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Prolactin Genotyping of Najdi Cattle Breed Using PCR-RFLP

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Abstract: Prolactin is a peptide hormone synthesized by lactotropes of the anterior pituitary. prolactin plays an important regulatory function in milk production and reproduction. Genomic DNA was isolated from blood samples of 84 Najdi cattle. A 156 bp PRL gene exon III segment was amplified by PCR using bovine specific primers. RFLPs in this segment was studied using RsaI restriction enzyme. The frequencies of genotypes were as follows: 0.2857-AA, 0.5714-AB, 0.1429-BB; Frequencies of allele A and B were 0.571 and 0.429 in Najdi Cows.

Key words: Prolactin gene, exon III, Najdi cows, PCR-RFLP, RsaI, genotype

INTRODUCTION

Lactation is under the physiological influence of the endocrine system. The milk protein and hormone genes are excellent candidate genes for linkage analysis with Quantitative Trait Loci (QTL) because of their biological significance on the quantitative traits of interest. Among several hormones that regulate lactation and reproduction in bovines, prolactin is an important anterior pituitary hormone.

Prolactin (PRL) is one of the multi-purpose hormones of the pituitary gland in terms of biological actions. More than 100 different and distinct effects of the hormone have been documented. This hormone consists of 197-199 amino acids in most mammalian species (Sinha, 1995). Bovine PRL consists of 199 amino acids (Wallis, 1974). Prolactin is necessary 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 of milk proteins lactose and lipids all major components of milk (Le Provost *et al*., 1994). PRL secretion is maintained during lactation by suckling, the most powerful natural stimulus for PRL release (Mural and Ben-jonathan, 1987) PRL regulating reproductive and immunological functions fluid balance, cellular growth and differentiation (Nicoll, 1980; Loretz and Bern, 1982; Kelly *et al*., 1991). Prolactin (PRL) 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 Prolactin (PRL) gene is localized in chromosome 23 (Brym *et al*., 2005) and consists of five exons separated by interval introns (Camper *et al*., 1984). Present study was undertaken to detect polymorphism at Prolactin (PRL) 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 PRL-RsaI genotypes were analysed using the PCR-RFLP method (Mitra *et al*., 1995). Crude DNA was isolated from whoole blood samples using DIAtom DNAprep 100 kit (Iso Gene Mmoscow).

A 156-base pair (bp) fragment of the PRL gene was amplified by Polymerase Chain Reaction (PCR) using forward(5'-CGAGTCCTTATGAGCTTGATTCTT-3')and reverse (5'GCCTTCCAGAAGTCGT TTGTTTTC-3') primers. The following cycles were applied: denaturation -94°C/5 min, followed by 30 cycles: denaturation -94°Cfor 30 sec, primer annealing -58°C for 40 sec, PCR products synthesis -72°C for40 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× PCR buffer (MBI Fermentas), 0.75 μL MgCl2, 0.5 μL dNTP and 0.2 μL Taq-polymerase in a total volume of 15 μL. Amplified DNA was digested by RsaI enzyme at 37°C for 12 h with the following

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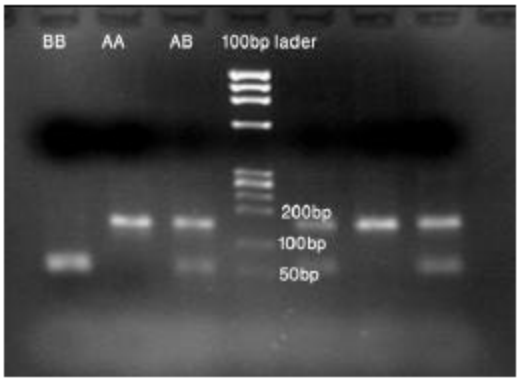

Fig. 1: PCR amplified prolactin gene digested with Rsa I in Najdi cows

reaction mixture: PCR product 10 μL, buffer 2 μL, RsaI 1 μL and dH2O 18 μL. The digestion products were separated by electrophoresis in 3% agarose gels in 1×TBE and 2 μM ethidium bromide.

The 100 bp Ladder was used as molecular size marker. The bands were visualized under UV light and photograplled.

