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REGULAR ARTICLE

SNP ANALYSES OF THE BOVINE GROWTH HORMONE AND LEPTIN GENES BY PCR – RFLP METHOD

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ABSTRACT

The aim of this study was detection of polymorphism in the bovine growth hormone and leptin genes with using PCR-RFLP method. A polymorphic site of the growth hormone gene (*AluI* loci) that results in amino acid change at position 127 of the protein chain (leucine, L to valine, V) has been linked to differences in circulating metabolites, metabolic hormones and to milk yield. The polymorphism in bovine leptin gene situated in the intron between two exons results in amino acid change at position 2059 of the protein chain (cytosine, C to thymine, T). The polymorphisms were studied in a group of 58 bull's Slovak spotted breed. A strategy employing PCR was used to amplify a 428 bp (GH gene) and 422 bp (LEP gene) products from blood samples. Digestion of PCR products with restriction enzymes *AluI* and *Sau3AI* revealed alleles: L and V; A and B for GH gene and LEP gene, respectively. The growth hormone gene is a candidate gene for body weight gains in cattle since plays a fundamental role in growth regulation. Leptin also plays an important role in the regulation of feed intake, energy metabolism, growth and reproduction of cattle, therefore, animals with higher leptin gene expression will probably have lower daily weight gain than others with similar forage offer and nutritional condition and probably will also have longer calving interval.

Keywords: cattle, growth hormone gene, leptin gene, PCR-RFLP, polymorphism

INTRODUCTION

Bovine growth hormone (GH) is a single chain polypeptide with 190 or 191 amino acids and molecular weight 22 kDa. This hormone is produced in the anterior pituitary gland under the hypothalamic controls of two hormones: growth hormone releasing factor, which increases the secretion of GH, and somatotropin release-inhibiting factor which inhibits its secretion **(Nicoll et al., 1986)**. Growth hormone exerts its effects on growth and metabolism by interacting with specific receptor on the surface of target cells. Changes in the functional regions of the GH receptor can affect its binding capacity and signal pathway, and therefore alter the activity of GH in the target tissue **(Stasio et al., 2005)**. The biological effects of GH involve a variety of tissue and metabolism of nutrient classes: carbohydrates, lipids, proteins and minerals. In ruminants, GH is known to be responsible for galactopoesis and for the persistency of lactation **(Svennersten-Sjaunja and Olsson, 2005)**. Because it's necessary for tissue growth, fat metabolism and homeorhesis, thus, it has an important role in reproduction, lactation and normal body growth **(Burton et al., 1994)**. GH plays a vital role in regulating body weight by decreasing the synthesis of lipids and, therefore, decreased concentrations of GH would increase synthesis of lipids **(McMahon et al., 2001)**. Therefore it has this important relationship; GH can be used as a candidate gene marker for improving growth, meat or milk production and for marked-assisted selection programs in cattle either. The GH gene with approximately 1800 bp length, five exons and four introns is a part of multiple gene family that contains prolactin and placental lactogenes and assigned with chromosome region 19q26 in bovine genome. Flanking repeat sequences of GH gene regulate the expression of a gene **(Hediger et al., 1990)**.

Leptin (LEP) is a 16-kDa polypeptide hormone produced by adipocytes, and its expression is regulated by body fatness, energy balance, insulin, growth hormone and glucocorticoids **(Leury et al., 2003)**. Leptin is expressed in a variety of other tissues, including placenta, mammary gland, skeletal muscle, gastric mucoa, brain and pituitary **(Passos et al., 2007)** and may also orchestrate and coordinate the reproductive status of an animal by acting as an intermediate molecule between nutrition and reproduction, monitoring the changes of nutritional states and probably recruiting additional molecules and hormones to regulate energy metabolism and reproduction **(Chehab et al., 2002)**. LEP binds to a receptor mainly localized on Neuropeptid – Y – neurons, which in hypothalamus also appear to play a key role in the integration of feeding behaviour with internal signals of body energy status. NPY is also involved in the control of reproductive function **(Wayne et al., 1995)**. Body

condition in cattle has a high heritability and current research is focused on identifying candidate genes influencing this complex phenotype. The influence of LEP on the regulation of food intake and energy expenditure, as well as the location of his receptors, indicate its role in the growth processes **(Barb et al., 1998)**. Receptors of LEP are expressed in a variety of cells and tissues, including the immune cells. In cattle, the LEP gene is located on chromosome 4. It consists out of tree exons and two introns of which only 2 exons are translated into protein. The coding region of the LEP gene (501 nucleotide length) is contained in second exon and 3 which are separated by introns of approximately 2 kb. The LEP gene promoter regions spans are approximately 3 kb. This gene inself is considered a potentional QTL, influencing different production traits in cattle **(Javanmard et al., 2010)**.

