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ASSOCIATIONS BETWEEN POLYMORPHISMS IN THE LEPTIN GENE AND MILK PRODUCTION TRAITS IN PINZGAU AND SLOVAK SPOTTED CATTLE

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ABSTRACT

The aim of this study was to evaluate the relationship between the polymorphism in the leptin gene and milk production traits in Pinzgau and Slovak Spotted cattle. A total of 85 Pinzgau and 110 Slovak Spotted cows were used to investigate how leptin gene polymorphisms affect milk production traits. The polymorphism of leptin gene in intron 2 on bovine chromosome 4 was genotyped by using the polymerase chain restriction fragment length polymorphism. A strategy employing PCR was used to amplify a 422 bp product from DNA samples. Digestion of PCR products with restriction enzyme *Sau*3AI revealed two alleles: allele A gave two fragments,390 and 32 bp in length and allele B gave three fragments of 303, 88, 32 bp. The predominant allele was A with observed frequency 0.69 (Pinzgau) and 0.83 (Slovak Spotted). In both populations were observed all three genotypes AA, AB and BB. Results from the statistical analysis between *Sau*3AI polymorphism and milk production parameters – milk, protein and fat yield (kg) were not significant. The comparison of animals from both breeds with the same genotype shows significant effect in Pinzgau cows with AA genotype on milk and protein yield ($P \leq 0.05$).

Key words: cattle / molecular genetics / leptin gene / polymorphism / milk production

1 INTRODUCTION

Leptin and its receptor can be used as a genetic marker for enhancing the productivity in livestock and are also potential candidates for marker assisted selection (Agarwal *et al*., 2008). Variation at DNA level contributes to the genetic characterization of livestock populations. Molecular genetics techniques are currently available that allow direct genotyping for candidate genes using PCR. Leptin is a hormone predominantly secreted from white adipose tissue and performs important roles in controlling body weight, milk production, feed intake, immune function and reproduction (Block *et al*., 2001). As the hormone leptin is involved in regulation of nutritional status and reproductive function, this hormone is an interesting protein to investigate during the periparturient period in dairy cattle (Liefers *et al*., 2005). Leptin binds to a receptor mainly localized on Neuropeptid – Y – neurons, which also appear in hypothalamus to play a key role in the integration of feeding behaviour with internal signals of body energy status (Wayne *et al*., 1995). In dairy cattle, the increase in milk yield has been accompanied by more negative energy balance during early lactation and a decrease in fertility. Leptin hormone concentrations were high during late pregnancy and declined to a lowest point at parturition. This indicates that the fall in circulating leptin levels towards and during lactation is due to the energetic costs of milk production (Liefers *et al*., 2005). The leptin gene (LEP) is highly conserved across species, and is located on bovine chromosome 4q32. Its DNA sequence has more than 15,000 base pairs and contains three exons, which are separated by two introns (Stone *et al*., 1996). Leptin is encoded by a single transcript of ~4.5 bp expressed primarily by adipose tissue. In cattle, leptin is expressed in the rumen,

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abomasums and duodenum before weaning but only in the duodenum after weaning. The ruminant mammary epithelial cells also synthesize leptin during pregnancy and during established lactation (Leury *et al.*, 2003). Several polymorphisms in the leptin gene have been associated with milk performance (Liefers *et al.*, 2002; Madeja *et al.*, 2004; Javanmard *et al.*, 2010), increased perinatal mortality in dairy (Brickell *et al.*, 2010), calf birth and weaning weights in beef and dairy (Almeida *et al.*, 2003; Nkrumah *et al.*, 2005) and reproductive performance in dairy cattle (Nkrumah *et al.*, 2005). The *Sau*3AI polymorphism detected inside the intron 2 targeting a cytosine to thymine transition results in amino acid change (arginine to cysteine) at position 2059 of the protein.

The aim of this study was to investigate the effect of polymorphism in the leptin gene on milk production traits in Slovak Spotted and Pinzgau cows.

2 MATERIAL AND METHODS

2.1 ANIMALS AND DNA EXTRACTION METHOD

Samples were collected from 85 of Pinzgau cows and from 110 cows of Slovak Spotted breed. Genomic DNA for genotyping was extracted from hair roots according to Gábor (2009) and from blood samples with isolation kit NucleoSpin Blood (Macherey-Nagel). DNA concentrations were estimated by spectrophotometer measuring the optical density at wave length of 260 nm.

