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PCR- RFLP GENOTYPING OF GROWTH HORMONE AND GROWTH HORMONE RECEPTOR IN THREE BREEDS OF INDIAN BUFFALO

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Abstract: Bovine Growth hormone (bGH) and growth hormone receptor loci (bGHR) plays an important regulatory function in growth and milk production.The study was conducted to find out polymorphism of different bGH and bGHR loci by using PCR-RFLP technique and to study the association of different polymorphic bGH and bGHR loci with milk production in Jaffarabadi, Surti and Mehsana buffalo. Genomic DNA was isolated from blood samples of 52 Jaffarabadi, 72 Surti and 52 Mehsana buffaloes. DNA samples were subjected to PCR amplification using bGH and bGHR specific primers GH1, GH2, GH3 and GHR1. The PCR products of GH1 (436 bp), GH2 (891 bp), GH3 (441bp) and GHR1 (approx 640 bp) loci were digested with *Alu 1*, *Msp 1, Hae III* and *Mae II* restriction enzymes, respectively. Only "AA", "CC", "FF" and "RR" genotypes were found in Jaffarabadi, Surti and Mehsana buffalo. All the bGH and bGHR loci studied were monomorphic.
Keywords: Buffalo, Growth hormone, Growth hormone receptor, PCR-RFLP.

Introduction

Improvement of important livestock through selective breeding has received more attention so that annual optimum selective breeding programs may achieve improvement in most of the economic traits of different farm animals (Pawar *et al*., 2007). Buffalo (Bubalus bubalis) is one large ruminant that has its own advantages for development because it can survive with low quality of feed, tolerant to local parasites and high productivity of meat and milk (Andreas *et al*., 2010).

Current advances in molecular genetics are leading to the discovery of individual genes or candidate genes with substantial effect on traits of economic importance like meat, milk production and milk quality.

Hormone genes regulate the metabolism and thereby the performance of animals. The Growth hormone (GH) gene is located on 19th chromosome and it is a major regulation gene for postnatal growth and metabolism in mammals. Growth hormone is a polypeptide hormone secreted by somatotroph of the anterior pituitary. Biologically it helps in body

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growth through rapid cell division and skeletal growth. It also influences metabolism (Neathery *et al*., 1991), mammogenesis, galactopoiesis, lypolysis etc (Bauman and McCutchean, 1986).

The Growth Hormone Receptor (GHR), gene is located on 20th chromosome and it is a mediation gene of the biological actions of growth hormone on target cells. Growth hormone receptor a single-pass trans membrane protein of the cytokine receptor superfamily and is required to regulate the action of growth hormone.

Significant association of polymorphic candidate gene with economic traits will help the breeders to search out some genetic marker for economic traits. Several studies proved that the genetic polymorphism of growth hormone and growth hormone receptor genes correlated with preweaning growth traits. In other words, the genetic polymorphism for both genes was found responsible to improve economic traits. The use of this information from two genes in breeding programs has potential to substantially enhance rates of genetic improvement. There is extensive literature on the genetic polymorphism of GH and GHR in cattle, but perusal of literature has indicated paucity of information on these two genes in buffalo.

Considering the above mentioned points, the present study was undertaken in three breeds of buffaloes to find out polymorphism at different bGH and bGHR loci i.e. GH1 (Growth Hormone1), GH2 (Growth Hormone2), GH3 (Growth Hormone 3) and GHR1 (Growth Hormone Receptor) using PCR-RFLP technique and their association with milk production.

Materials and Methods

Animals:

Experimental materials for the present study comprised of 52 blood samples of Jaffarabadi buffalo, 72 blood samples of Surti buffalo and 52 blood samples of Mehsana buffalo, maintained at Cattle Breeding Farm, Junagadh Agriculture University, Livestock Research Station, Navsari Agricultural University and Livestock Research Station, Sardar Krushinagar Agricultural University of Gujarat respectively. All the animals studied under experiment are genetically unrelated.

