



**EFFECT OF POLYMORPHISMS IN THE BOVINE GROWTH HORMONE GENE
AND IMPACT OF INBREEDING DEPRESSION ON MILK PERFORMANCE
TRAITS IN SLOVAK SPOTTED CATTLE**

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ABSTRACT

The aim of this study was to evaluate the relationship between the polymorphism in the growth hormone (GH) gene, inbreeding and long-life milk performance traits in Slovak Spotted cows. Genetic diversity was evaluated based on pedigree analysis. DNA was isolated from 110 blood samples. The polymorphism of GH gene was genotyped using PCR – RFLP method. Digestion of PCR products with restriction enzyme *AluI* revealed two alleles L and V, with L as a dominant (0.6818). The most frequent genotype was LV with frequency 0.509. The differences in average long-life milk production traits by different GH genotypes were not significant. We found significantly higher the average long-life milk, protein and fat yield in outbred animals with genotype LL and LV than in the groups of inbred cows. Therefore, animals with heterozygote genotypes might have potentially positive effect on long-life milk performance traits.

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

INTRODUCTION

Slovak Spotted is a dual – purpose breed with a good milk and meat production, which belongs to the Simmental type of cattle (**Bujko, 2011**). Growth hormone (GH) belongs to a family of somatotrophic hormones included prolactin, placental lactogen and number of haematopoietic growth factors (**Cosman et al., 1990**). Through the biological effect of GH are coordinated changes in tissue metabolism including nutrient partitioning and thus play a key role in increasing growth performance or milk yield (**Etherton and Bauman, 1998**). In ruminants, GH is known to be responsible for galactopoiesis and for persistency of lactation and it coordinates physiological processes so that nutrients are partitioned for milk synthesis (**Svennersten-Sjaunja and Olsson, 2005**). Selection of dairy cattle for milk yield interacted with the function of the growth hormone-insulin-like growth factor-I axis (**Baumgard et al., 2002**). 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 (**McMahon et al., 2001**). Moreover, the gene encoding GH was considered a promising candidate as a marker for selection purposes (**Parmentier et al., 1990**). Potentially, the genetic marker assisted selection can enhance progress in economic traits like lactation yield. Genetic variation at molecular level is pervasive in all breeding programs and these variants can be a potential marker gene resource. With the use of modern genetic selection programs and the development of new reproductive technologies, the best animals accumulate in pedigree. This leads to increasing in inbreeding (**Croquet et al., 2006**). Inbreeding is defined as the probability that 2 alleles at any locus are identical by descent (**Falconer and Mackay, 1996**) and occurs, when related individuals are mated. Inbreeding results in an increase in the number of homozygous loci (**Wright, 1922**), which may lead to an increase in the accumulation of recessive alleles (**Mc Parland et al., 2009**). One of the main economic consequences of inbreeding is inbreeding depression, the reduction of the phenotypic value for economically important quantitative traits (**Croquet et al., 2006**). Inbreeding negatively affects milk production (**Smith et al., 1998**), fertility (**Wall et al., 2005**) and survival (**Sewalem et al., 2006**). The inbreeding depression is generally greater in traits associated with fitness and survival (**Falconer and Mackay, 1986**).

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 which increases the secretion of GH, and somatotropin release-inhibiting factor which inhibits its secretion (**Nicoll et al., 1986**).

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 (**Hediger et al., 1990**). Flanking repeat sequences of GH gene regulate the expression of a gene (**Ferraz et al., 2006**). Several polymorphisms were detected in the GH gene. **Lucy et al. (1993)** reported a polymorphic site for *AluI* restriction endonuclease, localized in the exon 5 in bovine GH gene and characterized by 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 our study was to detection *AluI* GH gene polymorphism, estimation genotype and allele frequencies of this gene and evaluation of the impact of this polymorphic site and inbreeding depression in observed population of Slovak spotted cows on milk performance traits.