2.2 ANALYSES OF POLYMORPHISMS

A 422 bp fragment of intron 2 in bovine leptin gene was amplified by PCR using forward and reverse primers according to Liefers *et al.* (2002). The polymerase chain reaction was performed in a 25 µl reaction mixtures, containing: 1 × PCR buffer $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 2 mM dNTPs, 8 pM primers (Generi-Biotech), 1 U Taq DNA polymerase (Fermentas), 6 µl BSA (Fermentas), 50 ng genomic DNA. PCR amplification was carried out in C1000™ thermal cycler (Biorad). Thermal cycling conditions included: an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 sec, 55 °C for 20 sec, 72 °C for 30 sec and a final extension at 72 °C for 7 min. Genotype analyses were performed using PCR-RFLP method. The PCR products of LEP gene were digested with 1 µl of FastDigest *Sau*3AI (Fermentas) restriction enzyme at 37 °C for 10 minutes. The digestion products were separated by horizontal electrophoresis in 3% agarose gels in 0.5 × TBE (130 V for 40 min) stained with GelRed (Biotium) prior to visualization under UV light.

Table 1: *Primer sequences of LEP Sau3AI loci*

Locus	Primer sequence
LEP *Sau*3AI[1]	F 5´ -TGG AGT GGC TTG TTA TTT TCT TCT- 3´
	R 5´-GTC CCC GCT TCT GGC TAC CTA ACT- 3´

Note: F = Forward, R = Reverse. 1 Liefers *et al.* (2002)

2.3 STATISTICAL ANALYSIS

The allele and genotype frequencies of *Sau*3AI polymorphism were examined for deviation from Hardy – Weinberg equilibrium using χ^2 test. The effect of LEP *Sau*3AI genotype on long-life milk production traits – milk, protein and fat yield were analysed using SAS 9.1 software.

3 RESULTS AND DISCUSSION

A single nucleotide polymorphism (SNP) inside the intron 2 of the bovine leptin gene based on the use of restriction fragment length polymorphism was detected.. The digested AA PCR product exhibited two fragments of 390 and 32 bp, whereas the BB genotype gave three fragments 303, 88 and 32 bp (32 bp bands not detected on the gel). Figure 1 shows PCR product size and restriction patterns of the three genotypes AA, AB and BB.

On the basis of the Hardy-Weinberg rule, the expected frequencies of A and B alleles were in population of Pinzgau cows 0.69 and 0.31, respectively. The observed frequencies of genotypes were 0.447 (n = 38), 0.494 (n = 42), 0.59 (n = 5) for AA, AB and BB genotype, respectively. The expected frequencies of the three genotypes were 0.482 (AA), 0.425 (AB) and 0.93 (BB). In population of Slovak Spotted cows were frequencies of A allele 0.83 and B allele 0.17. The number of individuals with three genotypes and allele frequencies in Slovak spotted cows were observed with frequencies 0,691 (n = 76), 0.282 (n = 31) and 0.027 (n = 3) for AA, AB and BB, respectively. The most frequent genotype in Pinzgau cattle population was AB and in Slovak Spotted cow's population the genotype AA. Table 2 shows frequencies of the three detected genotypes AA, AB and BB in both breeds. The calculated χ^2 test values indicate Hardy-Weinberg genetic equilibrium in both populations.

Pomp *et al.* (1997) verified that the frequency of a restriction fragment length poly-

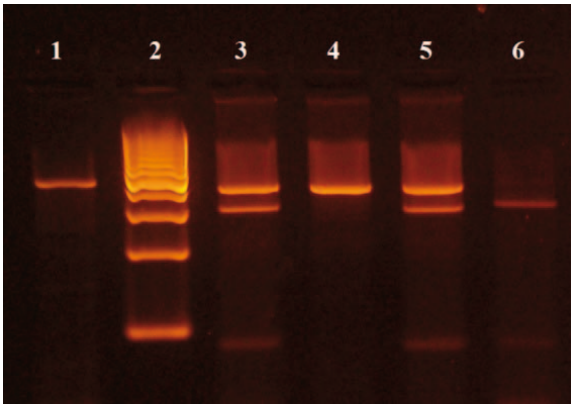

Figure 1: *Representative result of PCR-RFLP analysis LEP Sau3AI loci on 3% agarose gel. Line 1 is PCR product (422 bp), line 2 is a marker of molecular weight (Fermentas, 100 bp), line 3 is AB genotype (390, 303 and 88 bp), line 4 is AA genotype (390 bp), line 5 is AB genotype (390, 303 and 88 bp) and line 5 is BB genotype (303 and 88 bp)*

morphism (*Sau*3AI) in bovine LEP gene was different between *Bos taurus* and *Bos indicus*, being possible that genotype differences in leptin could explain some of the phenotypic variation observed between breeds of cattle. Rasor *et al*. (2002) detected higher frequency of A allele in *Bos indicus* breed group of cattle compared with *Bos taurus*. Our findings were similar to results reported for Holstein-Friesian cows (Liefers *et al*., 2002), Black-and-White cows (Kulig, 2005), Holstein bulls (Javanmard *et al*., 2010) and different meat producing breeds of cattle (Passos *et al*., 2007).

