The Association of Bovine Growth Hormone Gene Polymorphism with Milk Performance Traits in Slovak Spotted Cows

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Abstract

The aim of this work was to study polymorphism of the bovine growth hormone gene, which is a part of the multiple gene family that contains prolactin and placental lactogen. Genetic variations of this polymorphism have potential effect as genetic markers and could help in the genetic improvement of cattle population. A total of 80 cows of Slovak Spotted breed were genotyped for the bovine growth hormone *Alu*I polymorphism in exon 5 on chromosome 19 by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and associated with milk production parameters. The genotype and allele frequencies for observed population were determinated and tested to be in Hardy-Weinberg equilibrium. Frequencies of allele L and V were 0.694 and 0.306, respectively. The most frequent was the heterozygous genotype LV. Results from the statistical analysis between *Alu*I polymorphism and milk production parameters – milk, protein and fat yield (kg) were no significant.

Keywords: cattle, growth hormone gene, milk production, PCR-RFLP, polymorphism

1. Introduction

Growth hormone (GH) belongs to a family of somatolactogenic hormones that have included prolactin, placental lactogen and a number of haematopoetic growth factors [1].This hormone is essential for postnatal somatic growth and maintenance of lean tissue at maturity. In animal industries, increased concentrations of GH are economic importance because they are associated with faster growth, less fat stores and, in the dairy industry, more efficient milk production in dairy cows [2]. Gene coding bovine growth hormone is a candidate gene for body weight and weight gain in cattle since it plays a fundamental role in growth regulation. In ruminants GH is known to be responsible for galactopoesis and for persistency of lactation and it coordinates physiological processes so that nutrients are partitioned for milk synthesis [3]. The growth hormone protein is a single-chain polypeptide consisting of 191 amino acids and is synthesized and secreted by the anterior pituitary gland under the hypothalamic control of two hormones, GH-releasing hormone (GHRH), which increases the secretion of GH, and somatotropin release-inhibiting factor which inhibits its secretion [4].

Growth hormone gene is a member of multiple family approximately 1800 bp in length with five exons separated by interval introns and assigned with chromosome region 19q26 in bovine genome [5]. Flanking repeat sequences of GH gene regulate the expression of a gene [6]. Several polymorphisms were detected in the GH gene. Hilbert et al. [7] and Cowan et al. [8] identified a polymorphic site using restriction endonuclease *Msp*I, the polymorphism was localized in the intron 3 of the GH gene in the position 1547 [9]. Lucy et al. [10] reported a polymorphic site for *Alu*I restriction endonuclease, localized in the exon 5 in bovine GH gene and characterized by

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the substitution of cytosine for guanine at position 2,141 caused an amino acid change from leucine to valine at residue 127.

The objective of the study was to detection *Alu*I GH gene polymorphism as possible marker of production potential of Slovak Spotted cattle by evaluating the individual and combined effects associated with this marker on milk performance traits.

2. Materials and methods

Sampling and DNA extraction

The total numbers of blood samples were taken from 80 samples of Slovak spotted cows. Genomic DNA was extracted from whole blood samples according to Miller et al. [11]. DNA concentrations were calculated by spectrophotometer by taking the optical density at wave length of 260 nm.

PCR amplification

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. [12]. The polymerase chain reaction was performed in a 25 µl reaction mixtures, containing: 1 x PCR buffer $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 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.

Table 1. Primer sequence of GH *AluI* loci

Locus	Primer sequence
GH *AluI*[1]	F 5′-CGGACCGTGTCTATGAGAAGCTGAAG-3′ R 5′-GTTCTTGAGCAGCGCGTCGTCA-3′

Note: F= Forward, R= Reverse. [1]Balogh et al. [12]

Restriction reaction

Genotype analyses were performed using the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method. The PCR products of GH gene were digested with 1 µl of FastDigest *Alu*I (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.

Statistical analysis

The allele and genotype frequencies of *AluI* polymorphism were examined for deviation from Hardy-Weinberg equilibrium using χ^2 test. The effect of polymorphism of growth hormone genotypes on milk performance trait – milk, protein and fat yield were analysed using SAS 9.1 software.

3. 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 2,141. 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 three genotypes LL, LV and VV.

The result of the present study showed that the GH *AluI* loci allele L was frequent than the V allele (0.6938 vs. 0.3063). The expected frequencies of three genotypes were 48.1% (LL), 42.5 (LV) and 9.4% (VV). The observed number of genotypes 41.25% (LL), 56.25% (LV) and 2.5 (VV) were different to the expected values. Most of cows were heterozygous, 33 individuals were homozygous for the leucine allele and only 2 cows were homozygous for the valine allele. Table 2 shows frequencies of three detected genotypes LL, LV and VV. Based on the observed vs. expected genotype frequencies the whole pool wasn't in Hardy-Weinberg genetic equilibrium.

