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RESEARCH ARTICLE

Molecular study of growth hormone and growth hormone receptor in Jaffarabadi 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 buffalo.Genomic DNA was isolated from blood samples of 52 Jaffarabadi buffalo. DNA samples were subjected to PCR amplification using bGH and bGHR specific primers GH1, GH2, GH3 and GHR1. The PCR products of GH1 (427 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 buffalo and allelic frequencies of A, C, F and R alleles were 1.00 with absence of B, D, E and S alleles, respectively.

Key words : Buffalo, Growth hormone, Growth hormone receptor, PCR-RFLP

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INTRODUCTION

Growth hormone (GH) is a polypeptide hormone secreted by somatotroph of the anterior pituitary. Biologically it helps in body growth through rapid cell division and skeletal growth. It also influences metabolism (Neathery *et al*., 1991), mammogenesis, galactopoiesis, lypolysis etc (Bauman and McCutcheon, 1986). Growth hormone receptor (GHR), a single-pass trans membrane protein of the cytokine receptor superfamily and is required to regulate the action of growth hormone.

Allelic variation in the structural or regulatory sequences of the GH and GHR genes would be of interest because of possible direct or indirect effects on milk production and growth performance.

Considering the limited studies that had been carried out in buffaloes using molecular genetic techniques, the present study was undertaken in buffaloes to find out polymorphism at different bGH and bGHR loci i.e. GH1 (Growth Hormone1), GH2 (Growth Hormone 2), GH3(Growth Hormone 3) and GHR1(Growth Hormone Receptor) by using PCR-RFLP technique and their association with milk production.

RESEARCH METHODOLOGY

Animals:

Experimental materials for the present study comprised of 52 blood samples of Jaffarabadi buffalo maintained at Cattle Breeding Farm, Junagadh Agriculture University in Gujarat.

DNA extraction:

The DNA was extracted by phenol- chloroform method as per method described by John *et al.* (1991). 5 ml of blood is 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) is added to lyse the cells. The solution is mixed well by inverting several times and mixture is spinned down. The supernatant is 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 ml. 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 & 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 427 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 (274, 96, 50 and 16 bp fragments), BB (274,146 and 16 bp) and AB (274, 146, 96, 50 and 16 bp) based on presence and absence of restriction site. In the present study, only AA genotype was found in all the animals (Fig. 1).

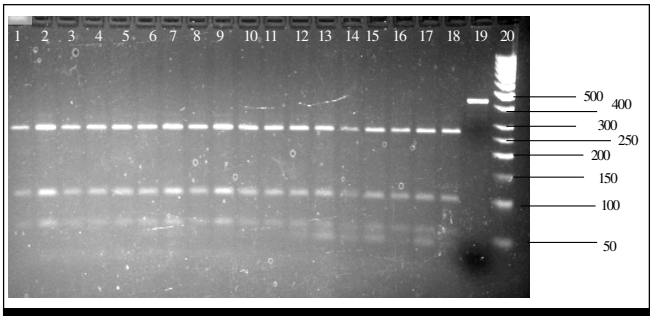

Fig.1. : GH1 gene 427 bp PCR fragment in Jaffarabadi buffalo digested by *Alu I*
Lanes: 1-18 Jaffarabadi (all AA genotype)
Lanes: 19- Positive control (undigested PCR product)
Lanes: 20- 50 bp DNA Ladder

As per Zhang *et al.* (1993) bovine GH gene RFLP for *Msp I* restriction enzyme, present within intron third were designated as 'C' and 'D' alleles, recorded three genotypes as CC (526, 193, 109 and 63bp fragments) DD (635, 193 and 63 bp) and CD (635, 526, 193 109 and 63bp) for presence and absence of restriction site. In the present study, only one type of genotype *i.e.* CC was found in all the animals (Fig. 2).

