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Variations in the Bubaline Growth Hormone Gene in the Coding and Non-Coding Regions

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ABSTRACT

Growth hormone is the major gene playing regulatory role in growth and metabolism of vertebrates. Several reports have identified mutations in GH gene that are associated with animal productivity. The bovine GH has been studied quite thoroughly but very little information regarding Buffalo growth hormone is available in literature. Buffalo is an important source of milk in Asia and there is a need to identify variations in the genes of buffalo GH and its possible effects in milk production. The present research was carried out to explore DNA polymorphism in buffalo growth hormone gene. In this study we amplified a 5' flanking region covering exon 1 from local specie of *Bubalus bubalis*. As the 5' region of the GH is very important in controlling the expression of the gene and minor changes in this sequence can affect its expression in blood. The PCR amplicon was sequenced and analyzed for homology with the help of BLAST search. Surprisingly, along with various point mutations in this region, we found that a considerable base sequence upstream exon 1 was similar to Bos mutus (yakQH1 chromosome 19) and did not align with reported *Bubalus bubalis* GH sequence. Only 49% of the sequenced product aligns with *Bubalus Bubalis* though 90% of the sequence aligns with *Bos mutus* and *Bos indicus* GH gene. A 46 bp inverted repeat sequence was also identified upstream exon 1. This report not only raises questions about the purity of the gene but also indicates mutations which may affect animal productivity like milk yield, growth regulation and carcass composition. There is a need to report these mutations so that their effects can be studied further. The complete animal history, means of semen supply or the methods used for its introduction can give clues about these findings.

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AJS was involved in the conceptualization, supervision and evaluation of the study. SB participated in data curation, writing, reviewing and editing of the manuscript. SAZ did sample collection and lab experimentation.

Key words
***Bubalus bubalis, Bos mutus, Bos indicus*, Growth hormone, Mutation**

INTRODUCTION

Growth hormone (GH) is polypeptide produced by pituitary gland which plays important role in growth and development in mammals (Sami, 2007; Wallis *et al*., 1985). With the increase in human population the demand for milk, eggs and meat is increasing which in turn increase demand of more livestock. As growth hormone plays vital role in regulating milk production and metabolism of farm animals, it is a focus of research by scientists from last two decades.

GH belongs to a multi gene family including about 1800 base pairs, consists of 4 intervening sequences, 5 exons and about 648 nucleotides. It has been observed that 90% of the primary structure of GH in bovine, ovine, caprine and bubaline is similar. GH1 and GH2 are present in bovine species. The buffalo growth hormone gene is quite similar to cattle (Gordon *et al*., 1983; Hediger *et al*., 1990; Fries *et al*., 1993).

A number of reports have been published on mutations in GH gene and their effects in productivity traits in dairy and milk production (Chikuni *et al*., 1991; Lucy *et al*., 1993; Zhang *et al*., 1993; Yao *et al*., 1996; Grochowska *et al*., 2001). Hecht and Gelderman (1996) studied the polymorphisms in the 5' flanking regions of the bovine GH gene. Scientists reported that mutations in region upstream TATA box or between the exon I and exon II is important as this region is involved in coding of signal peptide, and growth hormone is synthesized as pre GH with a signal peptide of 26 amino acid residues. Exon I consist of a short nucleotide sequence which has many transcription factor binding sites and is involved in controlling gene expression (Wallis *et al*., 1995). Sami *et al*. (2011) reported mutations in the 5' flanking region of the growth hormone of first exon in *Bos indicus*. Studies report that *Bubalus bubalis* growth hormone gene has structure quite similar with bovine growth hormone.

