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# IMPACT OF SNPs IN CANDIDATE GENES ON ECONOMICALLY IMPORTANT TRAITS IN PINZGAU CATTLE

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Original scientific paper

## SUMMARY

*The aim of this work was to evaluate the impact of polymorphisms in bovine genes encoding leptin (LEP), leptin receptor (LEPR) and pituitary transcription factor (Pit-1) on economically important traits in Pinzgau cattle. The genomic DNA samples were taken from the total of 85 Pinzgau cows. The detection of LEP/Arg2059Cys, LEPR/Thr945Met, and Pit-1/g.1178A>G single nucleotide polymorphisms were performed in order to evaluate their effect on long-life milk production traits. The genotyping of analysed animals was carried out using PCR-RFLP method. The observed allele frequencies showed that the LEP<sup>A</sup>, LEPR<sup>C</sup> and Pit-1<sup>B</sup> allele were the most distributed in the analysed population. The high proportion of homozygous genotype in case of each locus was transferred in to the relatively lower level of total observed heterozygosity which also confirmed the positive values of  $F_{IS}$  index. The results of statistical analyses indicated the significant effect ( $P < 0.05$ ) of the evaluated polymorphisms in LEP and LEPR genes on milk, protein and fat yield. The LEP<sup>AA</sup> and LEPR<sup>CC</sup> genotypes seem to be a desirable for milk production improvement. For the Pit-1 gene only tendency to increase the milk production for AA homozygous animals was found out. Our study was biased mainly by sample size and therefore the analysis of greater population is recommended.*

**Key-words:** LEP, LEPR, milk production, Pinzgau, Pit-1, SNP genotyping

## INTRODUCTION

The economically important production traits in cattle are influenced by many genes controlling the biological and physiological processes in organisms involved in formation of phenotypic traits. The candidate gene approach is based on a prior knowledge including the physiological mechanisms. Candidate genes are generally the genes with known biological function directly or indirectly regulating the developmental processes of the investigated traits, which could be confirmed by evaluating the effects of the causative gene variants in an association analysis (Zhu and Zhao, 2007). In practice, identification of the trait genes is achieved using a combination of genetic mapping, to localise the QTL region on a chromosome, and candidate gene or positional cloning approaches, to identify the trait gene within QTL region (Williams, 2005).

In dairy cattle many genes were evaluated as candidate for milk performance. In this study single nucleotide polymorphisms (SNPs) of three genes encoding leptin (LEP/Arg2059Cys), leptin receptor (LEPR/

Thr945Met) and pituitary transcription factor (Pit-1/g.1178A>G) were analysed in relation to the variability of milk production traits. The genes have been selected firstly for their biological function and secondly based on previous published association studies that confirmed their impact on milk production in different cattle breeds. Leptin as hormone synthesized and secreted primarily in the adipose cells is involved in the feed intake, energy expenditure, and reproductive performance (Liefers et al., 2005). Leptin is supposed to be a signal to the reproductive system that enough total energy is present to support the added energy demands of a successful conception and pregnancy (Lindersoon et al., 1998). Polymorphisms in the LEP gene in cattle have been associated with somatic cells count (Kulig et al., 2010), milk performance (Anton et al., 2012), reproduction traits (Almeida et al., 2003) and growth traits (Kulig and Kmiec, 2009). Effects of leptin are provided

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through six receptors which are glycoproteins with a single transmembrane - spanning region. The longest and fully active isoform (*LEPR-b*) is expressed mainly in hypothalamus, the centre of the energy homeostasis and the regulation of secretory organs activity (Matteis et al., 2012). The polymorphisms identified in *LEPR* gene were significantly associated with milk performance (Komisarek and Dorynek, 2006), reproduction traits (Clemson et al., 2011) and growth traits (Silva et al., 2012). The pituitary cells development and hormone secreting genes expression in mammals is provided by specific pituitary transcription factor gene (*Pit-1*). The *Pit-1* is responsible for activating growth hormone, prolactin and thyrotropin genes in the anterior pituitary gland (Tuggle and Trenkle, 1996). The effect of *Pit-1* gene polymorphisms was studied in relation to the body growth (Yang et al., 2010), milk composition and production (De Mattos et al., 2004) and reproduction traits (Edriss et al., 2009).

The aim of the present study was to determine *LEP*, *LEPR* and *Pit-1* genes allele and genotypes frequencies, population's genetic indices and evaluate the significance of their impact on long-life milk production traits in Pinzgau cattle that belongs to the animal genetic resources of the Slovak Republic.