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

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)**. The polymerase chain reaction was performed in a 25 µl reaction mixtures, containing: 1 x PCR buffer (NH₄)₂SO₄, 1.5 mM MgCl₂, 200 µM of dNTPs, 0.8 µM of primers, 1 U Tag DNA polymerase and 50 ng genomic DNA template. 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. The PCR products of GH gene were digested with 1 µl of FastDigest *AluI* (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 sequence of GH *AluI* locus

Locus	Primer sequence
GH <i>AluI</i> ¹	F 5'-CGGACCGTGTCTATGAGAAGCTGAAG-3'
	R 5'-GTTCTTGAGCAGCGCGTCGTCA-3'

Note: F= Forward, R= Reverse. ¹ Balogh et al. (2009)

Pedigree and statistic analysis

Genetic diversity was evaluated based on pedigree information. The data from Slovak Spotted cows were obtained from the database of the Breeding Services of Slovak Republic. The effects of genotypes and inbreeding coefficient were determined for long-life milk, protein and fat yield. The evaluated population consisted of 110 cows. The animals were divided into 4 groups, by genotypes and levels of inbreeding coefficient (F_i). Group 1 consisted of 25 outbred animals and group 2 consisted of 22 inbred animals of LL (dominant homozygote) genotype. Group 3 consisted of 35 outbred animals and group 4 of 21 inbred animals of genotype LV (heterozygote). Inbreeding coefficient basic statistics from the evaluated groups are published in Table 2. Software ENDOG v4.8 was used for estimation of inbreeding coefficient in population (Gutiérrez and Goyache, 2005). Software SAS Enterprise Guide V4.2, T-test was used for estimation of inbreeding depression in populations.

Table 2 The average values of inbreeding coefficient in the groups

Group	n	x F%	s	X _{min}	X _{max}
1	25	0.00	0.00	0	0
2	22	1.10	1.76	0.006	6.37
3	35	0.00	0.00	0	0
4	21	1.76	2.45	0.006	6.29

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. Amplified

PCR products of bovine GH gene (428bp) were digested using restriction enzyme *AluI*. 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.

The result of the present study showed that the GH *AluI* loci allele L was more frequent than the V allele (0.6818 vs. 0.3182). The expected frequencies of three genotypes were 46.5% (LL), 43.4% (LV) and 10.1% (VV). The observed number of genotypes 42.7% (LL), 50.9% (LV) and 6.4% (VV) were different to the expected values. Most of cows were heterozygous, 47 individuals were homozygous for the leucine allele and only 7 cows were homozygous for the valine allele. Table 3 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 ($P < 0.05$). The results of the present study were in agreement with previous observations achieved by **Lucy et al. (1993)**, which reported that the dairy breeds with the largest mature size (Holstein, Brown Swiss) had the highest frequency of L, whereas smaller breeds (Ayrshire, Jersey) had the highest frequency of V allele. This predominance of L allele was similar to those previously reported in the different population of dairy or beef cattle breeds (**Sabour et al., 1997; Kovács et al., 2006; Silveira et al., 2008; Balogh et al., 2009**).

Table 3 Frequency of alleles and genotypes of GH *AluI* locus

Cows (n=110)	LEP <i>Sau3AI</i> loci					χ^2
	Genotype			Allele		
	LL	LV	VV	L	V	
Number	47	56	7	0.6818	0.3182	4,615 [*]
Frequency	0.427	0.509	0.064			

$P < 0.05$

Based on statistical analysis in population non-significant differences between the average values of milk, protein and fat yield were observed. The average values of long-life milk performance traits of different GH genotypes are shown in Table 4. Milk, protein and fat yield were higher in animals with LV genotype in comparison with LL and VV genotype, but non-significant. Previously, in other studies was detected significant effect of *AluI* polymorphism on milk performance traits in cattle. **Lucy et al. (1991)** reported that in LL genotype, German Black-White cows GH releases more milk than the VV genotypes. In the study of **Dybus (2002)** had cows with LL genotype higher milk and protein yield. **Chung et al. (1996)** reported that cows with LL genotype had higher milk protein level. Any differences between leucine homozygous cows and valine allele in milk yield during the first 30 days

reported **Balogh et al. (2009)**. Similarly, **Lechniak et al. (2002)** could not establish a relationship between *AluI* polymorphism of the GH gene and genetic merit for milk production traits. In contrary **Kovács et al. (2006)** 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 genotype cows produced significantly higher values of fat and protein percent. Similarly **Sabour and Lin (1996)** reported that the V allele was preferred for increased milk production traits in Canadian Holstein bulls. **Eppard et al. (1992)** reported that the V variant of *AluI* polymorphism resulted in higher milk production in Holstein-Friesian cows. **Sabour et al. (1997)** reported GH gene *AluI* polymorphism in Holstein cows, when the cows with VV genotypes had better milk traits, especially higher milk protein yield. Based on achieved results, we can agree, that allele V might potentially positive effects on values of milk, protein and fat yield, if occurring in a heterozygous state. On the contrary the allele V may have potentially negative effect on the evaluated traits of milk production, if occurring as homozygous.

