WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 3, 270-280. Research Article ISSN 2277– 7105

IDENTIFICATION OF GH/*ALU*I AND GHR/*ALU*I GENE POLYMORPHISMS IN IRAQI CATTLE

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Article Received on
07 Jan 2015,

Revised on 28 Jan 2015,
Accepted on 19 Feb 2015

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ABSTRACT

Associations were analysed between the bovine growth hormone (GH) as well as growth hormone receptor (GHR) combined with their genotypes (GH/*Alu*I& GHR/*Alu*I). 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. This study aimed to evaluate the genetic polymorphism within growth hormone and its receptor genes in Iraqi buffalo using PCR-RFLP technique. Genomic DNA extracted from 100 healthy buffaloes and amplified using primers that were designed from the cattle GH and GHR gene sequences. All tested buffalo DNA amplified fragments at 211bp for GH 5th exon. The digestion with *AluI* endonuclease gave three types of genotypes. The Distribution of the three genotypes and allele frequency was calculated according to Hardy-Weinberg equation, were LL=94 (94.09%), LV=6 (5.82%) and VV=0 (0.09%), so that most of buffaloes was homozygous (LL) and less heterozygous was for the valine allele as compared with leucine allele homozygous. The amplified fragments of GHR gene obtained from all tested buffalo DNA at 342-bp were digested with *Alu*I endonuclease. The result showed that all tested buffaloes are genotyped as GG for GHR gene, where amplified fragments were digested into two digested fragments at 241and 101-bp due to the presence of *Alu*I restriction site at position (AG^CT).

KEYWORDS: Buffalo, GH, GHR, 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. Tambasco *et al.*, (2003) have proposed candidate gene strategy. In other words, the genetic variation of gene is affecting the physiological pathways and phenotype. The genetic variation of gene would be more likely to affect the phenotype variation than genes or chromosome regions that are chosen by chance. Several studies reported that the candidate genes have influenced for preweaning growth traits such as growth hormone and growth hormone receptor genes (Ge *et al.*, 2003; Kim *et al.*, 2004).

The growth hormone gene is located on 19th chromosome and it is a major regulation gene for postnatal growth and metabolism in mammals. The growth hormone receptor gene is located on 20th chromosome and it is a mediation gene of the biological actions of growth hormone on target cells.

Several studies proved that the genetic polymorphism of growth hormone and growth hormone receptor genes correlated with preweaning growth traits (Hale *et al.*, 2000; Stasio *et al.*, 2002). In other words, the genetic polymorphism for both genes was found responsible to improve economic traits.

The studies on candidate genes in beef cattle from other countries are carried out on growth (Tambasco *et al.*, 2003; Kim *et al.*, 2004).

The mutation of growth hormone gene on promoter or on the 5^{th} exon depicted one of changing phenotype since polypeptide chains that are translated from converting DNA. For example, the mutation on the 5^{th} exon. This substitution illustrated low body weight and average daily milk production (Lucy *et al.*, 1998). Studies concern with correlation between growth hormone receptor gene and growth were focused on mutations on promotor region, few studies reported the effect of exon nine mutations on economic traits (Hale *et al.*, 2000).

The detection of polymorphism makes it possible to identify differences between individual in genotype at growth hormone and growth hormone receptor genes. The use of this

information from two genes in breeding programs has potential to substantially enhance rates of genetic improvement.

Consequently, farmers and breeders could utilize the result of this research to genetically improve preweaning growth traits and use it as basic information for studying about genetic marker or marker assisted selection in the future.

MATERIALS AND METHODS

Genomic DNA extraction: The total numbers of blood samples were taken from *vena jugularis* of 100 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): Amplification of GH and GHR fragments were done by using polymerase chain reaction– restriction fragment length polymorphism (PCR-RFLP) methods. A 211 bp (GH gene), as well as 342 bp (GHR gene), were amplified by PCR using forward and reverse primers according to Reis *et al.,* (2001) and Di Stasio *et al.,* (2005) (Table1)**.**

Reagents used for amplification of both target fragments were 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 primer 1.3 µl and GHR was 1.25 µl, so far, the sterile water was 12.4, 12.5 µl respectively.

The reaction was cycled for 1 min. at 94°C, 2 min at an optimized annealing temperature that was determined for each primer (Table 1) and 2 min. at 72°C for 30 cycles.

The PCR products were electrophoresed on 1.5% agarose gel stained with ethidium bromide at constant voltage (10v/cm) for 30 minutes to test the amplification success (Othman *et al*., 2012).

Table1. The sequences and information of primers used in this study.

