Polymorphism of Prolactin and Growth Hormone genes in the Najdi Cattle Breed Using PCR-RFLP

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ABSTRACT: The objective of this study was to determine the polymorphisms of prolactin (PRL) and growth hormone (GH) genes in the Najdi cattle breed. Blood samples were collected from 84 Najdi cows from Khuzestan agricultural research centers in Ahvaz, Shushtar, Hendijan and Mahshahr. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique were used for identification of various genotypes. The results showed that allele frequencies of A and B of PRL gene were 0.571 and 0.429 and frequencies of AA, AB and BB genotypes were 0.2857, 0.5714 and 0.1429 respectively. Moreover, the frequencies of alleles L and V of GH gene were 0.8333 and 0.1667 and the frequencies of LL, LV and VV genotypes were 0.6905, 0.2857 and 0.2380 respectively. The allelic frequencies were intermediate and statistically similar as revealed by χ² test. The results showed that these two populations were in Hardy-Weinberg equilibrium and it is possible that didn’t carry out any selection for these loci in the populations.

Key words: Genotyping; Growth hormone; Najdi cattle; Polymorphism; Prolactin.

INTRODUCTION

Najdi cattle (Bos indicus) is a breed of Iran that its origin is not clearly known. The majority of the indigenous cows of the Khuzestan province in South-western Iran were comprised of the Najdi breed estimated 380,000 herds. The average of daily milk yield of this breed is 6-8 kg during 125-day lactation period with 5.8% milk fat that it provides %55 milk consumption of Khuzestan people (Khuzestan Agricultural research Center, 2006). This breed is adapted to the high temperatures of the region, which often reached to 50°C in the summer. Improvement of important indigenous breeds has obtained trough more attention to annual optimum selective breeding programs that may lead to improvement in the important economic traits of dairy cattle. Potentially, Marker Assisted Selection (MAS) can enhance the progress in the economic traits. Genetic variations at molecular level are pervasive in all breeding populations and they can be as potential marker gene resources.

Lactation is affected physiologically from the endocrine system. The genes of milk protein and hormones that regulate lactation are excellent candidate genes for linkage analysis with Quantitative Trait Loci (QTL) for their biological importance on the interest quantitative traits. Among several hormones that regulate lactation in bovines, prolactin (PRL) is an important anterior pituitary hormone. Prolactin is a multi-purpose hormone with several special biological actions. More than 100 different and distinct effects of this hormone have been recognized. This hormone consists of 197-199 amino acids in most mammalian species (Sinha, 1995). Bovine PRL consists of 199 documented amino acids (Wallis, 1974). Prolactin is essential for the initiation and maintenance of lactation. It acts on mammary alveoli to promote the synthesis and secretion of milk protein. This hormone is primarily responsible for the synthesis all major components of milk including proteins, lactose and lipids (Le Provost et al., 1994). Suckling is the most powerful natural stimulus for PRL release during lactation (Mural and Ben-Jonathan, 1987). Prolactin gene is expressed in the pituitary gland and at several other sites including the central nervous system, the immune system and the mammary gland. (Sinha, 1995; Ben-Jonathan et al., 1996). Bovine
PRL gene is localized in chromosome 23 (Brym et al., 2005) and includes five separated exons by interval introns (Camper et al., 1984).

In superior animals growth hormone (GH) plays a key role in nutrient utilization, mammary development and growth. The identification of mutations in GH permits selection at the DNA level (Khatami et al., 2005; Zhou et al., 2005; Ferraz et al., 2006). Moreover, GH gene is a member of multigene family approximately 1800 bp in length (Gordon et al., 1983) and assigned with chromosome region 19b26 in bovine genome (Hediger et al., 1990). For importance of PRL and GH genes, this study was performed to detect polymorphism at these loci using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique in Najdi breed of Khuzestan province in Iran.

MATERIALS AND METHODS

Blood samples (3 ml) were collected from 84 Najdi cows from Khuzestan agricultural research centers in Ahvaz (26 of 84 head), Shushtar (25 of 84 heads), Hendijan (14 of 84 heads) and Mahshahr (19 of 84 heads) into EDTA-containing tubes. All the animals were unrelated and selected randomly. DNA was isolated from whole blood samples using Diatom DNAprep 100 kit (Iso Gene Moscow). Genotypes of PRL and GH genes were analyzed using PRL-Rsal and GH-Alul restriction enzymes and PCR-RFLP method.

A 156 base pair (bp) fragment of the PRL gene was amplified by Polymerase Chain Reaction (PCR) using forward (5'- CGAGTCCTTAGGAGCTTGTT-3') and reverse (5' GCCTTCAGAAGTCGTTTGTT-3') primers. The following cycles were applied: denaturation 94°C/5 min, followed by 30 cycles: denaturation-94°C For 30 sec, primer annealing 58°C for 40 sec. PCR products synthesis 72°C for 40 sec and final synthesis 72°C/5 min. The PCR reaction contained 2 µl of genomic DNA, 1 µl of each primer, 2.5 µl 10 X PCR buffer (MBI Ferments), 0.75 µl MgC12, 0.5 µl dNTP and 0.2 µl Taq-polymerase in a total volume of 15 µl. Amplified DNA was digested by Rsa I enzyme at 37°C for 12 h with the following reaction mixture: PCR product 10 µl, buffer 2 µl, Rsal 1 µl and dH2O 18 µl. The digestion products were separated by electrophoresis in 3% agarose gels in 1X TBE and 2 µM ethidium bromide.

