value of expected heterozygosity. Analyzed locus showed based on the expected heterozygosity only median level of polymorphic information content. The effectiveness of loci allele impact in population has been expressed by effective allele numbers. Effective allele number demonstrated unbalanced activity of alleles in a analyzed population of cows.

Table 1

Distribution of SNP Pit-1/*HinfI* alleles and genotypes in Holstein cows

Genotype frequency			Allele frequency		χ^2 test	H _o	H _e	N _e	PIC
Pit-1/ <i>HinfI</i> ^{AA}	Pit-1/ <i>HinfI</i> ^{AB}	Pit-1/ <i>HinfI</i> ^{BB}	Pit-1/ <i>HinfI</i> ^A	Pit-1/ <i>HinfI</i> ^B	1.85	0.69	0.31	1.43	0.26
0.05	0.26	0.69	0.19±0.03	0.81±0.03					

$P < 0.05$

Table 2 shows average values of analyzed long-life milk production traits in whole population. Overall was found in cows population maximum of 5 completed lactations. The largest number of cows had completed only 1. lactation (38.20%). Number of completed lactation, sire, breed type, days of lactation and age at first calving were included as fixed effects in linear model (GLM procedure) for evaluation of Pit-1/*HinfI* genotypes influence on milk production traits. Table 3 shows average values of analyzed traits in relation to specific Pit-1/*HinfI* genotype and significance of selected fixed effect. The Pit-1/*HinfI* genotype affected the variability of analyzed traits only non-significant ($P > 0.05$). Based on the selected effect we were able to estimate the variability of traits on 79%. Statistically significant effect was found only for sire ($P < 0.01$), age at first calving ($P < 0.05$), number of completed lactation ($P < 0.01$) and days of lactation ($P < 0.0001$). In cows population was observed average age at first calving 743.98 ± 91.89 and days of lactation 310.03 ± 113.04 .

Table 2

Basic statistical variation measurements of milk production traits

	n	mean	SD	min	max
Milk yield (kg)	89	9977.54	3885.60	564.00	21584.00
Fat yield (kg)	89	356.67	132.67	27.00	772.00
Protein yield (kg)	89	320.87	125.24	23.00	656.00

Table 3

The association of Pit-1 gene polymorphism with milk production traits

	Pit-1/HinfI ^{AA}			Pit-1/HinfI ^{AB}			Pit-1/HinfI ^{BB}			Factors
	n	mean	SD	n	mean	SD	n	mean	SD	
MY (kg)	5	10324.00	1293.69	23	9457.49	3767.20	61	10132.05	3959.86	Breed type Sire**
FY (kg)	5	409.33	37.11	23	338.37	135.77	61	361.23	132.86	Age at 1. calving*
PY (kg)	5	359.33	37.74	23	303.76	125.35	61	325.36	126.37	Number of lactation** Days of lactation***

MY – Milk yield, FY – Fat yield, PY – Protein yield, *P<0.05, **P<0.01, ***P<0.0001

The development and function of mammary gland is mainly controlled by growth hormone and prolactin, two protein hormones secreted by the anterior pituitary gland. Their synthesis is under regulatory influence of pituitary factor 1 (PIT-1 or POU1F1), a protein factor produced in hypothalamic nuclei (Carsai *et al.*, 2012). In cattle, it was shown that a *HinfI* polymorphism located in exon 6 of PIT1 gene may have significant influence on milk quantity. In particular A allele was associated with a higher milk yield and could be a valuable genetic marker for improving milk quantity in cattle (Edriss *et al.*, 2009; De Mattos *et al.*, 2004; Selvaggi and Dario, 2011). In contrast with our study results was association between Pit-1 gene polymorphism (*HinfI*) and milk production traits found in several studies of Holstein cattle. In study Heidari *et al.* (2012) was similarly for associations relationship evaluation used GLM procedure, while the authors found in Holstein cows population significant (P<0.05) effect of Pit-1 genotype on milk yield. Animals with AB genotype produced more milk than BB genotype. Doosti *et al.* (2011) reported also for Holstein cattle, that the Pit-1/*HinfI* genotype could be use in fertility and create the next generation for increase in milk production and growth of animal. In contrary in study of Brown Swiss population (Ayetkinom and Boztepeom, 2013) was not observed the effect of Pit-1 polymorphism on milk yield and composition (P>0.05).

CONCLUSIONS

Selection for animals with some of the Pit-1/*HinfI* gene genotype could result in advantages for production traits of dairy cattle. The Pit-1 gene as a regulator of growth hormone and prolactin genes expression is necessary for mammary gland development and also for milk production traits. Because the results of our study doesn't suggest a potential significant effect of Pit-1 gene polymorphism on milk production traits, these associations should be validated in cattle population including the larger number of animals or involvement of other dairy cattle breeds. The animals breeding selection assisted with genetic markers can increase the production traits or optimize reproduction performance in dairy cattle.

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