DNA extraction:

The DNA was extracted by phenol- chloroform method as per method described by John *et al.* (1991). Five ml of blood was mixed with 5 ml of solution I (Tris 10mM pH 7.6; KCl 10mM; $MgCl_2$ 10mM) and 120 µl of Nonidet P-40 (BDH) was added to lyse the cells. The solution was mixed well by inverting several times and mixture was spinned down. The supernatant was discarded and nuclear pellet is resuspended gently in 400 µl of solution II

(Tris10mM pH 7.6, KCl 10mM, $MgCl_2$ 10mM, NaCl 5mM, EDTA 2mM and SDS 0.5%) to lyse the nuclei. The DNA was extracted from the supernatant by treating the supernatant with 400 µl saturated phenol, 400 µl of saturated phenol: chloroform: iso amyl alcohol (25:24:1) and equal volume of chloroform: iso amyl alcohol (24:1). The DNA was precipitated by two volumes of chilled absolute ethanol and washed with 70% ethanol. Pellet was dried properly and dissolved in 500 µl of Tris EDTA (TE) Buffer. DNA was kept for incubation at 55°C for 45 min to enhance the dissolution and then stored at 4°C.

Polymerase chain reaction:

Bovine GH gene specific primers (**GH1 F**: 5'-CCG TGT CTA TGA GAA GC-3' and **GH1 R**: 5'-GTT CTT GAG CAG CGC GT-3' , Lucy, 1991), (**GH2 F**: 5'- ATC CAC ACC CCC TCC ACA CAG T-3' and **GH2 R**: 5'-CAT TTT CCA CCC TCC CCT ACA G-3' , Zhang *et al.*, 1993), (**GH3 F**: 5'-ACG CGC TGC TCA AGA AC-3' **GH3 R**: 5'-GGC TGG AAC TAA GAA CC-3' , Unanian *et al*., 1994) and bovine GHR gene specific primer (**GHRI F**: 5'-GCG TAG CTA CTC AAC TCA TCA AAC TGC CCA TAC-3' and **GHR1 R**: 5'-AGC CAA CCC TGT GCC ATT CAA-3', Ge *et al.*, 2000) were custom synthesized at Sigma, India and were used to amplify different fragments.

PCR was carried out in a final reaction volume of 25 µl. Each reaction volume contained 12.5µl of MBI Fermentas 2X PCR Master Mix used at 1X concentration (Composition: Taq DNA polymerase (recombinant) 0.05units/µl, $MgCl_2$ 4mM, dNTPs 0.4mM of each, 1.0 µl of primer (10 pmole each), 3.0 µl template DNA (90ng) and 7.5 µl deionised water. The reaction mixture was subjected to 32 cycles of denaturation at 94°C, annealing at appropriate temperature (GH1 and GH3 loci- 60°C, GH2 loci – 64°C and GHR1 loci- 50°C) and extension at 72°C. Initial denaturation was carried out at 94°C for 5 minutes, while the final extension was performed at 72°C for 10 minutes.

Restriction Fragment Length Polymorphism and Agarose gel electrophoresis:

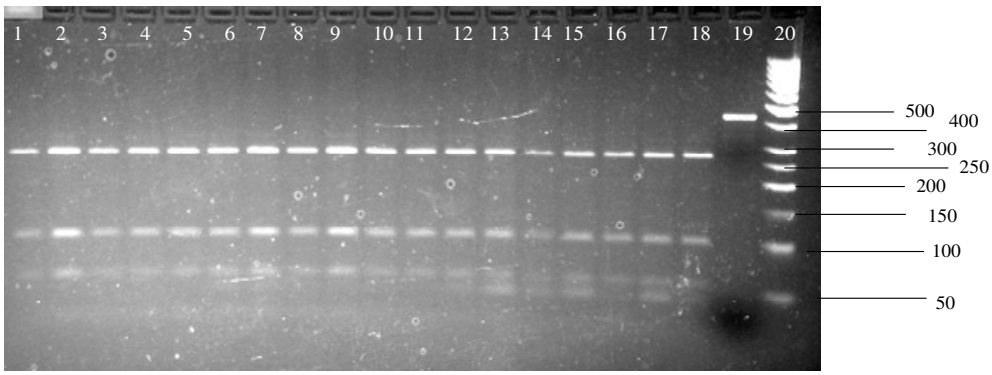
10 µl of PCR products were digested with *Alu I*, *Msp I, Hae III* and *Mae II* restriction enzymes, respectively by incubating them at 37°C for 2 hours (Mini Cycler) except for *Mae II* which was incubated at 65°C for 2 hours and electrophoresed on 2.5% agarose gel for 60-90 min (80 V) to reveal the restriction pattern. Single stained GelStar loading dye containing stock GelStar and Dimethylsulfoxide at the ratio of 1: 99 was used to load the digested PCR samples. 50bp DNA Ladder was used as a molecular size marker. The bands were visualized under UV light and documented by gel documentation system (Syngene, Gene Genius Bio Imaging).