RESULTS AND DISCUSSION

The PCR amplification generated a 156 bp segment from buffalo PRL genehomologous to the bovine PRL gene of similar length. Target sequence, which includes part of third exon of bovine PRL gene, has one polymorphic Rsa I site due to a silent A-G transition mutation at the codon for amino acid 103 (Lewin *et al*., 1992). Allele A of bovine PRL comprises of intact fragment of 156 bp with no internal Sile of Rsa I, while the B allele is having one internal site for Rsa I was represented by two fragments of 74 and 82 bp.

Genotype AA results 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 (Fig. 1).

In the present study, the amplified product when digested with Rsa I enzyme revealed three distinct genotypes. The allelic frequencies were intermediate and statistically similar as revealed by χ^2-test.

CONCLUSION

A 156 bp PRL gene exon III segment was amplified by PCR using bovine specific primers. RFLPs in this segment was studied using Rsa I restriction enzyme. The frequencies of genotypes were as follows: 0.2857-AA, 0.5714-AB, 0.1429-BB; Frequencies of allele A and B were 0.571 and 0.429 in Najdi Cows.

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REFERENCES

Brym, P., S. Kaminski and E. Wojcik, 2005. Nucleotide sequence polymorphism within exon 4 ofthe bovine prolactin gene and its associations with milk performance traits. J. Appl. Genet., 45 (2): 179-185.

Ben-jonathan, N., J.L. Mershon, D.I. Allen and R.W. Steinmetz, 1996. Extrapituitary prolactin: Distribution, regulation, functions and clinical aspects. Endocrine Rev., 17: 639-669. DOI: 10.1210/edrv-17-6-639.

Camper, S.A., D.N. Luck, Y. Yao, R.P. Woychik, R.G. Goodwin, R.H. Lyons and F.M Rootman, 1984. Characterization of the bovine prolactin gene. DNA, 3 : 237-249. DOI: 10.1089/dna.1.1984.3.237.

Kelly, P.A., J. Djiane, M.C. Postel-Vinay and M. Edery, 1991. The prolactin/growth hormone receptor family Endocrine. Rev., 12: 235-251. DOI:10.1210/edrv-12-3-235.

Le Provost, F., C. Leroux, P. Martin, P. Gaye and J. Dijane, 1994. Prolactin gene expression in ovine and caprine mammary gland. Neuroendocrinology, 60: 305-313. DOI: 10.1159/000126763. http://content.karger.com/ProdukteDB/produkte.asp?Aktion=ShowPDF&ArtikelNr=126763&Ausgabe=234605&ProduktNr=223855&filename=126763.pdf.

Lewin, H.A., K. Schmitt, R. Hubert, M.J.T. Van Eijk and N. Arnheim, 1992. Closelinkage between bovine prolactin and Bola Orbj genes: Genetic mapping in cattleby single sperm typing. Genomics, 13: 44-4. http://www.fao.org/agris/search/display.do?f=./1992/v1812/US9179549.xml;US9179549.

Loretz, C.A. and H.A. Bern, 1982. Prolactin and osmoregulation in vertebrates. Neuroendocrinology, 35: 292-304. http://content.karger.com/ProdukteDB/produkte.asp?Doi=123397.

Mitra, A., P. Schilee, C.R. Balakrishnan and F. Pirchner, 1995. Polymorph isms at growth hormone and prolactin loci in Indian cattle and buffalo. J. Anim. Breed. Genet., 112: 71-74. http://cat.inist.fr/?aModele=afficheN&cpsidt=3394310.

Mural, I. and N. Ben-Jonathan, 1987. Posterior pituitary lobectomy abolishes the suckling-induced rise in Prolactin (PRL): Evidence for a PRL-releasing factor in the posterior pituitary. Endocrinology, 121: 205-211. DOI: 10.1210/endo-121-1-205.

Nicoll, C.S., 1980. Ontogeny and evolution of prolactin's functions. FASEB J., 39: 2563-2566.

Sinha, Y.N., 1995. Structural variants of prolactin. occurrence and physiological significance. Endocrine Rev., 16: 354-369.

Wallis, M., 1974. The primary structure of bovine prolactin. Federation of European Biochemical Societies, Letters, 44: 205-208.