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REGULAR ARTICLE

HINFI POLYMORPHISM OF PIT-1 GENE IN SLOVAK SPOTTED CATTLE

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ABSTRACT

In this study has been Pit-1 gene detected as the pituitary specific transcription factor that regulates the expression of the growth hormone and prolactin genes in the anterior pituitary. A total of 110 Slovak Spotted cows were genotyped for polymorphism of Pit-1/*Hin*fI gene in exon 6 on bovine chromosome 1 by using polymerase chain reaction and restriction fragment length polymorphism methods. Digestion of PCR products with restriction enzyme *Hin*fI revealed two alleles: allele A gave one fragments, 260 bp in length and allele B gave two fragments of 190 and 70 bp. The predominant allele was B with observed frequency 0.7045. In population were detected all three genotypes AA, AB and BB. The most frequent was heterozygous genotype AB with observed frequency 0.417. The population was in Hardy-Weinberg equilibrium.

Keywords: cattle, PCR-RFLP, polymorphism, specific pituitary transcription factor

INTRODUCTION

Both genetic and environmental factors are known to influence production traits in cattle. Selection of animals with higher production or better reproductive performance is of great significance to breeders and consumers. Current technologies enable scientists to improve on the accuracy and efficiency of traditional selection methods by applying genetic

markers trough marker-assisted selection. Therefore, genetic polymorphisms that are significantly associated with certain traits of interest are very useful. Polymorphism detection in genes related to production traits and the identification of the allele which results in a phenotype of interest can allow for marker assisted selection (MAS) **(Zhao *et al.*, 2012; Gutiérrez-Gil *et al.*, 2008)**. Genes affecting polygenic traits characterizing production performance are difficult to identify. However, a number of potential candidate genes have been recognized. They may be selected on the basis of a known relationship between physiological or biochemical processes and production traits, and can be tested as quantitative trait loci (QTLs) or genetic marker **(Oprządek *et al.*, 2003)**.

Bovine Pit-1, a 291 amino acid protein with DNA binding POU domain **(De Mattos *et al.*, 2004)**, is a specific pituitary transcription factor that is responsible for pituitary development and hormone secreting gene expression in mammals **(Cohen *et al.*, 1997)**. Pit-1 is the cellular specific transcription factor for activating expression of growth hormone, prolactin and thyrotropin β-subunit genes in anterior pituitary gland **(Tuggle and Trenkle, 1996)** but also is a regulatory factor in differentiation and proliferation of cells of pituitary gland **(Hoggard *et al.*, 1993)**. An approximately 33 kDa pituitary specific protein contains two domains termed POU-specific and POU-homeo, which are both necessary for high affinity DNA binding to promoters of the growth hormone and prolactin genes **(Rosenfeld, 1991)** The gene encoding Pit-1 was chosen as a candidate gene to investigate its association with lactation performance, growth and carcass traits in several cattle breeds **(Renaville *et al.*, 1997; Woollard *et al.*, 1994; Moody *et al.*, 1995)**. The Pit-1 gene was located in centromeric region of bovine chromosome 1 **(Moody *et al.*, 1995)**. Its sequence is known and available in GenBank database at accession number AF453512 **(Showalter *et al.*, 2002)**. The Pit-1 gene is controlled by several factors that interact with its 5' regulatory the Pit-1 gene itself also occurs as there are two Pit-1 bindings site in the 5' flanking region. Pit-1 is also involved expression of gene coding for thyrotropin releasing hormone (TSH) **(Radovick *et al.*, 1992)**, a key hormone involved in thyroid gland activity. The inhibition of Pit-1 synthesis leads to a marked decrease of growth hormone, prolactin and TSH synthesis **(Beigi Nassiri *et al.*, 2010)** and therefore is considered a highly valuable genetic marker for improving milk production **(Renaville *et al.*, 1997)**. In the bovine Pit-1 gene, the restriction fragment length polymorphism (HinfI restriction enzyme) was detected **(Moody *et al.*, 1995)**. Molecular basis of this polymorphism was the silent mutation (G→A) located within the exon 6 of the Pit-1 gene **(Diekers *et al.*, 1998)**. HinfI polymorphism of Pit-1 gene was associated with growth **(Yang *et al.*, 2010, Carrijo *et al.*, 2008, Zhao *et al.*, 2004),** milk composition and production

(Renaville *et al.*, 1997; Dybus *et al.*, 2003; De Mattos *et al.*, 2004) and reproduction **(Edriss *et al.*, 2009)** traits.

The aim of this study was the detection of Pit-1 gene polymorphism and determination of their allele and genotype frequencies.

MATERIAL AND METHODS

Animals and DNA extraction method

The total numbers of blood samples were taken from 110 Slovak Spotted cows. DNA for genotyping was extracted from blood samples with standard phenol – chloroform extraction method **(Miller *et al.*, 1988)**. Concentrations of DNA were estimated by spectrophotometer measurement by the optical density at wave length of 260 nm.

Analyses of polymorphism

The PCR-RFLP method was used for the analysis of polymorphisms located in Pit-1 gene. A 260 bp fragment of the Pit-1 gene was amplified with using specific forward and reverse primers according to **Ozdemir (2012)**. The PCR reaction was performed in a 25 µl reaction mixtures, containing: 1 x PCR buffer $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 200 µM of dNTPs, 0.8 µM of primers, 1 U *Tag* DNA polymerase and 50 ng genomic DNA template. The following cycles were applied: denaturation at 94°C/5 min, followed by 30 cycles at 94°C/45 sec, primer annealing at 60°C/45 sec, PCR product synthesis at 72°C/45 sec, and final synthesis at 72°C/5 min using C1000^{TM} thermal cycler (Biorad). The PCR products of Pit-1 gene were digested with 1 µl of FastDigest *Hin*fI (G↓ANTC) (Fermentas) restriction enzyme at 37°C in time 5 min. The digestion products were separated by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (150 V for 45 min) stained with GelRed (Biotium) prior to visualization under UV light.