Scientists are introducing a number of strategies to increase milk and beef production and reproductive performances of farm animals. The use of recombinant growth hormones, artificial insemination and cross breeding is one of them. The use of recombinant growth hormone has been found to be a great source to increase the milk production and improving growth rates. Crossbreeding programs takes advantage of the combination of the better traits from two or more breeds (Cundiff *et al*., 1994). Artificial insemination is widely used as a mean to influence genetic change in a population through

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more selection pressure and has become one of the most important techniques in dairy and meat industry (Odde and Holland, 1994). The semen for artificial insemination is usually collected from bull and introduced in cattle. About 1.3 million beef cattle have been artificially inseminated currently. The main purpose of A.I is to introduce superior genetic traits in animals by looking for most desirable characteristics to increase yield in both dairy and beef cattle industry. However, these methods of horizontal gene transfer may affect structure of original gene and introduce deleterious mutations (Landaeta-Hernandez *et al*., 2002). The import of semen from US and Canada is very common in Pakistan and is sometimes used without check and balance, which raises questions about the purity of the sample. As a result, we are unable to achieve desired reproductive rates and improvements in milk production. Unchecked insemination may result in mutations which may alter the structure and function of growth hormone resulting in low milk and beef productions, loss of a natural sire and emergence of diseases.

The present study was aimed at identifying the silent mutation within the 5' flanking regions of Bubalis GH. In the present study we were able to amplify a region of Bubaline growth hormone covering exon I and exon II and 5'UTR. The results were astonishing as the sequence flanking exon 1 was present in Yak and cattle and was not found in Bubaline GH. There are a number of variations identified in the sequence and these results question the purity of the gene. The methods of horizontal gene transfer, complete animal history, means of semen supply or the methods used for its introduction should be checked to see if there is mixing of genes at genetic level. These practices can not only disturb GH genes at molecular level but also introduces mutations which may have drastic effects on animal genetics.

MATERIALS AND METHODS

Bubaline blood sample was collected from freshly slaughtered animal from a local butcher shop from suburbs of Lahore. The DNA was isolated by using DNA extraction Kit (Favorgen) according to the manual's instructions.

A set of primers was synthesized, as reported by Ferraz *et al*. (2006), to amplify a fragment of genomic DNA, which covers a part of the 5'-Flanking the first exon and the beginning of the first intron of GH gene. The sequence of forward primer was 5'-TCTCAAGCTGAGACCCTGTGT - 3' and reverse primer was 5'GGCCAAATGTCTGGGTGTAGA3'. Reaction mixture consisted of 25 µl of master mix for PCR as provided by Fermantas, 10 µl of DNA 4 µl of each forward and reverse primer and finally 7 µl of distilled water was added, to make a total volume of 40 µl. Conditions were set as follows: Denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s and then final extension at 72°C for 5 min, for 45 cycles. The amplified product was visualized on 1% agarose gel after electrophoresis using ethidium bromide. A fragment of ~450 bp was obtained which was purified and sent for sequencing. The obtained sequence (Fig. 1) was analyzed for homology with the other available sequences in the gene bank with the help of NCBI nucleotide BLAST search.

Fig. 1. Sequence of GH gene from bubaline samples, which cover a part of the 5'-flanking region of the first exon. The amplified product can be seen to be 501bp size. TATA box is shown in orange and sequence of exon 1 is shown in blue. The highlighted sequence in turquoise shows the inverted repeat sequence. A single base change in repeated sequence is highlighted in Yellow.

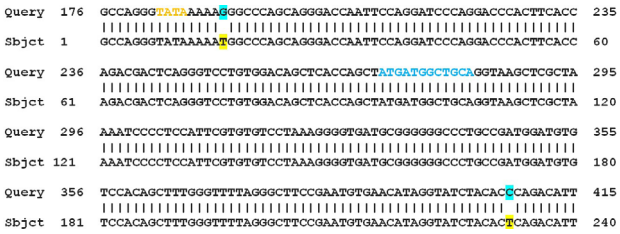

Fig. 2. Alignment of nucleotide sequence of product with *Bubalus bubalis* growth hormone (GH) gene (KC107770.1) using NCBI BLAST. The sequence aligned with position 1 to 246 of *Bubalus* GH reference sequence. Mutations are shown in turquoise color. Yellow shows the position of mutation in reference sequence. TATA box is shown in orange and sequence of exon 1 is shown in blue.