The aim of this study was conducted in order to identify the polymorphisms of growth hormone gene (*Alu*I loci) and leptin gene (*Sau*3AI loci) in population of Slovak spotted bulls.

MATERIAL AND METHODS

The total numbers of blood samples were taken from 58 samples of Slovak spotted bulls. Genomic DNA was extracted from whole blood samples with isolation kit NucleoSpin Blood (Macherey-Nagel). Genotype analyses were performed using the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method. A 428 bp fragment of exon 5 in bovine GH gene was amplified by PCR using forward and reverse primers according to **Balogh et al. (2009)** and PCR products of intron 2 in LEP gene with length 422 bp were carried according to **Liefers et al. (2002)**. PCR is a sensitive method by which is possible to amplify the DNA segment with a low concentration **(Kačániová et al., 2005; Kunová et al., 2011; Pochop et al., 2011)**. The polymerase chain reaction for the GH and LEP gene was performed in a 25 µl reaction mixtures, containing: 10 x PCR reaction buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 8 pM primers (Generi-Biotech), 1 U Tag DNA polymerase (Fermentas) and 50 ng genomic DNA. PCR amplification was carried out in C1000^{TM} thermal cycler (Biorad). Thermal cycling conditions included: an initial denaturation step at 94°C for 1 min, followed by 30 cycles of 94°C for 30 sec, 53°C for 30 sec, 72°C for 30 sec and a final extension at 72°C for 5 min; at 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 62 °C for 20 sec, 72°C for 30 sec and extension at 72°C for 7 min for GH and LEP genes, respectively. The PCR products of GH gene were digested with 1 µl of FastDigest *AluI* (Fermentas) restriction enzyme at 37°C in time 15 min and products of LEP gene were digested with 1 µl of FastDigest *Sau3AI* (Fermentas) restriction enzyme at 37°C in time 10

min. The digestion products were separated by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (130 V for 40 min) stained with GelRed (Biotium) prior to visualization under UV light.

Table 1 Primer sequences of *GH AluI loci* and *LEP Sau3AI loci*

Locus	Primer sequence
GH *ALUI*[1]	F 5′-CGGACCGTGTCTATGAGAAGCTGAAG-3′
	R 5′-GTTCTTGAGCAGCGCGTCGTCA-3′
LEP *Sau*3AI[2]	F 5′ -TGG AGT GGC TTG TTA TTT TCT TCT- 3′
	R 5′-GTC CCC GCT TCT GGC TAC CTA ACT- 3′

Legend: F= Forward, R= Reverse. [1]**Balogh et al. (2009)**, [2]**Liefers et al., (2002)**

RESULTS AND DISCUSSION

Single nucleotide polymorphism (SNP) in the exon 5 of the bovine GH gene based on the use of restriction fragment length polymorphism was detected. The SNP in exon 5 (at codon 127) changes leucine to valine (GTC to GTG) in the mature GH molecule. It is a point mutation in position 2141. Amplified PCR products bovine GH genes (428bp) were digested using restriction enzyme *Alu*I. The digested LL PCR product exhibited four fragments of 265, 96, 51 and 16. For the VV genotype were exhibited 265, 147 and 16 bp. Figure 1. shows PCR product size and the restriction patterns of the tree genotypes LL, LV and VV. The results show, that the most frequent genotype for growth hormone gene in observed population was LV. The frequency of the L allele was 0.6404 and for B allele was 0.3596 in group of 57 Slovak spotted bulls. The number of individuals with different genotypes and allele frequencies for this polymorphism of GH gene so that three patterns were observed and frequencies were 0.404 (n=23), 0.473 (n=27) and 0.123 (n=7) for LL, LV and VV, respectively. Based on the observed vs. expected genotype frequencies the whole pool was in Hardy-Weinberg genetic equilibrium (expected genotype frequencies were LL=0.410, LV=0.4606 and VV=0.1294).

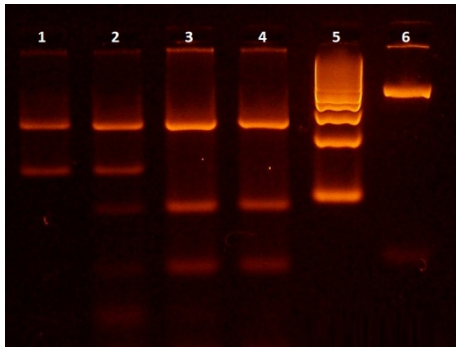

Figure 1 Representative results PCR-RFLP analysis *GH AluI loci* on 3% agarose gel

Line 1 is VV genotype (265, 147 and 16 bp), line 2 is LV genotype (265, 147, 96, 51 and 16 bp), line 3 and 4 are LL genotype (265, 96, 51 and 16 bp), line 5 is a marker of molecular weight (Fermentas, 100 bp) and line 6 is PCR product (428 bp)

