Identification of the Bovine Growth Hormone Gene and AluI Loci Polymorphism by PCR – RFLP Method

Nina Moravčíková, Anna Trakovická

Slovak University of Agriculture in Nitra, 949 76 Nitra, Tr. A. Hlinku 2, Slovakia

Abstract
The aim of this study was detection of polymorphism in the bovine growth hormone gene using PCR – RFLP as an efficient method for genetic analysis of polymorphism. A polymorphic site of the growth hormone gene (*Alu*I polymorphism) that results in an amino acid change at position 127 of the protein chain (leucine, L to valine, V) has been linked to differences in circulating metabolites and metabolic hormones and to milk yield traits. The growth hormone gene is a candidate gene for body weight and weight gain in cattle since it plays a fundamental role in growth regulation. The polymorphism of growth hormone gene was studied in a group of 58 bulls of Slovak spotted breed. A strategy employing PCR was used to amplify 428 bp products from blood samples. Digestion of PCR products with restriction enzyme *Alu*I revealed two alleles: allele L was 265, 96, 51, 16 fragments and allele V was 265, 147 and 16. Three patterns were observed and with frequencies 0.404, 0.473 and 0.123 for LL, LV and VV, respectively. The frequency of alleles L was 0.6404 and V was 0.3596.

Keywords: Cattle, growth hormone gene, PCR-RFLP, polymorphism.

1. Introduction

The biological effects of growth hormone (GH) involve a variety of tissue and the metabolism of all nutrient classes: carbohydrates, lipids, proteins, and minerals. It is known that GH is the main regulator of postnatal somatic growth, stimulating anabolic processes such as cell division, skeletal growth and protein synthesis and is involved in nutrient partition by way of regulating the oxidation rate of fats (lipolytic activity), inhibition of glucose transport to pheripheral tissues and the regulation of ribosomal activity involved in translation, which, in turn influences protein synthesis [1]. Therefore coordinated changes in tissue metabolism alter nutrient partitioning and thus play a key role in increasing growth performance or milk yield [2]. In ruminants, growth hormone is known to be responsible for galactopoesis and for the persistency of lactation [3]. Because it's necessary for tissue growth, fat metabolism and homeorhesis, thus, it has an important role in reproduction, lactation and normal body growth [4]. Therefore it has this important relationship; GH can be used as a candidate gene marker for improving growth, meat or milk production and for marker-assisted selection programs in cattle either. GH has wide physiological activates, which are usually selected because of their biological significante on the quantitative traits of interest. Growth hormone has wide physiological activities, which include the regulation of growth, lactation and mammary gland development, gluconeogenesis, the activation of lipolysis, and the enhancement of amino acid incorporation in to muscle protein [4]. Bovine growth hormone is a single chain polypeptide with 190 or 191 amino acids and molecular weight 22 kD. This hormone is produced in the anterior pituitary gland under the hypothalamic control of two hormones: GH-releasing hormone, which increases the secretion

* Corresponding author: Nina Moravčíková, E-mail: nina.moravcikova1@gmail.com

of GH, and somatotropin release-inhibiting factor which inhibits its secretion [5]. Growth hormone exerts its effects on growth and metabolism by interacting with a specific receptor on the surface of the target cells. Consequently, changes in the functional regions of the growth hormone receptor can affect its binding capacity and signaling pathway, and therefore alter the activity of the GH in the target tissue [6].

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 lactogens and assigned with chromosome region 19q26 in bovine genome [7]. Flanking repeat sequences of GH gene regulate the expression of a gene [8]. The effect of some GH gene polymorphisms have been widely studied in beef cattle and the proximately between some of these polymorphisms, which can by characterized using different restriction enzymes, suggests a strong linkage between them [9]. Although, a number of polymorphisms have been determinate in the GH gene of cattle up to date, two polymorphisms located in the intron 3 and exon 5 were found significant for their effects on milk and meat yield parameters by restriction length polymorphisms (RFLP) with using *Msp*I and *Alu*I restriction enzymes respectively [10].