RESULTS AND DISCUSSION

Genomic DNA from blood was isolated using Invitrogen Genomic DNA extraction kit from Thermo Fisher Scientific. Gene amplification of bubaline growth hormone was done by conventional PCR. Gene specific primers were used for amplification. Amplified product was visualized on 1.5 % agarose gel. The pcr product was sent for sequencing. The obtained sequence was aligned with sequence of Bubalus bubalis growth homone (Accession no. KC107770.1). A region covering TATA box

and first exon aligned with Bubalus GH sequence (Fig. 3). In this sequence two point mutations were identified, one transition and one transversion (Table I).

Table I. Mutations recorded in sequence as compared to *Bubalus bubalis* growth hormone (GH) gene (Accession No: KC107770.1), to *Bos mutus* (Accession No: CP027087.1) and *Bos indicus* (Accession No: AY662651.1).

S/N	Comparison to GH gene	Position	Replacement
1	Bubaline GH	151	T to G
		191	T to C
2	Yak GH	29	C to A
		150	G to A
		151	A to G
		184	G to T
		229	C to G
		312	T to C
		328	G to A
		357	C to T
		360	C to G
		370	T to C
		460	TGG missing
3	Cattle GH	150	G to A
		151	A to G
		191	G to T
		229	C to G
		312	T to C
		328	G to A
		345	C to T
		357	C to T
		370	T to C
		457	TGG missing

A sequence of about 170 bp did not align with Bubalus GH, instead complete sequence was found to be highly similar with Yak and Cattle (Figs. 3, 4). The region upstream TATA box and region after 420 nucleotides that did not align with *Bubalus bubalis* aligned with YakQH1 chromosome 19, which may be the region of Yak GH, as it hasn't been reported yet, but Bubalus GH is also located on Chromosome 19, so there is an equal possibility that this region belongs to Yak GH sequence (Fig. 2). There was one transversion at position 29 (Fig. 3A). In second region there are 9 point mutations; 5 transitions and 3 transversions (Fig. 3B) and in region 3 TGG were missing at position 460 (Fig. 3C).

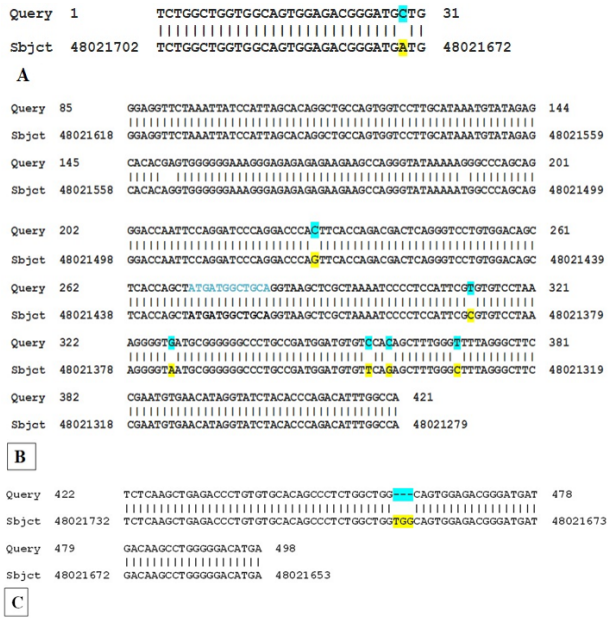

Fig. 3. Alignment of nucleotide sequence of product with *Bos mutus* isolated yakQH1 chromosome 19 (CP027087.1) using NCBI BLAST. 90% of the sequence aligned with three regions of Yak. A, showing range 1 aligned with first 31 nucleotides. B, showing range 2 aligned with nucleotides from 85 to 421 nucleotides. C, showing range 3 aligned from position 422 to 478.Mutations are shown in turquoise color. Yellow shows the position of mutation in reference sequence.

Table II. Comparison of sequenced product with bubaline (*Bubalus bubalis*) and bovine sequences (*Bos mutus, Bos indicus*) showing the number of nucleotides of sequenced product aligned with reference sequences. The percentage similarity of the aligned sequences with each other and rate of mutation within the aligned sequence are shown.