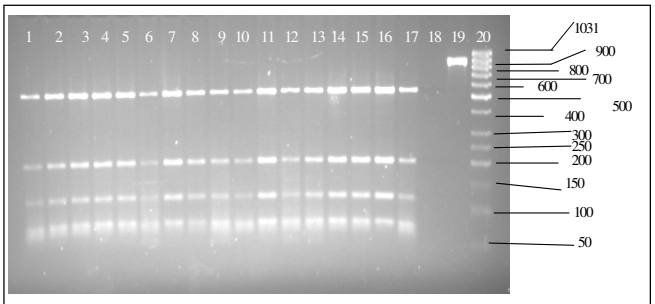

Fig. 2 : GH2 gene 891 bp PCR fragment in Jaffarabadi buffalo digested by *Msp I*
Lanes:1-17 Jaffarabadi (CC genotype)
Lanes : 19- Positive Control (undigested PCR product)
Lanes: 20- 50 bp DNA Ladder

As per Unanian *et al.* (1994) bovine GH gene RFLP for *Hae III* restriction enzyme, present within 3' flanking region were designated as 'E' and 'F' alleles, showing three genotypic patterns: EE (268bp, 102bp, and 71bp fragments), FF (268bp, 102bp, and 50bp) and EF (268bp, 102bp, 71bp and 50 bp).In the present study, only genotype FF was found in all the animals (Fig. 3).

Table 1 : Genotypic and allelic frequencies for different growth hormone and growth hormone receptor loci

GH Loci	No. of Animals	Genotype frequency			Allele frequency	
GH1	49	1.00	0.00	0.00	1.00	0.00
		(AA)	(AB)	(BB)	(A)	(B)
GH2	50	1.00	0.00	0.00	1.00	0.00
		(CC)	(CD)	(DD)	(C)	(D)
GH3	49	0.00	0.00	1.00	0.00	1.00
		(EE)	(EF)	(FF)	(E)	(F)
GHR1	50	1.00	0.00	0.00	1.00	0.00
		(RR)	(RS)	(SS)	(R)	(S)

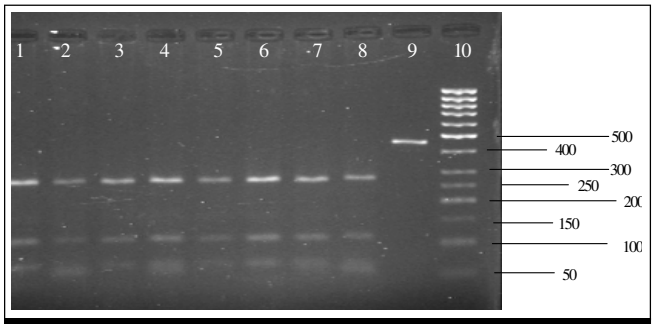

Fig. 3 : GH3 gene 441 bp PCR fragment in Jaffarabadi buffalo digested by *Hae III*
Lanes: 1—8 Jaffarabadi (FF genotype)
Lanes: 9- Positive control (undigested PCR product)
Lanes: 10- 50 bp DNA Ladder

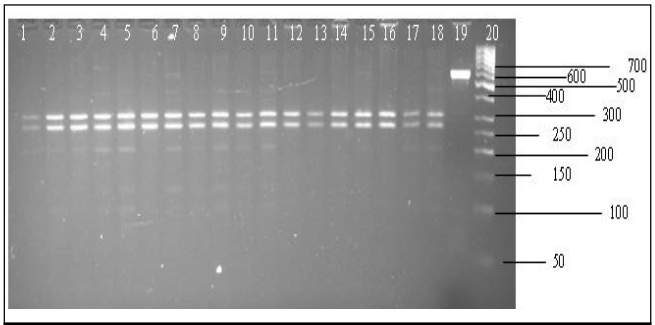

Fig. 4 : GHR1 gene 640 bp PCR fragment in Jaffarabadi buffalo digested by *Mae II*
Lanes: 1-18 Jaffarabadi (RR genotype)
Lanes: 19- Positive control (undigested PCR product)
Lanes: 20- 50 bp DNA Ladder

Mitra *et al.* (1995) studied 57 Indian Sahiwal cattle, 53 Murrah, 19 Nili-Ravi, 11 Egyptian buffaloes for GH *Alu I and* GH *Msp I* polymorphism resulted in monomorphic pattern for all buffaloes in contrast to presence of polymorphism in cattle. Biswas *et al.* (2003) carried out a study in Sahiwal, Holstein-Friesian, Jersey and crossbred cattle and Murrah, Bhadwari, Jaffarabadi, Nagpuri and Surti buffaloes for *Alu I* polymorphism also showing monomorphism in buffaloes and polymorphism in cattle. Ge *et al.* (2000) have observed four SNP (single nucleotide polymorphisms) at the position of 76 (T → C), 200 (G →A), 229 (T→ C), and 257 (A→G) bp from the 5' end of the growth hormone receptor fragment amplified with GHR1 primer sets.