The primer	5′ -------- 3′	PCR (35 cycles)	product size	R.E	References
GH	GCT GCT CCT GAG GGC CCT TC CAT GAC CCT CAG GTA CGT CTC CG	95°C 1 min 62°C 2 min 72°C 2 min	211 bp	*Alu*I	Reis *et al* (2001)
GHR	GCT AAC TTC ATC GTG GAC AAC CTA TGG CAT GAT TTT GTT CAG	94°C 1 min 53°C 2 min 72°C 2 min	342 bp	*Alu*I	Di Stasio *et al* (2005)

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 50 v for 2hours, staining with ethidium promide 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 AND DISCUSSION

Growth hormone (GH) actions on target cells depend on the GH receptor (GHR) (Burton *et al.*, 1994). The GH binding to GHR causes its dimerization, activation of the GHR-associated JAK2 tyrosine kinase, and tyrosyl phosphorylation of both JAK2 and GHR (Zhu *et al*., 2001). These events activate a variety of signaling molecules, including MAP kinases, protein kinase C, and STAT transcription factors (Maj *et al.*, 2004).

The primers GH, GHR 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 (GH, GHR) were visualized as a single band of expected size under the UV with the marker, which were 211bp for GH and 342bp for GHR (Figure 1 and 2).

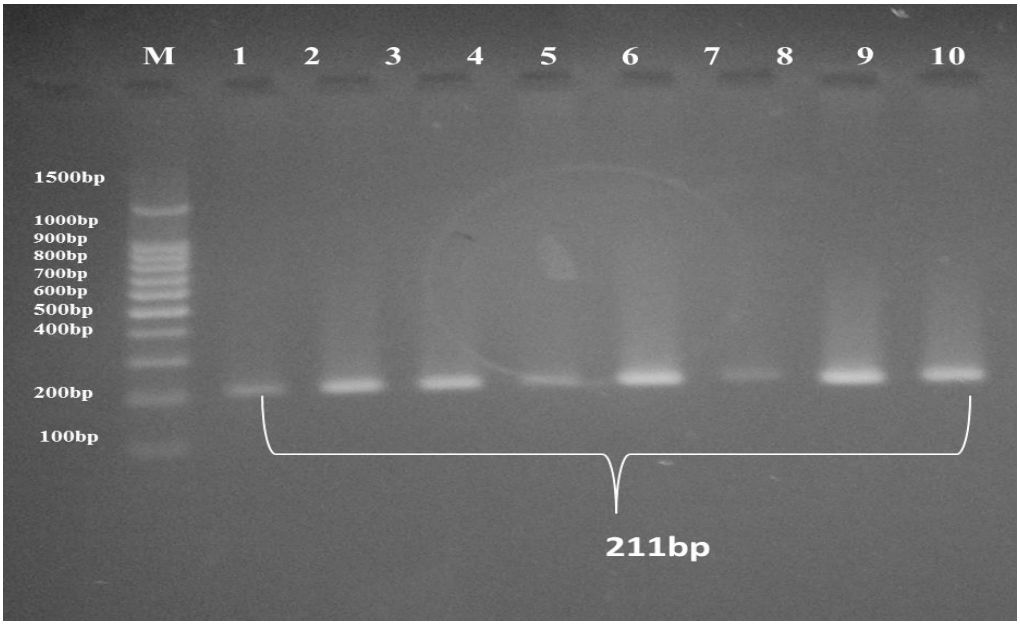

Figure (1): PCR products of bovine GH gene with size of 211 bp, amplified with primer GH. The product was electrophoresis on 1.5% agarose gel at5 volt/cm^2 for 1hour.Lane M DNA ladder (100-1000), Lane (1-10) PCR products was visualized under U.V light after stain with Ethidium Bromide.