## MATERIAL AND METHODS

The genomic DNA for genotyping animals was obtained from total of 85 hair roots samples of Pinzgau cows. The analysed individuals were selected based on stratification method. Genomic DNA from each sample was extracted according to Gábor (2009). After extraction of DNA the concentration was controlled by the spectrophotometry measurement using NanoPhotometer™ (IMPLEN). The genotyping of each individual was carried out using PCR-RFLP methods. A 422 bp fragment of *LEP* gene was obtained by PCR with time and temperature conditions in accordance with Liefers et al. (2002). The amplification of a 197 bp fragment of *LEPR* gene was performed with appropriate reaction condition proposed by Almeida et al. (2008) and a 260 bp fragment of the *Pit-1* gene was amplified according to Ozdemir (2012). Subsequently, the PCR products of *LEP*, *LEPR* and *Pit-1* genes were digested at 37°C for 10 min with 1 µl of FastDigest restriction

enzymes *Sau3AI*, *BseGI* and *Hinfl*, respectively. The PCR products and restriction fragments were separated and visualised by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (130 V for 50 min) stained with dye GelRed.

The determination of population structure in order to estimate the allele and genotype frequencies were done using Popgene32 software (Yeh et al., 2000). The differences between observed and expected genotype frequencies were tested using Chi-square ( $\chi^2$ ) analysis. The population genetic parameters, including observed and expected heterozygosity ( $H_e$ ) and homozygosity ( $H_o$ ), effective allele numbers ( $N_e$ ), polymorphic information content (*PIC*), and Wright's fixation index ( $F_{IS}$ ) were calculated using the PowerMarker software according to Liu and Muse (2005). The impact of analysed single nucleotide polymorphisms in *LEP* (*Arg2059Cys*), *LEPR* (*Thr945Met*) and *Pit-1* (*g.1178A>G*) genes on the long-life milk production traits (milk, protein, and fat yield) in analysed population were determined using SAS software. The normality test showed non-significant results and therefore the significance of genotypes effect on production traits were tested by parametric statistic.

## RESULTS AND DISCUSSION

For the *LEP* gene (SNP *Arg2059Cys*), across analysed population, the prevalence of heterozygous animals was found. The lowest proportion was observed for homozygous BB animals. The high distribution of AA and AB animals in population was reflected in the A allele frequency increase. In the *LEPR* gene (SNP *Thr945Met*), CC genotype gained absolute ascendancy because the observed frequency of CC genotype was high (82.4%). This indicated that the observed frequency of C allele is much higher than of T allele (Table 1). In case of *Pit1/g.1178A>G* locus heterozygous AB genotype was the most frequent. The B allele reached higher frequency in population than A allele. The differences between the observed and expected genotype frequencies were non-significant and in the analysed population the Hardy-Weinberg equilibrium was found for each locus. The heterozygosity, effective allele numbers and polymorphism information content of analysed bovine loci are shown in Table 1.

**Table 1. The allelic and genotypic frequencies of *LEP*, *LEPR* and *Pit-1* genes in the analysed population**

Locus	Genotypes			Alleles		$\chi^2$ test	$H_o$	$H_e$	$N_e$	PIC	$F_{IS}$
	AA	AB	BB	A	B						
<i>LEP/Arg2059Cys</i>	0.447	0.494	0.059	0.694	0.306	2.234	0.57	0.43	1.73	0.33	0.16
<i>LEPR/Thr945Met</i>	0.071	0.565	0.364	0.353	0.647	4.745	0.84	0.16	1.19	0.15	0.09
	CC	CT	TT	C	T						
<i>Pit1/g.1178A&gt;G</i>	0.824	0.176	-----	0.9118	0.0882	2.28	0.54	0.46	1.84	0.35	0.24

$H_o$  – observed homozygosity,  $H_e$  – observed heterozygosity,  $N_e$  – effective allele number, PIC – polymorphic information content,  $F_{IS}$  – fixation index

Observed Wright's fixation indexes showed positive values across all loci indicating slight deficiency of heterozygote animals compared to the Hardy-Weinberg equilibrium expectations. The  $F_{IS}$  reflects the average deviation of the population genotypic proportions from Hardy-Weinberg equilibrium for a locus. The  $F_{IS}$  can be considered also as the inbreeding coefficient of an individual with respect to the local subpopulation. In the analysed population the positive value of  $F_{IS}$  may indicate the increased homozygosity resulting from inbreeding level. According to the classification of *PIC*, the analyses showed low (*LEPR/Thr945Met*) or median (*LEP/Arg2059Cys* and *Pit1/g.1178A>G*) level of polymorphic information content across loci (Table 1). The effectiveness of loci allele impact in populations has been expressed by effective allele numbers. Comparison of loci  $N_e$  showed the highest effective allele numbers in *Pit1/g.1178A>G* locus. The observed  $N_e$  indicates a good level of genetic variability in Pinzgau cows population at the considered loci.