In bovine, a single nucleotide polymorphism 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 [11]. This hormone was shown to be polymorfphic in many breeds, being that the distribution of GH variants (LL, LV and VV) and their frequencies differ among each breed. Although there are many reports investigating the role of GH genotype (*Alu*I polymorphism) in affecting milk production traits, the association is not consistent. Current knowledge on the interrelation of *Alu*I polymorphism and reproduction is limited [12]. GH genotype may directly or indirectly (e.g. milk yield) affect the interval from calving to resumption of cyclicity, although this relationship has not yet been investigated [13].

The aim of this study was conducted in order to identify the polymorphism of growth hormone gene of *Alu*I loci in Slovak spotted breed.

2. Materials and methods

The total numbers of blood samples were taken from 58 samples of Slovak spotted breed. 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 [13]. DNA was amplified in a total volume of 25 µl containing: 10 x PCR reaction buffer, 1.5 mM $MgCl_2$, 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). PCR conditions were at 94°C for 1 min, followed by 30 cycles of 94°C for 30 s, 53°C for 30 s and 72°C for 30 s. After 30 cycles, reactions were completed by an extension at 72°C for 5 min.

The PCR product for each sample was digested with 1 µl of FastDigest *Alu*I (Fermentas) restriction enzyme at 37°C in time 15 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) and fragments typical of either leucine (L; 265, 96, 51 and 16 bp) or valine (V; 265, 147 and 16 bp) alleles were visualized under UV light.

Table 1. Primer sequences of GH *Alu*I loci

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

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

3. Results and discussion

Single nucleotide polymorphism in the exon 5 of the bovine growth hormone gene based on the use of restriction fragment length polymorphism was detected. 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.

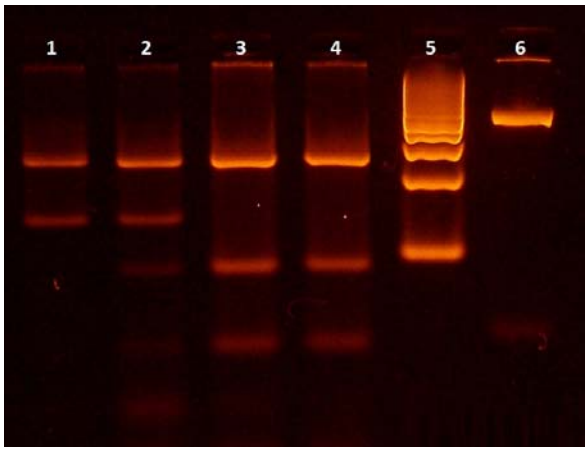

Figure1. Representative results PCR-RFLP analysis GH *Alu*I loci on 3% agarose gel
Line1 is VV genotype (265, 147 and 16 bp), line 2 is LV genotype (265, 147, 96, 51 and 16 bp), line 3 and 4 are LL genotype (265, 96, 51 and 16 bp), line 5 is a marker of molecular weight (Fermentas, 100 bp) and line 6 is PCR product (428 bp)

Table 2. Gene and genotypic frequencies GH *Alu*I loci

Bulls	Genotype			Allele	
(n=57)	LL	LV	VV	L	V
Number	23	27	7	0.6404	0.3596
Frequency	0.404	0.473	0.123		

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).
The leucine allele was frequent than the valine, 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 [13]. These findings on allele and genotype frequencies were similar reported in study Lucy et al. [10], Kovács et al. [14], Silveira et al. [9]. 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*. Differences were caused by size of the analyzed population and the differences in origin of cattle breeds, where have been detected *Alu*I polymorphisms of the GH gene.

4. Conclusions

By using PCR-RFLP method have been detected three genotypes in the polymorphic site of growth hormone gene (*Alu*I loci). In the studied population of 58 Slovak Spotted bulls were detected all three genotypes and the LL genotype (n=23), LV (n=27) and VV (n=7). The most frequent allele in this population was allele L with observed frequency 0.6404.

Acknowledgements

This work had financial support from project VEGA (project number 1/0061/10).