Table 4 Average values of long-life milk performance traits in Slovak spotted cattle of different GH genotypes

Genotypes	n	Traits		
		Milk yield (kg)	Protein yield (kg)	Fat yield (kg)
LL	47	13923.1 ± 6964.5	468.3 ± 225.5	549.2 ± 268.3
LV	56	14719.4 ± 7695.2	498.5 ± 263.5	602.3 ± 305.0
VV	7	10833.0 ± 7287.0	375.1 ± 238.2	452.6 ± 277.7

Further we evaluated milk, protein and fat yield dependence on different values of inbreeding coefficient. The results of average values of long-life milk performance traits to different GH genotypes and values of inbreeding coefficient are shown in Table 5. We found the average values of milk, protein and fat yield to be significantly higher (<0.05) for outbreed animals to genotype LL and LV contrast to the groups inbreed animals. Significant inbreeding depression for milk, fat and protein production has been found in a number of populations including North America Holsteins (**Miglior et al., 1995**), North America Jerseys (**Miglior et al., 1992**) and Swiss Braunvieh (**Casanova et al., 1992**). **Thompson et al. (2000)** reported losses in milk production per lactation of 35 kg when inbreeding was one percent. Losses in fat and protein production were proportional to losses in milk production. **Wiggans et al. (1995)** estimated reduction of 21.3 kg of milk, 1.03 kg of fat, and 0.88 kg of protein per

lactation for each one percent increase in inbreeding in the Jersey breed. The reduction on inbreeding depression easily generates enough profit to justify the use of computerized mating programs, because this service is usually offered by breeding companies for a negligible cost. **Weigel and Lin (2000)** showed that mating programs, after correct sire selection, could reduce inbreeding by 1.8 and 2.8 percent on the next generation of Holsteins and Jerseys, respectively. This reduction would increase \$37 and \$60 in lifetime net income for Holsteins and Jerseys, respectively. Compared were milk performance traits among animals with different genotypes and of inbreeding coefficient vice versa. The average values of long-life milk performance traits were non-significantly higher for inbred animals with heterozygote genotypes as for inbred dominant homozygous animals. The average values of milk and protein yield were non-significantly higher for animals of genotype LL ($F_i < 1.0$) in comparison to LV genotype ($F_i < 1.0$). Contrariwise average values of fat yield were non-significantly higher for animals of genotype LV. In the groups with values of inbreeding coefficient $F_i > 1.0$, we found significantly higher (<0.05) values of milk, protein and fat yield for animals of genotype LV as for animals of genotype LL, (+4106.2, +183.7 and +135.2 kg of milk, protein and fat respectively). Inbred animals ($F_i > 1.0$) with heterozygote genotypes have higher of long-life milk performance traits, in comparison to inbred ($F_i > 1.0$) dominant homozygous.

Table 5 Average values of long-life milk performance traits in Slovak spotted cattle of different GH genotypes and different values of inbreeding coefficient

Traits				
Group	n	Milk yield (kg)	Protein yield (kg)	Fat yield (kg)
1	25	17038.0 ± 7072.2	569.8 ± 223.2	670.7 ± 270.0
2	22	10383.1 ± 4940.0	353.0 ± 168.1	411.2 ± 192.0
3	35	17213.9 ± 8152.6	581.1 ± 282.8	698.1 ± 323.7
4	21	10561.9 ± 4562.4	360.7 ± 152.2	442.6 ± 186.7

CONCLUSION

The main economic consequence of inbreeding is depression expressing by reduction of the phenotypic values for economically important quantitative traits. The allele V might potentially have positive effects on values of long-life milk performance traits, if occurring in a heterozygous state. On the contrary the allele V in homozygous state may have potentially negative effect on long-life milk production traits. Based on results it can be concluded, that

animals with heterozygote genotypes might have potentially positive effects on long-life milk performance traits. Use of molecular markers based on growth hormone can have beneficial effect on genetic gain in future breeding.

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