INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

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EVALUATION OF DNA POLYMORPHISM IN BOVINE GROWTH HORMONE GENE BY PCR-RFLP METHOD

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ABSTRACT
Currently, the primary thrust of research in animal genetics is the identification of genes (so-called major genes), which affect the expression of quantitative traits markedly. One of these major genes is growth hormone (*GH*)) gene which is related to production and reproduction traits in livestock. There is extensive literature on the genetic polymorphism of *GH* in cattle, but perusal of literature has indicated paucity of information on this gene in buffalo. This study aimed to evaluate the genetic polymorphism within growth hormone gene in Iraqi buffalo using PCR-RFLP technique. Genomic DNA extracted from 50 healthy buffaloes and was amplified using primers that were designed from the cattle *GH* gene sequences. All buffalo animals investigated in this study are genotyped as LL for *GH1, GH2* fragments throughout all tested buffalo DNA amplified fragments at 211bp and 428bp respectively, were digested with *Alu*I endonuclease and gave two digested fragments at 159- and 52-bp in (*GH1,* 211bp) and four in the case of *GH2,* 428 bp due to the presence at least once or twice according to the amplicon length of the *Alu*I restriction site at position (AG^CT).

KEY WORDS: Buffalo, *GH*, PCR, RFLP.

INTRODUCTION

Molecular markers that reveal polymorphism at the DNA level are now key players in animal genetics. Recently, a number of significant candidate genes have been recognized (Dybus *et al*., 2004). Potentially, the genetic marker assisted selection can enhance progress in economic traits. Genetically superior animals are efficient in nutrient utilization and growth hormone exerts a key control in nutrient use, mammary development and growth (Pawar *et al*., 2007). On the other hand, animals that are genetically adapted to specific environmental condition would be more productive because it can be developed using low cost, supporting the diversity of food, agriculture and culture, as well as effective in achieving the objectives of food security (FAO, 2000). Growth hormone (GH) is necessary for tissue growth and fat metabolism, thus, it has an important role in reproduction, lactation and normal body growth (Beauchemin *et al*., 2006, Curi *et al*., 2006 and Yardibi *et al*., 2009). Because of these important biological roles, GH is considered a promising candidate gene for improving milk and meat production in cattle and buffalo through marker-assisted selection programs (Othman *et al*., 2012). The bovine growth hormone is a 22 KDa single-chain polypeptide hormone produced in the anterior pituitary gland. The encoding gene is approximately 1800 base pairs and consists of five exons and four introns (Zhou *et al*., 2005 and Sadeghi *et al*., 2008), and assigned to chromosome region 19q26 in bovine genome (Hediger *et al*., 1990). The polymorphism within bovine growth hormone gene was reported by and Curi *et al*. (2006) and Kovacs *et al*. (2006). Information of diversity using molecular approaches at the local buffalo in Iraq is still very rare, on the other hand, the diversity of functional genes has been widely used as an auxiliary marker selection on some livestock commodities, combined with optimal maintenance management. The objective of this study was to detect the genetic polymorphism within growth hormone gene in Iraqi buffalo using PCR-RFLP technique.

MATERIALS & METHODS

Genomic DNA extraction

The total numbers of blood samples were taken from *vena jugularis* of 50 different sex pubered local buffaloes and were accomplished by reserving them in EDTA tubes at -20˚C (Miller *et al.,* 1988). Genomic DNA was extracted from whole blood samples with isolation kit, QIA®mini, (QIAGENE, Germany). Moreover, the DNA concentration was estimated and the samples were diluted to 30ng/µl in TE at least 24 hours prior to the reaction.

Polymerase chain reaction (PCR)

Genotype analyses were performed using the polymerase chain reaction– restriction fragment length polymorphism (PCR-RFLP) method. A 211 bp, as well as 428 bp, fragments of exon 5 in bovine GH gene were amplified by PCR using forward and reverse primers according to Reis *et al*. (2001) and Balogh *et al*. (2009) (Table1). Amplification of fragments of the GH gene was done by using PCR (polymerase chain reaction) method. The polymerase chain reaction for the GH was performed in a 25 µl reaction mixtures(Promiga, USA), containing(2x PCR reaction buffer, 3 mM MgCl2, 400 µM dNTPs, 10 U Tag DNA polymerase), 5 µl template genomic DNA, while *GH*1primer 1.3 µl and *GH*2 was 1.25 µl, so far, the sterile water was 12.4, 12.5 µl respectively.