A 211 base pair (bp) fragment of the GH gene was amplified by Polymerase Chain Reaction (PCR) using forward (5, GCTGCTTGAGGGGCCTTCG 3) and reverse (5, GGCGGGCGACTTC ATGACCTC3) primers. The following cycles were applied denaturation 95°C 4 min, followed by 1 cycles denaturation 94°C for 20 sec, primer annealing 59°C for 30 sec, followed by 35 cycles, PCR products synthesis 72°C for 30 s and final synthesis 72°C 4 min, followed by 1 cycles. The PCR reaction contained 2.5 µl of genomic DNA, 1.25 µl of each primer, 2.5 µl 10 X PCR buffer (MBI Ferments), 0.75 µl MgC12, 0.5 µl dNTP and 0.2 µl Taq-polymerase in a total volume of 16.8 µl. Amplified DNA was digested by Alul enzyme at 37°C for 16 h with the following reaction mixture: PCR product 10 µl, buffer 2 µl, Alul 1 µl and dH2O 18 µl. The digestion products were separated by electrophoresis in 2% agarose gels in 1X TBE and 2µM ethidium bromides.

The 100 pub Ladder was used as molecular size marker. The band were visualized under UV light and photographed.

RESULTS

Results for PRL gene were revealed two alleles, A and B as shown in table 2, and three genotypes were observed, in table 1. Statistical analyses were performed by PopGene 32 and Excel softwares. The frequencies of allele A and B were 0.571 and 0.429 and frequencies of AA, AB and BB genotypes were 0.2857, 0.5714 and 0.1429 respectively. Moreover, for GH gene were revealed two alleles, L and V as shown in table 4, and three genotypes were observed, in table 3 as follows. The frequencies of allele L and V were 0.8333 and 0.1667 and the frequencies of LL, LV and VV genotypes were 0.6905, 0.2857 and 0.2380 respectively.

The allelic frequencies were intermediate and statistically similar as revealed by χ2 test. This showed that this two populations were in Hardy-Weinberg equilibrium and it is possible that any selection done for these loci in the populations.

DISCUSSION

In this study, polymorphisms of 156 bp PRL gene exon III and 211 bp GH gene exon IV segments were studied by PCR-RFLP technique using Rsal (PRL) and Alul (GH) restriction enzymes, respectively, and bovine specific primers.

The PCR amplification produced, respectively, 156 bp and 211 bp segments from buffalo PRL and GH genes that they were homologous to the bovine PRL and GH genes with similar lengths. Target sequence of PRL gene, has one polymorphic Rsal site due to a silent A-G
transition mutation at the codon for amino acid 103 (Lewin et al., 1992). The A Allele of bovine PRL gene includes of whole fragment of 156 bp without internal site for RsaI, while the B allele is having one internal site for RsaI is represented by two fragments of 74 and 82 bp. Genotype AA was determined in a single fragment of 156 bp, AB in three fragments of 74, 82, 156 bp and BB in two fragments of 74, 82 bp on electrophoresis.

Target sequence of GH gene has one polymorphic AluI site due to a silent A-G transition mutation at the codon for amino acid 103. Allele V of bovine GH gene consists of intact fragment of 211 bp without internal site of Alu, while the L allele is having one internal site for Alu was represented by two fragment 159 and 52 bp. Genotype LL was determined in two a single fragment of 159 and 52 bp, LV in three fragment of 211, 159, 52 bp and VV in fragments of 211 bp on electrophoresis.

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Table 1. Genotype frequencies of prolactin for Najdi breed.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of observation</th>
<th>Frequency</th>
</tr>
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<tbody>
<tr>
<td>AA</td>
<td>24</td>
<td>%29</td>
</tr>
<tr>
<td>AB</td>
<td>48</td>
<td>%57</td>
</tr>
<tr>
<td>BB</td>
<td>12</td>
<td>%14</td>
</tr>
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</table>

Table 2. Allele frequencies of prolactin gene for Najdi breed.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of observation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>43</td>
<td></td>
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</table>

Table 3. Genotype frequencies of growth hormone gene for Najdi breed.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of observation</th>
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<tbody>
<tr>
<td>LL</td>
<td>58</td>
<td>%69/05</td>
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<tr>
<td>LV</td>
<td>24</td>
<td>%28/57</td>
</tr>
<tr>
<td>VV</td>
<td>2</td>
<td>%2/38</td>
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</tbody>
</table>

Table 4. Allele frequencies of growth hormone gene for Najdi breed.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of observation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>83/33</td>
<td>16/67</td>
</tr>
<tr>
<td>V</td>
<td></td>
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REFERENCES