A higher frequency of L allele (0.896) GH gene was reported for Holstein-Friesian [12].

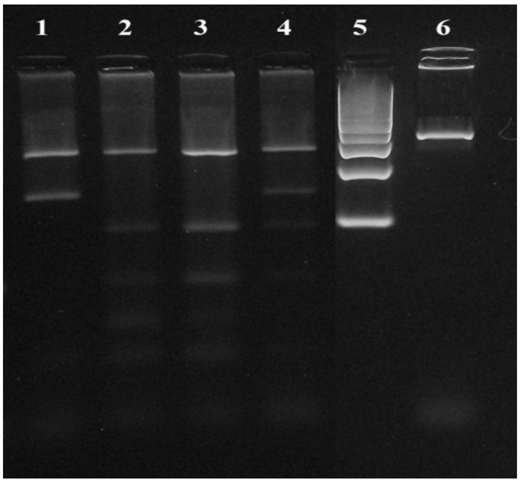

Figure 1. Representative results PCR-RFLP analysis GH AluI loci on 3% agarose gel
Line1 is VV genotype (265, 147 and 16 bp), line 2 and 3 are LL genotype (265, 96, 51 and 16 bp), line 4 is LV (265, 147, 96, 51 and 16 bp) genotype, line 5 is a marker of molecular weight (Fermentas, 100 bp) and line 6 is PCR product (428 bp)

These findings on allele and genotype frequencies were similar reported in study [10, 13, 14]. Lucy et al. [10] 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. [15] reported, that the L allele frequency of GH *Alu*I loci was higher for cattle with origin in *Bos indicus* than *Bos taurus*.

The results of statistical analysis show statistically non-significant differences between the mean values of milk, protein and fat yield in studied population with different GH *AluI* genotypes.

Table 3 represents the differences between GH genotypes in milk production characterized by some considerable traits. The cows with genotypes AA and AB showed a higher milk yield compared cows with BB genotype. The mean of protein and fat yield in milk were higher in cows with heterozygous genotype.

In other studies was detected significantly effect of *AluI* polymorphism on milk performance traits in cattle. Kovács et al. [13] found significantly differences of test milking data and milk composition traits in Hungarian Holstein-Friesian bull dam population, when cows with LV and VV genotype had higher test milking data than LL cows and LL genotyped cows produced significantly higher values of fat and protein percent. Similarly Sabour and Lin [16] reported that the V allele was preferred for increased milk production traits in Canadian Holstein bulls. Eppard et al. [17] reported that the V variant of *AluI* polymorphism resulted in higher milk production in Hostein-Friesian cows. Shariflou et al. [18] shown in population Australian Holstein Friesian cows higher milk production associated with L allele. Dybus [19] found higher milk fat and protein yield in cows with LL genotype compared to LV individuals. Lucy et al. [10] reported that L allele is closely related with higher milk production traits for Holstein cattle, whereas in Jersey the V allele is associated with higher milk yield. Factors effecting milk production in Slovak spotted cattle were also evaluated by Bujko et al. [20]. Significance of observed differences was affected by number of data available for particular trait.

Table 2. Allele and genotype frequencies of GH *Alu*I loci

Cows (n=80)	GH *AluI* loci					χ^2
	Genotype			Allele		
	LL	LV	VV	L	V	10.7^{+}
Number	33	45	2	0.6938	0.3063	
Frequency	0.481	0.425	0.094			

$P < 0.05$

Table 3. Means and standard deviations of milk performance trait in cows of different GH *AluI* genotypes

Genotypes	n	Traits (means±SD)			
		Milk yield (kg)	Protein yield (kg)	Fat yield (kg)	N
AluI					
LL	33	6222.8±1206.6	3.3716±0.1881	3.9432±0.3383	88
LV	45	6113.4±1308.5	3.4114±0.1900	4.0220±0.4074	123
VV	2	5915.3±1410.4	3.3571±0.1618	3.9143±0.3579	7

4. Conclusions

By using PCR-RFLP method have been detected genotypes in the polymorphic sites of growth hormone gene (*AluI* loci). In the studied population of 80 Slovak Spotted cows were detected genotypes LL (n=33), LV (n=45) and VV (n=2). Allele L was frequent (0.6938) than the V allele. Based on the statistical analysis GH *AluI* polymorphism had in observed population non-significant effect of milk, protein and fat yield.

Acknowledgements

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