The PCR products were electrophoresed on 1.5% agarose gel stained with Syber safe at constant voltage (10v/cm) for 30 minutes to test the amplification success (Otaviano, *et al.*, 2005).

TABLE1: The sequences and information of primers used in this study

Primers	Sequences 5′ -------- 3′	PCR conditions (35 cycles)	PCR product size	Restriction enzyme used	References
*GH*1	CGGACCGTGTCTATGAGAAGCTGAAG GTTCTTGAGCAGCGCGTCGTCA	94°C 1 min 53°C 2 min 72°C 2 min	428 bp	*Alu*I	Balogh *et al*. (2009)
*GH*2	GCTGCTCCTGAGGGCCCTTC CATGACCCTCAGGTACGTCTCG	95°C 1 min 62°C 2 min 72°C 2 min	211 bp	*Alu*I	Reis *et al*. (2001)

Restriction fragment length polymorphism (RFLP) technique

The PCR products for the two tested fragments were digested with the restriction enzyme *Alu*I. The restriction mixture for each sample was prepared by adding 2 µl of 10 × restriction buffers to 7 units of the appropriate restriction enzyme and 0.2 µl BSA; the volume was completed to 20 µl by sterile water. This restriction mixture was mixed with PCR product (~10µl) and incubated at 37˚c for 3 hours in water bath. The digested PCR products were electrophoresed on 3% agarose gel at 50v for 2hours, staining with Ethidium Bromide to detect the different genotypes of the two tested sequences by UV-transilluminator and finally documented in gel doc system(Otaviano *et al.*, 2005).

RESULTS & DISCUSSION

The primers GH1, GH2 were amplified with DNA fragment which is used as a template for PCR reaction. The PCR amplification was confirmed by running 7µl of PCR product along with 100bp DNA marker in 1.5 agarose gel. The amplified PCR products (GH1, GH2) were visualized as a single band of expected size under the UV with the marker, which were 428bp, 211bp respectively (Figure 1 and 2). Determination of GH gene (211- bp, 428bp) genotypes in this study was done by PCR-RFLP method using *Alu*I which depending on the presence or absence of the restriction site at position 52^53 (AG^CT).

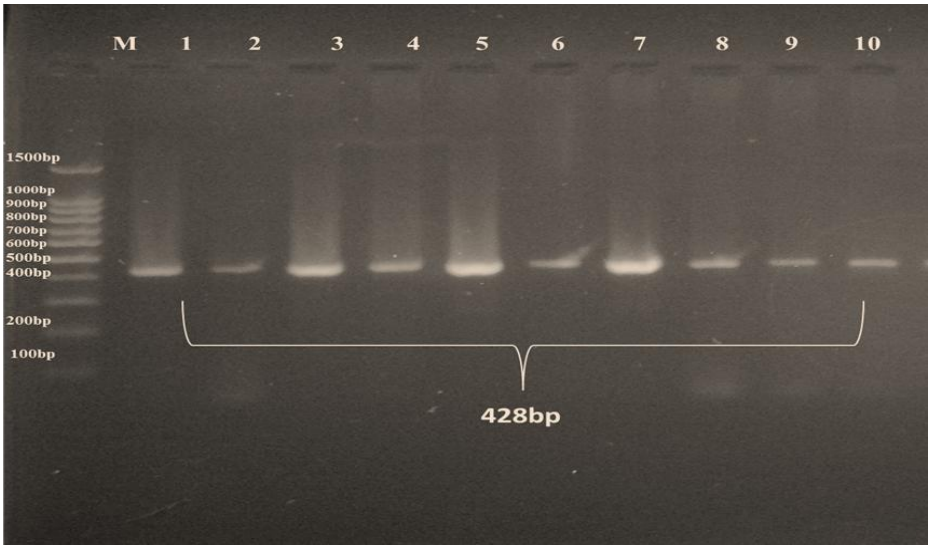

FIGURE 1: PCR products of bovine GH gene with size of 428 bp, amplified with primer GH1. The product was electrophoresis on 1.5% agarose gel at5 volt/cm^2 for 1hour.Lane M DNA ladder (100-1000), Lane (1-10) PCR products was visualized under U.V light after stain with Ethidium Bromide.