In the analysed population of Pinzgau cows comparable average value of milk production traits to the results of milk recording control in the Slovak Republic for years 2013/2014 was observed. The statistical analysis of *LEP/Arg2059Cys* locus impact showed significant effect on evaluated long-life milk production traits, with A as a desirable allele (Table 2). But the statistically significant differences ( $P < 0.05$ ) were found only in comparison of cows with AA and AB genotypes. Milk, protein and fat yield were significant by higher in homozygous AA cows. Similarly the analysis of *LEP/Arg2059Cys* genotype effect showed its significant influence on the evaluated traits ( $P < 0.05$ ). The CC homozygous cows had significantly more milk, protein and fat yield in long-life production compared to heterozygous individuals. The *Pit1/g.1178A>G* locus affected the average values of milk, protein and fat yield in relation to the each genotype group only non-significant. The cows with AA genotype had only slight tendency to produce more milk in the analysed production season.

**Table 2. Average values of the evaluated production traits in relation to the different *LEP/Arg2059Cys*, *LEPR/Thr945Met* and *Pit1/g.1178A>G* genotypes**

Genotypes	N	Traits (average)						
		Milk yield, kg	P	Protein yield, kg	P	Fat yield, kg	P	n
<i>LEP/Arg2059Cys</i>								
AA	38	20408.0	+	711.2	+	776.0	+	36
AB	42	14919.6	+	520.9	+	565.2	+	42
BB	5	15199.4		552.2		610.2		5
<i>LEPR/Thr945Met</i>								
CC	70	18384.8	+	642.6	+	699.9	+	69
CT	15	12054.1	+	422.0	+	459.4	+	14
<i>Pit1/g.1178A&gt;G</i>								
AA	6	23844.2		789.9		868.1		6
AB	48	17386.8		607.1		658.9		46
BB	31	15950.0		567.0		619.5		31

+ significance of difference at  $P < 0.05$

The impact of selected loci on the economically important traits was evaluated in many recently published studies. On the contrary to our results, Liefers et al. (2002) reported in study of 613 Holstein cattle the association between the leptin genotype and milk production when the AB genotype was associated with higher milk yield. Heravi et al. (2006) also evaluated the association of genetic differences in *LEP* gene, milk production and reproduction traits. A significant association in total of 230 Iranian Holstein cows was detected between the *LEP/Arg2059Cys* polymorphism and 305-d milk yield. Javanmard et al. (2010) found in Iranian cattle significant association between *LEP/Arg2059Cys* polymorphism and milk fat. Significant effect of SNP *Arg2059Cys* on calving interval and weight at first calving was reported by Almeida et al. (2003) in study of 160 beef cows. Significant association between fat and

protein content in milk and *LEPR/Thr945Met* genotype in 219 Jersey cows was confirmed by Komisarek and Dorynek (2006), when animals with TT genotype were characterized by the lowest values of both traits. On the contrary, Giblin et al. (2010) reported no significant effect of the analysed SNP on milk production and reproduction performance in 848 Holstein cattle, but significant associations were found between other polymorphisms in *LEPR* gene and energetically expensive process of lactogenesis, energy storage and fertility performance. Clempson et al. (2011) reported only a weak association of *LEPR/Thr945Met* polymorphism with milk yield and days to first service in 509 Holstein cows. On the contrary to our results the significant impact of *Pit-1* gene polymorphism was confirmed by Edriss et al. (2009) for reproduction performance and by Yang et al. (2010) for body growth. The effect of *Pit1/g.1178A>G*

locus on milk production was found by De Mattos et al. (2004) when the heterozygous AB genotype was superior for fat milk production. Viorica (2007) reported associations between *Pit1/g.1178A>G* allele A and better milk performance in Simmental cattle. Negative influence of BB genotype on fat and protein yield and positive on birth weight was found in the study by Edriss et al. (2009).

## CONCLUSION

The selection of animals based on the *LEP/Arg2059Cys*, *LEPR/Thr945Met* or *Pit1/g.1178A>G* genotypes could result in production traits improvement in dairy cattle. It is generally accepted that the leptin and its receptor has important role during pregnancy, in feed intake, energy expenditure, growth and reproduction and therefore can be considered as a strong candidate gene for economically important production traits. The results of present study confirmed mainly the significant impact of *LEP* and *LEPR* genes on milk production traits. Since our study was biased by small sample size, the analysis of bigger population is recommended. That would increase the significance of the statistical analysis and also the reliability of the obtained results.

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