Results and Discussion

PCR amplification generated segments of 436 bp, 891 bp, 441 bp and approx 640 bp for GH1, GH2, GH3 and GHR1 loci respectively which is homologous to the cattle GH gene of similar length. As per Zhang *et al.* (1992) bovine GH gene RFLP for *Alu I* restriction enzyme, present within exon fifth results in three genotypes: "AA", "BB" and "AB". As per Zhang *et al.* (1993) bovine GH gene RFLP for *Msp I* restriction enzyme, present within intron third were recorded in three genotypes as "CC", "DD" and "CD". As per Unanian *et al.* (1994) bovine GH gene RFLP for *Hae III* restriction enzyme, present within 3' flanking region were showing three genotypic patterns: 'EE", "FF" and "EF'. Studies carried out by Mitra *et al.* (1995) and Biswas *et al.* (2003) also resulted in monomorphic pattern for all buffaloes (Murrah, Nili-Ravi, Bhadwari, Jaffarabadi, Nagpuri, Surti and Egyptian buffaloes). Results obtained for growth hormone receptor gene in the present study are in accordance with the results of Pawar (2005) i.e. cattle GHR1 gene comprising of two fragments of around 280 and 300 bp with only one internal restriction site for *Mae II*.

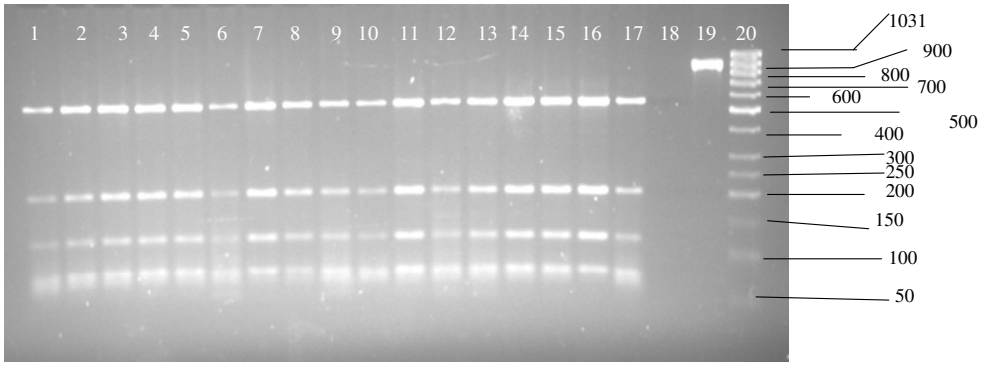
In the present study, only "AA" (Fig.1), "CC" (Fig.2), "FF" (Fig.3) and "RR" (Fig.4) genotypes was found in all the animals.

Fig.1. Monomorphic bands of GH1 gene (436 bp PCR fragment) revealed by PCR-RFLP using *Alu I* RE in three breeds of Indian buffalo



