

Growth Hormone Genotyping of Najdi Cattle Breed Using PCR-RFLP

¹A. Rastegari, ¹H. Roshanfekar, ¹M. Mamouie and ²S.R. Khatami

¹Department of Animal Science,

Ramin Agricultural and Natural Resources University, Ahvaz, Iran

²Department of Genetic Science of Ahvaz University, Ahvaz, Iran

Abstract: Growth Hormone is a peptide hormone synthesized by lactotropes of the anterior pituitary. It is well known that it plays an important role in biological processes such as mammary development, lactation, growth and metabolism regulation, being therefore a promising candidate gene marker for improving milk and meat production in cattle. Genomic DNA was isolated from blood samples of 84 Najdi cattle. A 211 bp GH gene exon IV segment was amplified by PCR using bovine specific primers. RFLP in this segment was studied using AluI restriction enzyme. The frequencies of genotypes were as follows, 0.6905 LL, 0.2857 LV, 0.2380 VV, frequencies of allele L and V were 0.8333 and 0.1667 in Najdi cows.

Key words: Growth hormone gene, exon IV, Najdi cows, PCR-RFLP, AluI, genotype

INTRODUCTION

Improvement of important indigenous breeds through selective breeding has received more attention so that annual optimum selective breeding programs may achieve improvement in most of the economic traits of dairy cattle. Potentially, the genetic marker assisted selection can enhance progress in economics. Genetic variation at molecular level is pervasive in all breeding population and these variants can be a potential marker gene resource. Genetically superior animals are efficient in nutrient utilization and growth hormone exerts a key control in nutrient use, mammary development and growth. The identification of mutation in growth hormone permits selection at the DNA level (Khatami *et al.*, 2005; Zhou *et al.*, 2005; Ferraz *et al.*, 2006). Moreover, growth hormone gene is a member of multigene family approximately 1800 bp in length with intervening sequences (Gordon *et al.*, 1983) and assigned with chromosome region 19b26 in bovine genome (Hediger *et al.*, 1990). Flanking repeat sequences of growth hormone gene (Ferraz *et al.*, 2006). Considering the importance of growth hormone, the study was undertaken in cattle with the objectives (BGH) loci by using PCR-RFLP technique and its association with production.

The study was undertaken to detect polymorphism at growth hormone locus using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in Najdi breed of Khozestan province in Iran.

MATERIALS AND METHODS

Experimental material for the present study comprised of 84 Najdi cows. All the animals were unrelated and selected at random. The GH-AluI genotypes were analysed using the PCR-RFLP method. Crude DNA was isolated from whole blood samples using D1Atom DNAprep 100 kit (Iso Gene Moscow). A 211 base pair (bp) fragment of the GH gene was amplified by Polymerase Chain Reaction (PCR) using forward (5' GCTGCTCCTGAGGGCCCTTCG 3') and reverse (5' GCGGCGGCACTTCATGACCCT 3') primers. The following cycles were applied denaturation 95°C 4 min, followed by 1 cycle 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 cycle. The PCR reaction contained 2.5 µL of genomic DNA, 1.25 µL of each primer, 2.5 µL 10 x PCR buffer (MBI Fermentas), 0.75 µL MgCl₂, .5 µL dNTP and 0.2 µL Taq-polymerase in a total volume of 16.8 µL. Amplified DNA was digested by AluI enzyme at 37°C for 16 h with the following reaction mixture, PCR product 10 µL, buffer 2 µL, AluI 1 µL and dH₂O 18 µL. The digestion products were separated by electrophoresis in 2% agarose gels in 1xTBE and 2 µM ethidium bromides. The 100 bp Ladder was used as molecular size marker. The bands were visualized under UV light and photographed.

RESULTS AND DISCUSSION

The PCR amplification generated a 211 bp segment from buffalo GH gene homologous to the bovine GH gene

of similar length. Target sequence which includes part of four exon of bovine 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 comprises of intact fragment of 211 bp with no internal site of AluI, while the L allele is having one internal site for AluI was represented by two fragments of 211, 159 and 52 bp. Genotype LL results in two a single fragment of 159 and 52 bp, LV in three fragments of 211, 159, 52 bp and VV in fragments of 211 bp on electrophoresis. In the present study, the amplified product when digested with AluI enzyme revealed three distinct genotypes. The allelic frequencies were intermediate and statistically similar as revealed by Chi Square test.

CONCLUSION

A 211 bp GH gene exon IV segment was amplified by PCR using bovine specific primers. RFLPs in this segment were studied using AluI restriction enzyme. The frequencies of genotypes were as follows, 0.6905-LL, 0.2857-LV, 0.2380-VV; Frequencies of allele L and V were 0.8333 and 0.1667 in Najdi Cows.

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