Based on statistical analysis in population of Pinzgau cows were differences between the average values of milk, protein and fat yield not significant. In the comparison was milk yield higher in animals with AA genotype. Also cows with genotype AA showed a higher fat and milk yield.

Even if the results were not significant, allele A may potentially have positive effects on the evaluated parameters of milk production. Significance of observed differences was affected by a number of biological samples and number of data available for particular trait. The results of statistical analysis in population of Slovak Spotted cows show statistically not significant differences between the average values of milk, protein and fat yield in studied population with different LEP *Sau*3AI genotypes. The cows with genotypes AB and BB showed a lower milk yield compared cows with AA genotype. The average yields of protein and fat in milk were comparable in cows with heterozygous and homozygous genotypes. Only the comparison across breeds shows significant effect in Pinzgau cows with AA genotype on milk yield and protein yield ($P \leq 0.05$). Table 3 represents the differences between LEP *Sau*3AI genotypes in some milk production traits.

Liefers *et al*. (2002) reported that heifers with the *Sau*3AI AB genotype produce 1.32 kg/day more milk compared with *Sau*3AI AA genotype. They suggested that B allele could yield a higher milk production without negatively affecting energy balance and fertility. Madeja *et al*. (2004) did not find any effect of *Sau*3AI polymorphism for milk production traits. Heravi *et al*. (2006) evaluated the association of genetic differences in the leptin gene and milk yield, reproduction, body condition score and plasma glucose level in Holstein cows. A significant association was detected between the AB genotype and 305-d milk yield. The results demonstrated that the B allele can yield a higher 305-d milk production with a trend to better reproductive performance. Javanmard *et al*. (2010) found association between LEP Sau*3AI* polymorphism and milk fat. In evaluated population animal with AA had lower milk fat production than AB genotype. No association was detected between bovine leptin gene and milk production. Factors effecting milk production in Slovak spotted cattle were also evaluated by Bujko *et al*. (2011) and Kasarda *et al*. (2007). In previous studies was observed association of LEP gene with higher milk, fat and protein yield. In contrary, observed differences in our studies show no significant associations between milk performance and LEP gene. Significance of observed differences was affected by number of data available for particular trait.

4 CONCLUSIONS

By using PCR-RFLP method have been detected genotypes in the polymorphic sites of the leptin gene

Table 2: *Leptin allelic and genotypic frequency of LEP Sau3AI loci*

		LEP *Sau*3AI genotypes					
		Genotye frequency			Allele frequency		
Breed	n	AA	AB	BB	A	B	χ^2
Pinzgau	85	0.447	0.494	0.059	0.6941	0.3059	4.19^-
Slovak Spotted	110	0.691	0.282	0.027	0.8318	0.1682	1.0065^-

$P > 0.05$

Table 3: *Average values of long-life milk performance trait in cows of different LEP Sau3AI genotypes*

			Traits (mean ± SD)		
Breed	Genotypes	n	Milk yield (kg)	Protein yield (kg)	Fat yield (kg)
Pinzgau	**LEP*Sau*3AI**				
	AA	38	19333.9 ± 12111.9	673.8 ± 406.3	735.1 ± 437.3
	AB	42	14919.6 ± 10510.2	520.9 ± 349.1	565.2 ± 392.4
	BB	5	15199.4 ± 13197.6	552.2 ± 510.5	610.2 ± 597.3
Slovak Spotted	AA	76	14515.3 ± 7599.2	488.1 ± 256.0	582.3 ± 302.6
	AB	31	13308.8 ± 6932.1	455.2 ± 227.9	544.5 ± 259.8
	BB	3	12921.7 ± 6889.7	447.1 ± 226.5	526.2 ± 259.6

(*Sau*3AI). In the studied population of 85 Slovak Pinzgau and 110 Slovak Spotted cows were detected genotypes AA, AB and BB. The most frequent genotype in Pinzgau cows population was AB and in Slovak Spotted cows was AA. Allele A was in both observed population more frequent than the B allele. Based on the statistical analysis SNP *Sau*3AI had no significant effect on evaluated milk production parameters. The mutual comparison of both breeds with same genotype shows significant effect in Pinzgau cows with AA genotype on milk and protein yield ($P \leq 0.05$).

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