PCR-RFLP Analyses for Studying the Diversity of GH and Pit-1 Genes in Slovak Simmental Cattle

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Abstract
The aim of this study was evaluation of growth hormone (GH) and specific pituitary transcription factor (Pit-1) genes diversity in population of 353 Slovak Simmental cows. The analyses were based on single nucleotide polymorphisms GH/AluI and Pit-1/Hinfl detections. A polymorphic site of GH gene (AluI) has been linked to differences in circulating metabolites, metabolic hormones and milk yield. Bovine Pit-1 is responsible for pituitary development and hormone secreting gene expression, including GH gene. The Pit-1/Hinfl locus was associated with growth, milk production and reproduction performance in cattle. Samples of genomic DNA were analyzed by PCR-RFLP method. Digestion of GH gene PCR products with restriction enzyme AluI revealed allele L and V with frequency 0.695 and 0.305, respectively. The digested Pit-1 gene PCR products with enzyme Hinfl revealed alleles A (0.249) and B (0.751). Dominant genotypes were for GH gene heterozygous LV (0.47) and for Pit-1 gene homozygous BB (0.56) animals. The observed heterozygosity, effective allele numbers and polymorphism information content of GH/AluI and Pit-1/Hinfl bovine loci population were 0.42/0.37, 1.73/1.59 and 0.33/0.30, respectively. The median polymorphic information content of loci was also transferred to the higher observed homozygosity in population (0.58/0.63).

Keywords: cattle, growth hormone, leptin, PCR, Pit-1, polymorphism.

1. Introduction
Genes encoding growth hormone (GH) and pituitary specific transcription factor (Pit-1) are considered promising candidates genes of economically important quantitative traits. The biological effects of growth hormone involve variety of tissue and the metabolism of all nutrient classes: carbohydrates, lipids, proteins and minerals and therefore is a great interest in using GH gene as a promising candidate for selection purposes in breeding program of animals [1]. Associations of GH gene polymorphism with production traits in dairy or beef cattle have been studied by many researchers [2-5]. 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 the gene. Lucy et al. [6] reported a polymorphic site for AluI restriction endonuclease, localized in the exon 5 of bovine GH gene and characterized by the substitution of cytosine for guanine at position 2,141 causing an amino acid change from leucine to valine at residue 127. Pit-1 is the cellular specific transcription factor for activating the expression of growth hormone, prolactin and thyrotropin β-subunit genes in anterior pituitary gland [7]. Moreover, is a regulatory factor for the differentiation and proliferation of cells of pituitary gland [8]. The gene encoding Pit-1 was chosen as a candidate gene to investigate its association with lactation performance, growth and carcass traits in several cattle breeds. The

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effects of bovine Pit-1 genetic variants on production traits in cattle have been reported by many studies [9-11]. The Pit-1 gene was located in centromeric region of bovine chromosome 1. In the bovine Pit-1 gene, the restriction fragment length polymorphism (HinfI polymorphic site), was detected [12]. The aim of this study was the evaluation of the genetic diversity in growth hormone and specific pituitary transcription factor genes in population of 353 Slovak Simmental cows.

2. Materials and methods

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. [13]. The polymerase chain reaction was performed in a 25 µl reaction mixtures, containing: 1 x PCR buffer (NH₄)₂SO₄, 1.5 mM MgCl₂, 2 mM dNTPs, 0.8 µM of primers, 1 U Tag DNA polymerase and 50 ng genomic DNA. Thermal cycling conditions included: an initial denaturation step at 94°C/1 min, followed by 30 cycles of 94°C/30 sec, 53°C/30 sec, 72°C/30 sec and a final extension at 72°C/5 min. A 260 bp fragment of the Pit-1 gene was amplified with using specific forward and reverse primers according to Ozdemir [14]. The PCR reaction was similarly performed in a 25 µl reaction mixtures, containing: 1 x PCR buffer (NH₄)₂SO₄, 1.5 mM MgCl₂, 2 mM dNTPs, 0.8 µM of primers, 1 U Tag DNA polymerase and 50 ng genomic DNA template. The following cycles were applied: denaturation at 94°C/5 min, followed by 30 cycles at 94°C/45 sec, primer annealing at 60°C/45 sec, PCR product synthesis at 72°C/45 sec, and final synthesis at 72°C/5 min. The PCR products of bovine genes were digested with FastDigest restriction enzymes (Fermentas): GH gene – 1 µl AluI and Pit-1 gene – 1 µl HinfI 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>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences</th>
<th>Size (bp)</th>
<th>Restriction enzyme</th>
<th>Genotype/Digestion patterns (bp)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| GH   | F 5’-CGGACCGTGTTATGAGAAGCTGAAG-3’  
R 5’-GTTCTTGAGCAGCGCGTCA-3’ | 428 | Alu | LL 265, 96, 51, 16  
LV265, 147, 96, 51, 16  
VV 265, 147, 16 | [13] |
| Pit-1| F 5’-CTG TCTGTTAC ACAATA GGA GAG  
R 5’-TCC TGG CAA CTC ACC TCC C - 3’ | 260 | HinfI | AA 260  
AB 260, 190, 70  
BB 190, 70 | [14] |