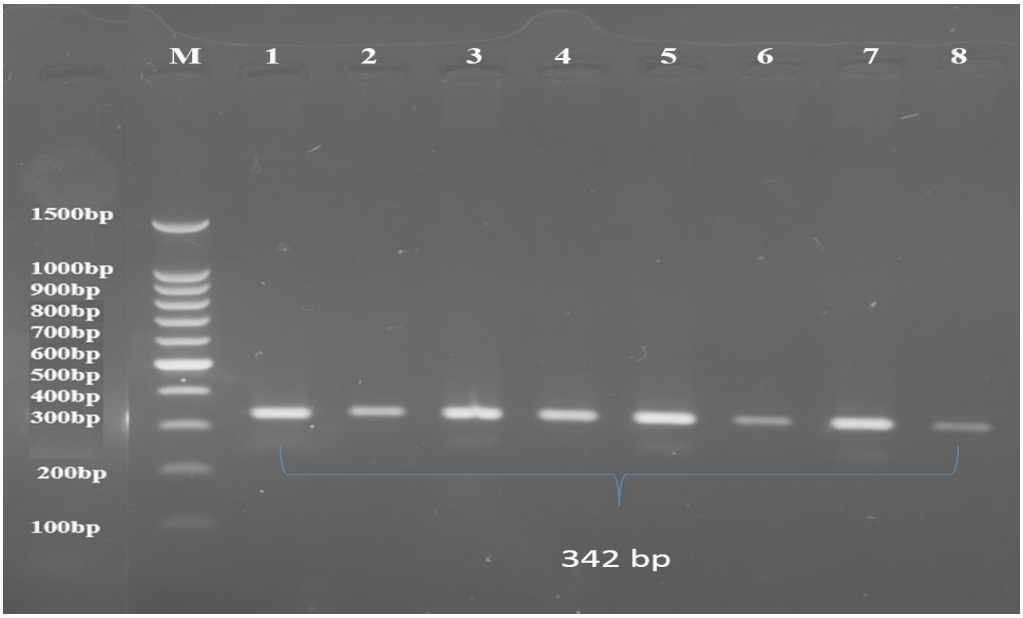

Figure (2): PCR products of bovine GHR gene with size of 342 bp, amplified with primer GHR. The product was electrophoresis on 1.5% agarose gel at5 volt/cm^2 for 1hour.Lane M DNA ladder (100-1000), Lane (1-8) PCR products was visualized under U.V light after stain with Ethidium Bromide.

The PCR amplified fragments (211bp) of the primer GH that located on the 5th exon at the position 2141, which consider the single SNP were lay on the coding region. We can easily differentiate between 3 different genotypes, VV with undigested one fragment at 211bp, LL with two digested fragments at 159bp and 52bp, LV with three digested fragments at 211bp,

159bp and 52bp. Most of buffalo animals investigated in this study are genotyped as LL. As were tested buffalo DNA, amplified fragments were digested with *Alu*I endonuclease and gave two digested fragments at 159bp and 52bp (Figure 3). As a result of the presence of the restriction site at position 52^53 (AG^CT) for the PCR product, 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.

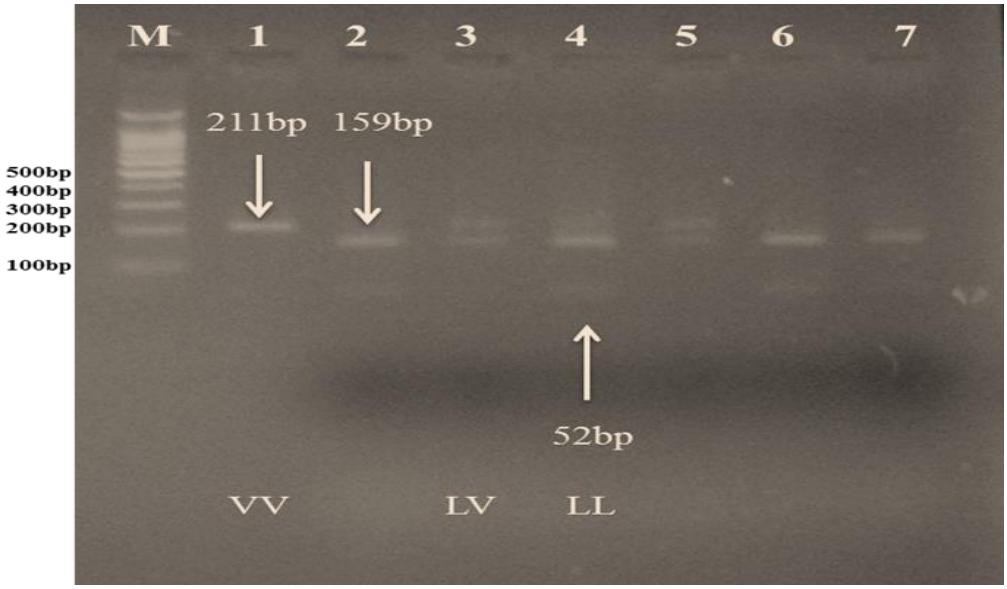

Figure (3): The digestion of PCR products (211) of GH gene with *AluI* enzyme. The product was electrophoresis on 3% agarose gel at 5 volt/cm^2 for 1.5 hour, Visualized under U.V light after stain with Ethidium Bromide. Lane M DNA ladder (100-1000), Lane (1): the undigested PCR products of the GH gene 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 and allele frequency was calculated according to Hardy-Weinberg equation as shown in table (2), Were LL=94 (94.09%), LV=6 (5.82%) and VV=0 (0.09%), so that most of buffaloes was homozygous (LL) and less heterozygous was for the valine allele as compared with leucine allele homozygous (Hussain *et al*., 2014) . However Kovacs *et al*. (2006), have obtained same result. 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).