Organism	Position of aligned sequences	% Similarity of the sequence	% of Mutation with the aligned sequence
Bubalus bubalis (Water buffalo)	176-421	49%	1%
Bos mutus (Wild Yak)	1-31 85-421 422-498	90%	9%
Bos indicus (Cattle)	1-28 85-421 422-498	87%	7%

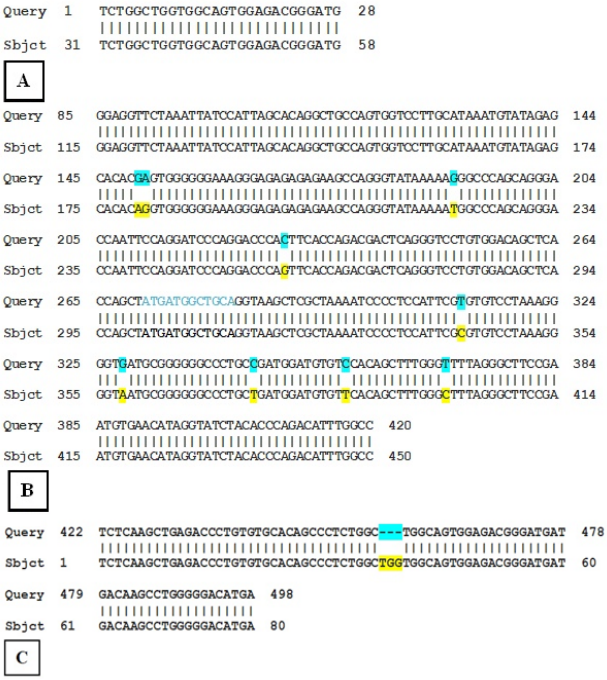

Fig. 4. Alignment of nucleotide sequence of product with Bos indicus bovine growth hormone (GH2) gene, GH2.1 allele (AY662651.1) using NCBI BLAST. 87% of the sequence aligned with three regions of Cattle. A, showing range 1 from position 31 to 58 aligned with first 28 nucleotides. B, showing range 2 from position 115 to 450 aligned from 85 to 420 nucleotides. C, showing range 3 from position 1 to 80 of gene aligned with position 422 to 478.Mutations are shown in turquoise color. Yellow shows the position of mutation in reference sequence.

The same three regions aligned with *Bos indicus* (cattle). The aligned region was 97% identical with cattle. In second region there were 7 transitions and 2 transversions. Mutations at positions 150,151,229,312,328, 357, 370 and 457 were identical to mutations found in Yak (Table I).

Only 49% of the sequence aligned with *Bubalus* although 90% of the sequence was identical to yak and 87% with cattle (Table II). The amino acid sequence of Yak GH is 100% similar to cattle and is quite similar to other bovine species (Wang *et al*., 2009). There is a possibility that the semen that was used for fertilization was obtained from Yak and the altered gene sequence is a result of cross breeding or artificial insemination. The effects of these mutations should be evaluated on animal productivity and carcass composition as this region is involved in binding of trans-acting factors hence very important in regulating the gene expression (Hecht and Geldermann, 1996).

CONCLUSION

The primary focus of animal genetics is the identification of genes which have an important role in the expression of quantitative traits. The goal of this study was to identify the mutation within the 5' flanking regions and exon I of Bubalis GH. The present study showed that the obtained sequence of gene was a mixture of bovine and bubaline GH with many point mutations, insertions and deletions. The sequence is more similar with Yak GH than bubaline GH, and there is also an inverted repeat sequence. These changes may be the result of cross breeding or artificial insemination of Yak and *Bubalus*. The methods of horizontal gene transfer, complete animal history, means of semen supply or the methods used for its introduction should be checked to see if there is mixing of genes at genetic level. These practices can not only disturb GH genes at molecular level but also introduces mutations which may have drastic effects on animal genetics.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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