3. Results and discussion

Single nucleotide polymorphism in the exon 5 of the bovine GH gene based on the use of restriction fragment length polymorphism was detected. 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 three fragments of 265, 147 and 16 bp. The results of the present study showed that the GH/AluI locus allele L was frequent than the V allele (0.695 vs. 0.305). The observed frequencies of genotypes were 0.462, 0.467 and 0.071 for LL, LV and VV genotypes, respectively (Table 2). Similar allele frequencies
we have found in population of Slovak Simmental bulls [17]. These findings on allele and genotype frequencies were similarly reported in other study [6, 13, and 18]. In the exon 5 of the bovine Pit-1 gene using digestion of PCR fragment with restriction enzyme Hinfl was detected a restriction fragment length polymorphism. The digested AA PCR product exhibited one fragment of 260 bp. For the BB genotype exhibited fragments of 190 and 70 bp. The frequencies for A and B alleles were 0.249 and 0.751, respectively. The most frequent genotype for Pit-1 Hinfl locus in observed population was BB. The observed number of individuals and frequencies of three genotypes in Pit-1/Hinfl locus were 0.054, 0.391 and 0.555 for AA, AB and BB, respectively (Table 2).

Table 2. Distribution of the allelic and genotypic frequencies of GH and Pit-1 genes

<table>
<thead>
<tr>
<th>Breed</th>
<th>N</th>
<th>Gene/Locus</th>
<th>Genotypes</th>
<th>Alleles</th>
<th>χ² test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slovak Simmental</td>
<td>353</td>
<td>GH/AluI</td>
<td>LL (n=163) LV (n=165) VV (n=25) L (n=169) V (n=174)</td>
<td>0.462 0.467 0.071 0.695 0.305</td>
<td>3.783*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pit-1/Hinfl</td>
<td>AA (n=19) AB (n=138) BB (n=196) A (n=165) B (n=168)</td>
<td>0.054 0.391 0.555 0.249 0.751</td>
<td>0.699*</td>
</tr>
</tbody>
</table>

n: observed number of animals; P>0.05

The high frequency of predominant B allele was investigated in other studies of different cattle breed populations [10, 11, 14, and 19]. In population of Slovak Simmental cattle were also detected genes encoding caseins and beta-lactoglobulins with PCR-RFLP method [20]. Based on the observed vs. expected genotype frequencies of both analyzed genes the whole pool was in Hardy-Weinberg genetic equilibrium.

Table 3. Genetic diversity parameters evaluated in population of 353 Slovak Simmental cows

<table>
<thead>
<tr>
<th>Locus</th>
<th>H_o</th>
<th>H_e</th>
<th>N_e</th>
<th>PIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH/AluI</td>
<td>0.58</td>
<td>0.42</td>
<td>1.73</td>
<td>0.33</td>
</tr>
<tr>
<td>Pit-1/Hinfl</td>
<td>0.63</td>
<td>0.37</td>
<td>1.59</td>
<td>0.30</td>
</tr>
</tbody>
</table>

H_o: expected homozygosity. 
H_e: expected heterozygosity. 
N_e: effective allele numbers. 
PIC: polymorphism information content.

The expected heterozygosity, effective allele numbers and polymorphism information content of GH/AluI and Pit-1/Hinfl bovine loci in analyzed population were 0.42/0.37, 1.73/1.59 and 0.33/0.30, respectively (Table 3). The decrease of heterozygosity was probably caused as a result of inbreeding level. According to the classification of PIC (low polymorphism if PIC value < 0.25, median polymorphism if 0.25 < PIC value < 0.5, and high polymorphism if PIC value > 0.5) (Botstein et al., 1980), analyzed loci in Slovak Simmental cows population belonged to the median polymorphism level (Table 3). The effectiveness of loci allele impact in populations has been expressed by effective allele numbers. The value 2,000 is limit of effective allele number in biallelic genetic system. Comparison of loci N_e showed higher effective allele numbers in GH/AluI locus (Table 3). This was due to higher values of heterozygosity for this locus. In this study we have detected higher frequency of alleles L for GH/AluI and B for Pit-1/Hinfl loci. We have also detected relative lower frequency of heterozygote animals in the analyzed population of Slovak Simmental cows. The low heterozygosity values indicate that inbreeding probably may be a potential problem at the population level, therefore should be included pedigree information in further population’s analyses. In other studies were found significant associations between GH/AluI and Pit-1/Hinfl polymorphisms and production traits in different cattle breeds. Detection of polymorphism in genes related to production traits and the identification of the allele which results in a phenotype of interest can allow for marker assisted selection in
the cattle breeding program. These markers are probably useful for association analyses of production traits as candidate gene, therefore should be included also parameters of milk production in a further after statistical analyses. Selection of animals with higher production of traits of interest based on the molecular data including genetic markers can be very valuable to cattle breeders.

4. Conclusions

Single nucleotide polymorphisms in bovine genes encoding growth hormone and specific pituitary transcription factor in population of 353 Slovak Simmental cows were detected. Genotyping was carried by the PCR-RFLP analyses. In population of cows was dominant of GH/Alu locus allele L with frequency 0.695 and heterozygous LV genotype (0.47). In case of Pit-1/Hinfl locus was frequent the B allele (0.751) and homozygous BB genotype with frequency 0.56. The values of polymorphic information content of GH/AluI and Pit-1/Hinfl loci were median, 0.33 and 0.30, respectively. These values were determined on the basis of expected heterozygosity in population and also signalized higher observed homozygosity. In the further analysis of this population should be included the pedigree information for confirm high level of inbreeding.

Acknowledgements

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