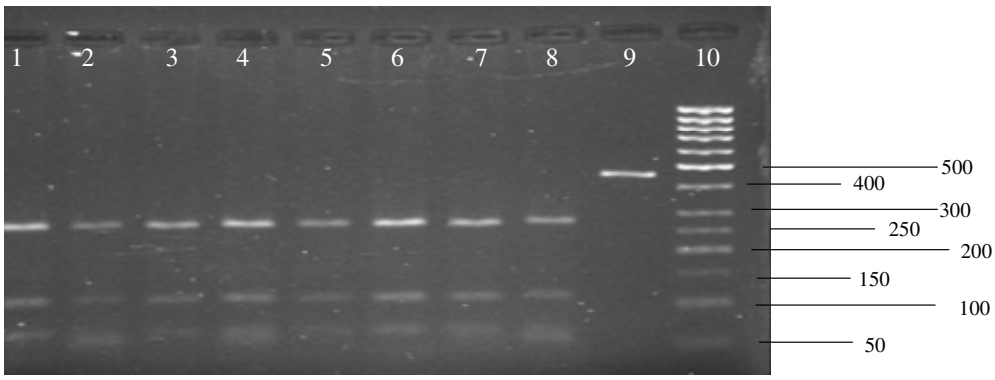
Lanes: 1-6 Jaffarabadi buffalo (all "AA" genotype)
Lanes: 7-12 Surti buffalo (all "AA" genotype)
Lanes: 13-18 Mehsana buffalo (all "AA" genotype)
Lanes: 19- Positive Control (undigested PCR product)
Lanes: 20- DNA Ladder (50 bp)

Fig.2. Monomorphic bands of GH2 gene (891 bp PCR fragment) revealed by PCR-RFLP using *Msp I* RE in three breeds of Indian buffalo



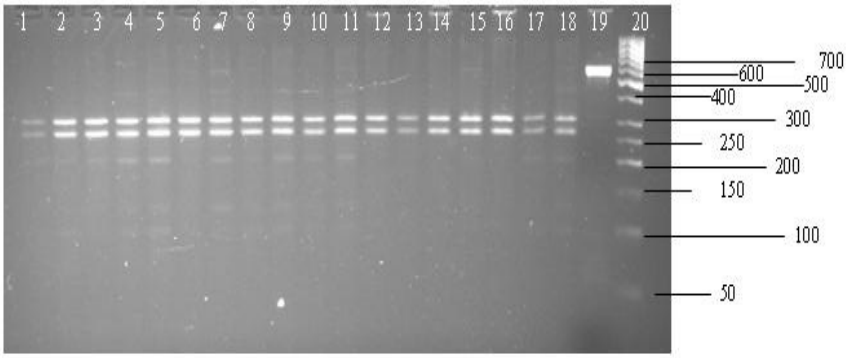
Lanes: 1-6 Jaffarabadi buffalo (all "CC" genotype)
Lanes: 7-12 Surti buffalo (all "CC" genotype)
Lanes: 13-17 Mehsana buffalo (all "CC" genotype)
Lanes: 19- Positive Control (undigested PCR product)
Lanes: 20- DNA Ladder (50 bp)

Fig.3. Monomorphic bands of GH3 gene (441 bp PCR fragment) revealed by PCR-RFLP using *Hae III* RE in three breeds of Indian buffalo



Lanes: 1--3 Jaffarabadi buffalo (all "FF' genotype)
Lanes: 4- 6 Surti buffalo (all "FF" genotype)
Lanes: 7-8 Mehsana buffalo (all "FF" genotype)
Lanes: 9- Positive control (undigested PCR product)
Lanes: 10- DNA Ladder (50 bp)

Fig.4. Monomorphic bands of GHR1 gene (640 bp PCR fragment) revealed by PCR-RFLP using *Mae II* RE in three breeds of Indian buffalo



Lanes: 1-6 Jaffarabadi buffalo (all "RR" genotype)
Lanes: 7-12 Surti buffalo (all "RR" genotype)
Lanes: 13-18 Mehsana buffalo (all "RR" genotype)
Lanes: 19- Positive Control (undigested PCR product)
Lanes: 20- DNA Ladder (50 bp)

Conclusions

Only "AA", "CC", "FF" and "RR" genotypes were found in Jaffarabadi, Surti and Mehsana buffalo at GH1, GH2, GH3 and GHR1 loci, respectively. In conclusion, it can be concluded that the diversity of GH and GHR genes in buffalo was very low and showed no polymorphisms were detected in these genes. Buffalo GH and GHR gene loci cannot be used as genetic markers for selection purpose. It was not possible to correlate them with the economic traits due to absence of polymorphism in this study.

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