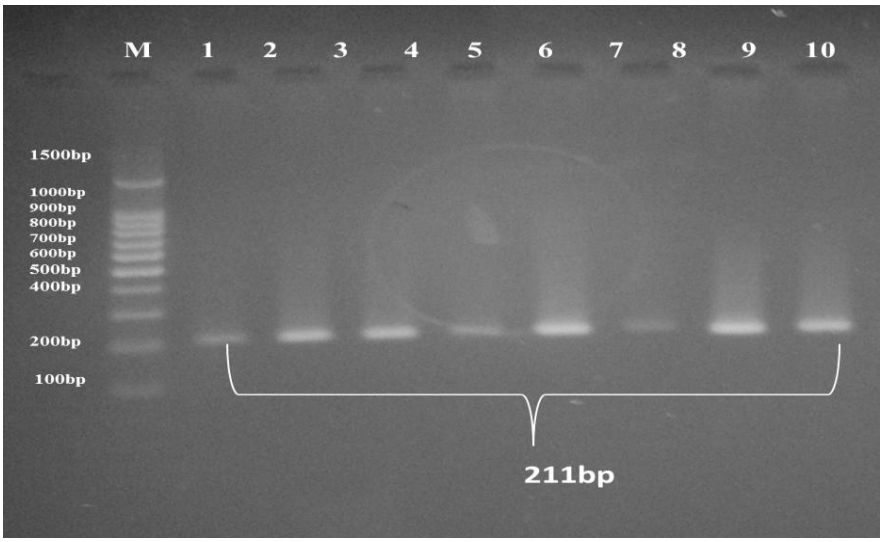

FIGURE 2: PCR products of bovine GH gene with size of 211 bp, amplified with primer GH2. The product was electrophoresis on 1.5% agarose gel at5 volt/cm^2 for 1hour.Lane M DNA ladder (100-1000), Lane (1-10) PCR products was visualized under U.V light after stain with Ethidium Bromide.

Single nucleotide polymorphism (SNP) in the exon 5 of the bovine GH gene based on the use of restriction fragment length polymorphism was detected. The SNP in exon 5 (at codon 127) changes leucine to valine (GTC to GTG) in the mature GH molecule. It is a point mutation in position 2141. Amplified PCR products of bovine GH gene (428bp) using GH1 primer were digested using also restriction enzyme *Alu*I. The digested LL PCR product exhibited four fragments of 265, 96, 51 and 16. For the LV genotype was exhibited 265, 147, 96, 51 and 16. VV genotype (265, 147 and 16 bp) were not observed (Fig. 3). This result supported by Balgh *et al*. (2009) and Andreas *et al*. (2010) through their studying on GH/ *Alu*I gene polymorphisms in Indonesian buffalo. In contrary, this result was partially disagree with Moravčíková *et al.* (2012), with the presence of VV genotype through their studying on GH/ *Alu*I gene polymorphisms in Slovak cattle, otherwise the digested give more than band this may be hard to detect this is may be due to product size which consider large and has to more than one restriction site for the same enzyme, so for farther accuracy another primer were used to shortening the PCR product giving only one site for the *Alu*I enzyme GH2.

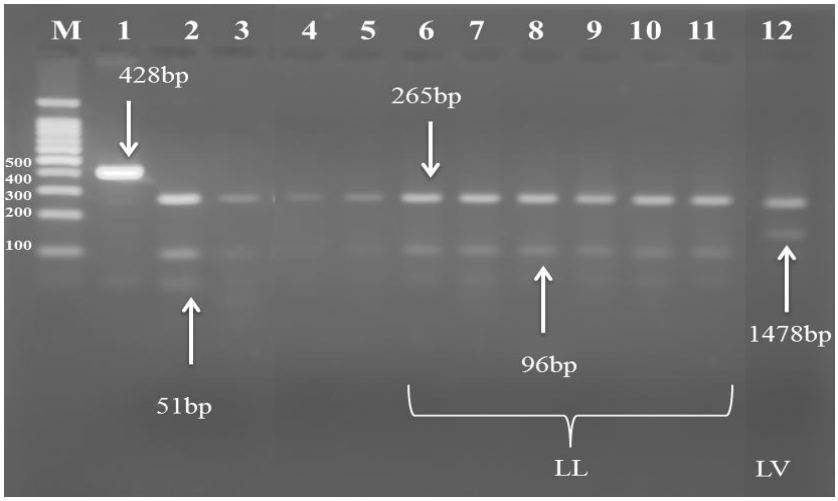

FIGURE 3: The digestion of PCR products (428) of GH gene with *AluI* enzyme. The product was electrophoresis on 3% agarose gel at 5 volt/cm^2 for 1.5 hour, Visualized under U.V light after stain with Ethidium Bromide. Lane M DNA ladder (100-1000), Lane (1): PCR products of the GH gene with size of 428 bp, Lane 2-11: the digested form which represented the dominant LL genotype. Lane 12: digested form which represented the dominant heterogeneous LV genotype.