Results obtained for growth hormone receptor gene in the present study are contrary to the earlier report of Ge *et al.* (2000) i.e. the C allele had been recognized using *Mae II* at positions 76 and 229 bp but 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* (Fig. 4).

The allelic and genotypic frequencies for different GH and GHR loci are given in Table 1.

Since all the loci studied were monomorphic in all the animals, indicating monomorphism at these loci may be a species characteristic of buffalo.

Conclusion:

Only AA, CC, FF and RR genotypes were found in Jaffarabadi buffalo at GH1, GH2, GH3 and GHR1 loci, respectively. The allelic frequencies of A, C, F and R alleles were 1.00 with absence of B, D, E and S alleles, respectively. Since all the growth hormone and growth hormone receptor loci studied were monomorphic in buffalo, indicating monomorphism at these loci may be a species characteristic of buffalo, probably due to absence of any mutation and high degree of sequence conservation. As buffalo GH and GHR gene loci studied at present are monomorphic, they can not be used as genetic markers for selection purpose.

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LITERATURE CITED

Bauman, D.E. and McCutcheon, S.N. (1986). The effects of growth hormone and prolactin on metabolism. In: *Control of digestion and metabolism in ruminants* (Ed. L. P.Miligan, W. I. Groven and A. Dobson). Prentice-hall, England Cliffs.N.J. 436 pp.

Biswas, T.K., Bhattacharya, T.K., Narayan, A.D., Badola, S., Kumar, P. and Sharma, A. (2003). Growth hormone gene polymorphism and its effect on birth weight in cattle and buffalo. *Asian Australasian J. Animal Sci.,* **16** (4): 494-497.

Ge, W., Davis, M. E., Hines, H. C. and Irvin, K. M. (2000). Rapid communication: Single nucleotide polymorphisms detected in exon 10 of the bovine growth hormone receptor gene. *J. Animal Sci*., **78**: 2229–2230.

John, S.W.M., Weitzner, G., Rozen, R. and Scriver, C.R. (1991). A rapid procedure for extracting genomic DNA from leucocytes. *Nucleic Acid Res.,* **19**: 408.

Mitra, A., Schlee, P., Balakrishnan, C.R and Pirchner, F. (1995). Polymorphisms at growth hormone and prolactin loci in Indian cattle and buffalo. *J. Animal Breed. & Genet.,* **112**(1): 71-74.

Neathery, M.W., Crowe, C.T., Hartnell, G.F., Veensuizen, J.J., Reagean, J.O. and Blacmon, D.M. (1991). Effects of sometribove on performance, carcass composition and chemical blood characterstics of dairy calves. *J. Dairy Sci.,* **74**: 3933- 3939.

Pawar, R. S. (2005). Growth hormone and growth hormone receptor gene polymorphism in relation with milk production in dairy cattle. M.V.Sc Thesis, Sardarkrishinagar Dantiwada Agriculture University, Sardarkrishinagar, GUJARAT (INDIA).

Unanian, M.M., DeNise, S.K., Zhang, H.M. and Ax, R.L. (1994). Rapid communication: polymerase chain reaction-restriction fragment length polymorphism in the bovine growth hormone gene. *J. Animal Sci.,* **72**(8): 2203.

Zhang, H.M., Brown, D.R., DeNise, S.K. and Ax, R.L. (1992). Nucleotide sequence determination of a bovine somatotropin allele. *Animal Genetics,* **23**: 578.

Zhang, H.M., Brown, D.R., DeNise, S.K. and Ax, R.L. (1993). Rapid communication: Polymerase chain reaction-restriction fragment length polymorphism analysis of the bovine somatotropin gene. *J. Animal Sci.,* **71**: 2276.

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