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Investigation of Prolactin Polymorphism in Buffalo Population of Khuzestan-Iran by PCR-RFLP

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Abstract: Prolactin is a polypeptide hormone with multiple functions, secreted mainly by the anterior pituitary gland this experiment conducted to investigate of prolactin polymorphism in buffalo population in Khuzestan of Iran. DNA was isolated from blood samples of 85 buffalos of different areas. In this study, used Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique for amplified a 156 fragment consisting of part of exon 3. The amplified fragment digested with RsaI restriction endonuclease and subjected to electrophoretic separation in ethidium bromide-strained 3% agarose gel. All the samples studied comprised of single fragment of 156 bp indicating the monomorphic nature of the locus, showing AA genotype.

Key words: Prolactin gene, polymorphism, buffalo, PCR-RFLP, blood sample, Iran

INTRODUCTION

Genes influencing polygenic traits characterizing milk or meat production are difficult to identify. However, a number of potential candidate genes have been recognized. They may be selected on the basis of a known relationship between physiological or biochemical processes and production traits and can be tested as quantitative trait loci (Oprzadek *et al*., 2003).

Lactation is under the physiological influence of the endocrine system. Among several hormones that regulate lactation and reproduction in bovines, prolactin is an important anterior pituitary hormone (Bole-Feysot *et al*., 1998). Bovine prolactin consists 199 amino acids (Wallis, 1974) and is essential for the initiation and maintenance of lactation. Bovine prolactin gene is localized in chromosome 23 (Barendse *et al*., 1997) and consists of 5 exons separated by interval introns (Camper *et al*., 1984). This hormone is, primarily responsible for the synthesis of milk proteins, lactose and lipids, all major components of milk (Le Provost *et al*., 1994). Prolactin secretion is maintained during lactation by suckling, the most powerful natural stimulus for prolactin release (Mural and Ben-Jonathan, 1987). Prolactin plays important roles in reproductive and immunological functions, fluid balance, cellular growth (Kelly *et al*., 1991). The aims of this study were:

- Investigation of prolactin polymorphism in buffalo population of Khuzestan-Iran
- Determination of allelic and genotypic frequency of prolactin gene in noteworthy population
- Investigation Hardy-Weinberg equilibrium about prolactin gene in buffalo population of Khuzestan-Iran

MATERIALS AND METHODS

This study was conducted on a total of 85 buffalos from five different regions as follows: 25 buffalos from Shadegan region, 20 buffalos from Shoshtar region, 15 buffalos from Ahwaz region, 14 buffalos from Dezful region and 11 from Susangerd region (Khuzestan, Iran). From each animal, about 3-5 cc of blood was collected from the jugular vein with vacuum tubes coated with EDTA and transported in the laboratory and stored at -22°C, until DNA extraction. Genomic DNA was isolated by using DNA Extraction Kit and was based on Boom's method. Spectrophotometer was used to investigate the quality and quantity, samples show an Optical Density (OD) ratio (260/280 nm) of between 1.6 and 1.8. PCR primer sequences was amplified using forward 5-CGA GTC CTT ATG AGC TTG ATT CTT-3 and reverse 5-GCC TTC CAG AAG TCG TTT GTT TTC-3 primers (Lewin *et al*., 1992; Mitra *et al*., 1995). The PCR reaction volume of 25 μL contained approximately 50 ng of genomic DNA, 1.25 mM Taq DNA polymerase, 2.5 μL of 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP and 10 pM of each primer. Amplification conditions included an initial denaturation at 94°C for 4 min, followed by 35 cycles at

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94°C for 40 sec, 58°C for 40 sec and 72°C for 40 sec, followed by a final extension at 72°C for 10 min. The PCR products were separated by 1.2% (w/v) agaros gel electrophoresis. The amplified fragment of prolactin was digested with 10 units of RsaI restriction enzyme and 10 µL of PCR product at 37°C overnight in a water bath. The digested PCR products were subjected to 3% (w/v) agarose gel electrophoreses and stained with ethidium bromide, the banding being visualized and documentation system. Popgene software was used to estimate the gene and genotype frequencies, the heterozygosity and effective number of alleles (Yeh *et al*., 1999). Expected theoretical heterozygosity from Hardy-Weinberg assumption was calculated.

RESULTS AND DISCUSSION

Allele A of bovine prolactin comprises of intact fragment of 156 bp with no internal Site of RsaI, while the B allele is having one internal site for RsaI 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 and 82 bp on electrophoresis. PCR amplification with special forward and reverse primers generated a 156 bp fragment from prolactin gene. PCR products after digestion by RsaI enzyme generated a 156 bp fragment of DNA that indicating AA genotype of prolactin in buffalo. All the samples studied from the five regions comprised of single fragment of 156 bp indicating the monomorphic nature of the locus, showing AA genotype (Fig. 1).

Results of this experiment agreement with Kumar (2004) and in contrast to the earlier reports (Ladani *et al*., 2003) indicating the polymorphic nature of the same locus in buffalo.

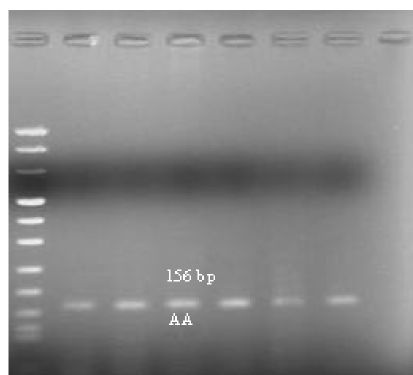

Fig. 1: PCR amplified prolactin gene digested with RsaI in buffalo's Khuzestan

CONCLUSION

Findings presented in this study indicated that the 156 bp prolactin gene fragment was monomorphic in this population. This population not followed to Hardy-Weinberg equilibrium.

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