The PCR amplified fragments (211bp) of the primer GH2, we can easily differentiate between 3 different genotypes, VV with undigested one fragment at 211bp, LL with two digested fragments at 159-and 52-bp and LV with three digested fragments at 211-, 159- and 52-bp. All buffalo animals investigated in this study are genotyped as LL, as were all tested buffalo DNA amplified fragments were digested with *Alu*I endonuclease and gave two digested fragments at 159- and 52-bp (Fig. 4) due to the presence of the restriction site at position 52^53 (AG^CT), this result supported by Biswas *et al*. (2003) and Othman *et al*. (2012) in their studying on Indian and Egyptian buffaloes as well as cattle consequently.

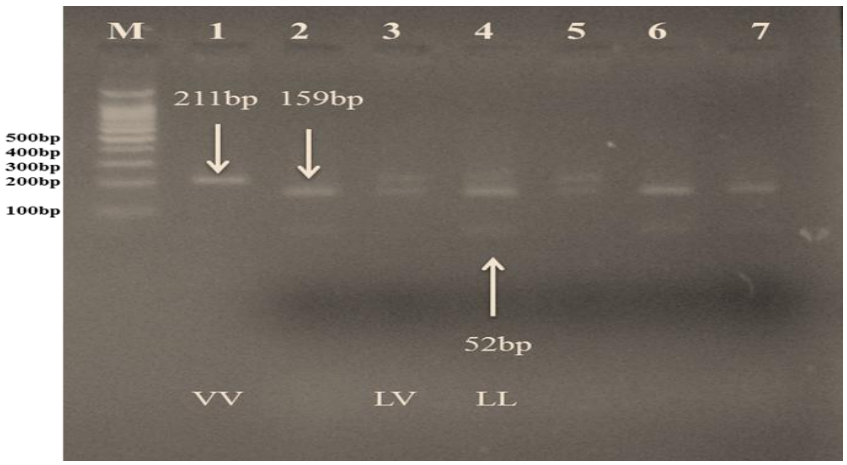

FIGURE 4: The digestion of PCR products (211) of GH gene with *AluI* enzyme. The product was electrophoresis on 3% agarose gel at 5 volt/cm^2 for 1.5 hour, Visualized under U.V light after stain with Ethidium Bromide. Lane M DNA ladder (100-1000), Lane (1): the undigested PCR products of the GH gene with size of 211bp which represented recessive VV genotype, Lanes 2, 4, 6, and 7: the digested form which represented the dominant LL genotype. Lanes 3, 5: digested form which represented the dominant heterogeneous LV genotype.

Distribution of the three genotypes was 94% (LL), 6% (LV) and 0.00 % (VV), so that most of buffaloes was heterozygous and less was homozygous for the leucine allele as compared with homozygous valine allele. However same result has been obtained by Kovacs *et al.* (2006), Silvia *et al.* (2008). On the basis of statistical analyses it can be found that LL genotyped dams produced milk with significantly higher milk fat and protein percent. The same association between LL genotype of *GH* gene with higher milk fat and protein percent was reported by Reis *et al*. (2001), Sadeghi *et al.* (2008) and Jakaria *et al.* (2009). These authors reported an association between LL and LV genotypes with the average live body weight in these cattle breeds. Moreover, Information on genetic diversity of a population using multiple loci, can be described by the value of heterozygosity (Nei and Kumar, 2000) GH *Alu*I loci in buffalo were very low. This was indicated by the value of one genotype percentage. Low diversity in buffalo can be caused by a limited number of males in the population, and the high inbreeding frequency. It can be concluded that the diversity of GH *Alu*I gene in Iraqi buffalo was very low and showed no polymorphisms were detected in these genes. All buffaloes tested had LL genotype for locus GH *Alu*I.

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