References

1. Goodman, H. M., Growth hormone and metabolism. In: The endocrinology of growth, Development and Metabolism in vertebrates. M. P. Schreibman, C. G. Scanes and P. K. T. Pang, Ed. Academic Press, 1993, pp. 93-115
2. Etherton, T. D., Bauman, D. E., Biology of somatotropin in growth and lactation of domestic animals, Physiol. Rev., 1998, 78, 745-761
3. Svennersten-Sjaunja, K., Olsson, K., Endocrinology of milk production, Domest. Anim. Endocrinol., 2005, 29, 241-258
4. Burton, J. L., McBride, B. W., Block, E. and Glim, D. R., A review of bovine growth hormone, Can. J. Anim. Sci., 1994, 74, 167-201
5. Nicoll, C. S., Mayer, G. L. and Russell, S. M., Structural features of prolactin and growth hormones that can be related to their biological properties, Endocrinology Revue, 1986, 7, 169-203
6. Stasio, L. D., Destefanis, G., Brugiapaglia, A., Alberta, A. and Rolando, A., Polymorphism of the GHR gene in cattle and relationship with meat production and quality, Animal Genetics, 2005, 36, 138-140
7. Hediger, R., Johnson, S. E., Barendse, W., Drinkwater, R. D., Moore, S. S. and Hetzel, J., Assignment of the growth hormone gene locus to

19q26-qter in cattle and to 11q25-qter in sheep by in situ hybridization, Genomics, 1990, 8, 171-174
8. Ferraz, A. L., Bortolossi, J. C., Curi, R. A., Ferro, M. I. and Furlan, L. R. Identification and characterization of polymorphisms within the 5´ flanking region, first exon and part intron of bovine GH gene, Journal of Animal Breeding and Genetics, 2006, 123, 208-212
9. Silveira, L. G. G., Furlan, L. R., Curi, R. A., Ferraz, L. J., Alencar, M. M., Regitano, L. C. A., Martins, C. L., Arrigoni, M. B., Suguisawa, L., Silveira, A. C. and Oliveira, H. N., Growth hormone 1 gene (GH1) polymorphisms as possible markers of the production of beef cattle using the Brazilian Canchim breed as a model, Genetics and Molecular Biology, 2008, 31, 874-879
10. Lucy, M. C., Hauser, S. D., Eppard, P. J., Krivi, G. G., Clark, J. H., Bauman, D. E. and Colier, R. J., Variants of somatotropin in cattle: gene frequencies in major dairy breeds and associated milk production, Domest. Anim. Endocrinol., 1993, 10, 325-333
11. Lucy, M. C., Hauser, S. D., Eppard, P. J., Krivi, G. G. and Collier, R. J., Genetic polymorphism within the bovine somatotropin (bST) gene detected by polymerase chain reaction and endonuclease digestion, J. Dairy Sci., 1991, 74, 284
12. Lechniak, D., Machnik, G., Szydlowski, M. and Switonski, M., Growth hormone gene polymorphism and reproductive performance of bulls, Theriogenology, 1999, 52, 1145-1152
13. Balogh, O., Kovács, K., Kulcsár, M., Gáspárdy, A., Zsolnai, A., Kátai, L., Péci, A. Fésüs, L., Butler, W. R. and Huszenicza, G., AluI polymorphism of the bovine growth hormone (GH) gene, resumption of ovarian cyclicity, milk production and loss of body condition at the onset of lactation in dairy cattle, Theriogenology, 2009, 71, 553-559
14. Kovács, K., Völgyi-Csík, J., Zsolnai, A., Györkös, I. and Fésüs, L., Associations between the AluI polymorphism of growth hormone gene and production and reproduction traits in a Hungarian Holstein-Friesian bull dam population, Arch. Tierz., 2006, 49, 236-249
15. Jakaria, R., Noor, R., Martojo, H., Duryadi, D. and Tappa, B., Identification of growth hormone (Gh) Gene mspI and AluI Loci Polymorphism in Beef Cattles. Proc. The 1st International Seminar on Animal Industry, Bogor, Indonesia, 2009, pp. 42-46