Statistical analysis

The allele and genotype frequencies of the candidate gene Pit-1 were estimated for deviation from Hardy-Weinberg equilibrium using χ^2 test.

Table 1 Primer sequences of Pit-1 *Hin*fI locus

Locus	Primer sequence
Pit-1 *Hin*fI [1]	F 5′ -ACT CGC TAT TAC ACA ATA GGA GAG CCT- 3′
	R 5′-TCC TGC CAA CTC CTC ACC TCC C - 3′

Note: F= Forward, R= Reverse. [1] **Ozdemir (2012)**

RESULTS AND DISCUSSION

In the exon 6 of the bovine Pit-1 gene using digestion of PCR fragment with restriction enzyme *Hin*fI was detected restriction fragment length polymorphism. The digested AA PCR product exhibited one fragment of 260 bp. For the BB genotype exhibited 190 and 70 bp. Figure 1 shows PCR product size and the restriction patterns of the tree genotypes AA, AB and BB and they confirmed G to A mutation.

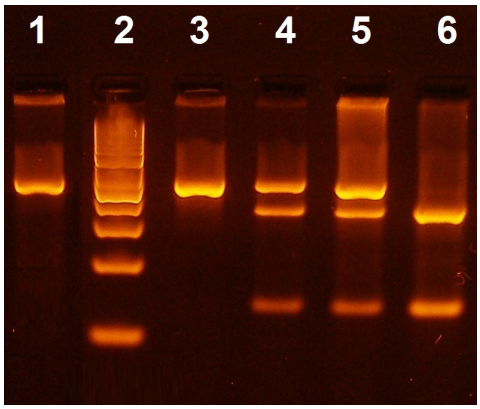

Figure 1 Representative result of PCR-RFLP analysis of Pit-1 *Hin*fI locus on 3% agarose gel Line 1 is PCR product (260 bp), line 2 is a marker of molecular weight (50 bp), line 3 is AA genotype (260 bp), line 4 and 5 are AB genotypes (260, 190 and 70 bp) and line 6 is BB genotype (190 and 70 bp)

Table 2 Frequency of alleles and genotypes of Pit-1 *Hin*fI locus

Frequency	Genotype (n=110)			Allele (n=220)		χ^2
	AA	**AB**	**BB**	A	B	*d.f = 2*
Absolute	*observed* 5	55	50	65	155	5.883-
	expected 8.73	41.64	49.63			
Relative	*observed* 0.0455	0.5	0.4545	0.2955	0.7045	
	expected 0.0873	0.4164	0.4963			

P>0.05

The frequencies for A and B alleles were 0.296 and 0.704, respectively. The most frequent genotype for Pit-1 *Hin*fI locus in observed population was AB. The number of individuals with three genotypes and allele frequencies in leptin gene were observed and frequencies were 0.087 (n=5), 0.417 (n=55) and 0.496 (n=50) for AA, AB and BB, respectively. Based on the observed vs. expected genotype frequencies the whole pool was in Hardy-Weinberg genetic equilibrium. Table 2 shows observed and expected allele and genotype frequency.

The high frequency of predominant B allele was confirmed in other studies of different cattle breed populations. In contrary with our results was in many cattle population analysis the most frequent BB genotype. **Woolard *et al.* (1994)** identified similarly allelic frequency for the allele A 0.10 and B 0.85 in group of 214 Holstein dairy cattle. In population of 130 Limousine calves found **Dybus *et al.* (2003)** high frequency of B allele (0.7269) that the most frequent was BB genotype. **Ozdemir (2012)** reported as a dominant in Anatolian Red cattle AB genotype and in Holstein cattle BB genotype. In the study **Edriss *et al.* (2009)** was associated genotypes of Pit-1/*Hin*fI locus in population of 268 Holstein cows with milk and reproduction performance. The most frequent was the homozygous BB genotype (0.519), which affects significant negatively fat and protein yield and positively birth weight with comparison AA and AB genotype. Also in the study **De Mattos *et al.* (2004)** was heterozygous AB genotype superior for fat milk production in relation to homozygous BB genotype. **Viorica (2006)** reported in Simmental cattle associations between allele A and better milk performance, that genotypes favourable for selections were AA and AB. Similarly reported **Yang *et al.* (2010)** in population associations analyse of different cattle breed with

growth traits as a dominant allele B and genotype BB with trend to higher body weight and body size. The Pit-1 gene was studied as candidate for genetic markers of growth and carcass traits in population of 417 Aberdeen Angus cattle, when the most frequent was a dominant homozygous genotype BB (0.45), but the associations with production traits were no significant **(Zhao *et al.*, 2004)**. **Oprządek *et al.* (2003)** found by evaluation growth, feed conversation and carcass quality significant effect of Pit1/*Hin*f1 gene polymorphism only on carcass dimension, when the most frequent was BB.

CONCLUSION

The goal of our study was the detection of Pit-1/*Hin*fI polymorphism in population of 110 Slovak Spotted cows. We have validated the dominance of B allele. The most frequent was in contrary compared to other studies heterozygous AB genotype. Until now has been confirmed potential effect of polymorphism in Pit-1 gene on cattle production performance. The results from many associations analysis of this effect shows potential positively effect of B allele occurrence on growth and negatively on milk production performance. Preferably average values for milk, growth and reproduction parameters had animals with heterozygous genotype AB and therefore were favourable for selection in breeding dairy or beef cattle programs. In the further would be appropriate for the next assessment to involve a similar association analysis between Pit-1/*Hin*fI polymorphism and production traits.

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