In the intron between two exons of the bovine LEP gene using digestion of PCR fragment with restriction enzyme *Sau3AI* was detected restriction fragment length polymorphism. There were two *Sau3AI* sites in 422 bp fragments. The digested AA PCR product exhibited two fragments of 390 and 32 bp. For the BB genotype exhibited 303, 88 and 32 bp. Fig 2. shows PCR product size and the restriction patterns of the tree genotypes AA, AB and BB and they confirmed mutation GAC»GAT (C to T) transversion. The frequencies of were 0.8017 and 0.1983 for A and B alleles, respectively. The most frequent genotype for *LEPSau3AI loci* in this observed population was AA. The number of individuals with different genotypes and allele frequencies for this polymorphism in leptin gene so that three patterns were observed and frequencies were 0.638 (n=37), 0.328 (n=19) and 0.034 (n=2) for AA, AB and BB, respectively.

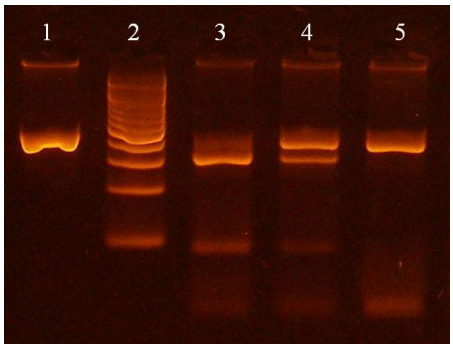

Figure 2 Representative results PCR-RFLP analysis *LEP Sau3AI loci* on 3% agarose gel

Line 1 is PCR product (422 bp), line 2 is a marker of molecular weight (Fermentas, 100 bp), line 3 is BB genotype (303, 88 and 32 bp), line 4 is AB genotype (390, 303, 88 and 32 bp) and line 5 is AA genotype (390 and 32 bp)

Table 2 Gene and genotypic frequencies of GH *AluI loci* and LEP *Sau3AI loci*

Bulls (n=57)	GH *Alu*I loci					LEP *Sau*3AI loci				
	Genotype			Allele		Genotype			Allele	
	LL	LV	VV	L	V	AA	AB	BB	A	B
Number	23	27	7	0.6404	0.3596	37	19	2	0.8017	0.1983
Frequency	0.404	0.473	0.123			0.638	0.328	0,034		

The result of the present study showed that the GH *Alu*I loci allele L was frequent than the V allele (0.6404 vs. 0.3596), so that most of bulls (47.3%) were heterozygous, 40.4% were homozygous for the leucine allele and only 12.3% were homozygous for the valine allele. A higher frequency of L allele (0.896) GH gene was reported for Holstein-Friesian cows **(Balogh et al., 2009)**. These findings on allele and genotype frequencies were similar reported in study **Lucy et al. (1993)**, **Kovács et al. (2006)**, **Silveira et al. (2008)**. **Lucy et al. (1993)** reported that the dairy breeds with the largest mature size (Holstein and brown Swiss) had the highest frequency of L allele, whereas smaller breeds (Ayshire and Jersey) had the highest frequency of V allele. **Jakaria et al. (2009)** reported, that the L allele frequency of GH *Alu*I loci was higher for cattle with origin in *Bos indicus* than *Bos taurus*.

The A and B genetic variants of the *Sau*3AI polymorphism in the LEP gene were observed in this study. The A allele was most frequent than the B allele (0.8017 vs. 0.1983), so that most of the bulls (63.8%) were homozygous for the A allele, 32.8% were heterozygous and 3.4% were homozygous for B allele. These findings were similar to those previously reported for Holstein-Friesian cows **(Liefers et al., 2002)**, Black-and-White cows **(Kulig et al., 2005)**, Holstein bulls **(Javanmard et al., 2010)** and different breeds of cows designed on meat yield **(Passos et al., 2007)**. **Pomp et al. (1997)** verified that the frequency of a restriction fragment length polymorphism (RFLP) (*Sau*3AI) in bovine LEP gene was different between *Bos Taurus* and *Bos indicus* cattle breeds, being possible that genotype differences in leptin could explain some of the phenotypic variation observed between breeds of cattle.

CONCLUSION

By using PCR-RFLP method have been detected genotypes in the polymorphic sites of growth hormone gene (*Alu*I loci) and leptin gene (*Sau*3AI loci). In the studied population of 58 Slovak Spotted bulls were detected all three genotypes of GH *Alu*I loci and the LL genotype (n=23), LV (n=27) and VV (n=7). The frequent allele in this population was allele L

with observed frequency 0.6404. Of the bovine LEP gene were identified also three genotypes and the AA genotype (n=37), AB genotype (n=19) and BB genotype (n=2). Allele A was in observed population most frequent (0.8017) than the B allele.

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