The mutation of GH 5th exon loci in buffalo were very low. This was indicated by the value of one genotype percentage. A limited number of males in the population, and the high inbreeding frequency can cause low diversity in buffalo.

It can be concluded that the diversity of GH gene in Iraqi buffalo was very low and showed no polymorphisms were detected in these genes. Most buffaloes tested had LL genotype for locus GH 5th exon loci.

Table (2):-The genotype of buffalo GH genotypes and allele frequency calculated according to Hardy-Weinberg equation distribution among samples.

Genotypes	**LL**	**LV**	**VV**
Observed	94	6	0
Expected H-W Freq.	94.09 94.09%	5,82 5.82%	0.09 0.09%
Allele Frequencies	L= 194 (97%) V= 6 (3%)		
	P-Value =0.7571		

In cattle, a single gene located on chromosome 20 encodes the GHR (Menon *et al*., 2001). The gene coding for bovine GHR consists of 9 exons (from 2 to 10) in the translated part and of a long 5'-noncoding region (Maj *et al*., 2004). So far, the primers used in this study (Table 1) flanked a 342-bp fragment from exon 9 of Iraqi buffalo GHR gene. The amplified fragments obtained from all tested buffalo DNA (100 animals) at 342-bp.

Depending on the presence or absence of the restriction site at position 101^102 (AG^CT) in these amplified fragments, we can easily differentiate between 3 different genotypes. AA with undigested one fragment at 342-bp, GG with two digested fragments at 241-and 101-bp and AG with three digested fragments at 342-, 241- and 101-bp.

All buffalo animals investigated in this study are genotyped as GG where all tested buffalo DNA amplified fragments were digested with *Alu*I endonuclease and gave two digested fragments at 241- and 101-bp (Figure 4) due to the presence of restriction site at position 101^102 (AG^CT**)** .

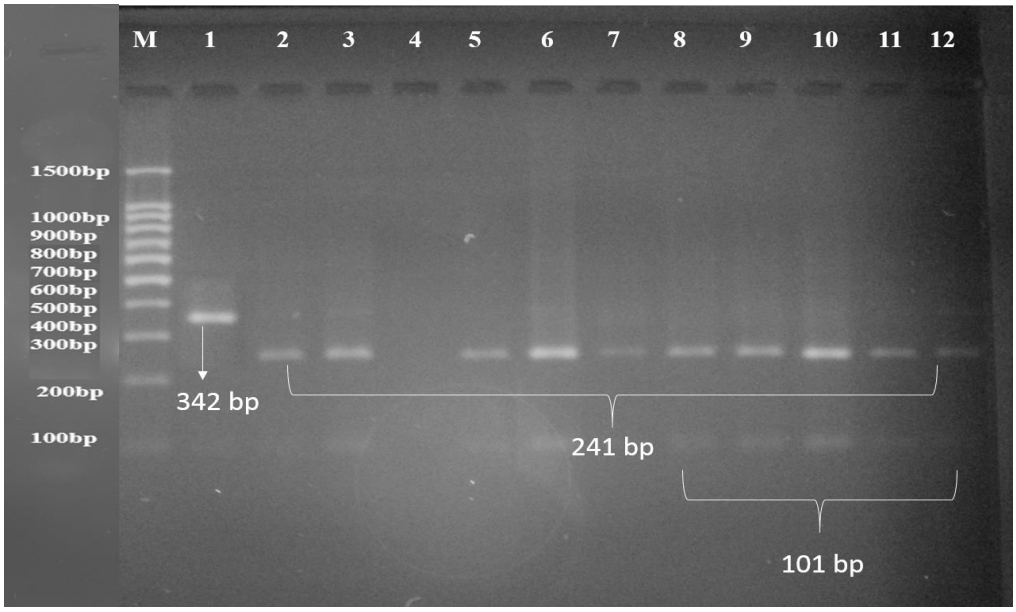

Figure (4): The digestion of PCR products (342) of GHR gene with *AluI* enzyme. The product was electrophoresis on 3% agarose gel at 5 volt/cm^2 for 1.5 hour, Visualized under U.V light after stain with Ethidium Bromide. Lane M DNA ladder (100-1000), Lane (1): the undigested PCR products of the GHR gene with size of 342bp, which represented recessive AA genotype, Lanes 2 to 12: the digested form which represented the dominant GG genotype.

These results supported by Andreas *et al*., (2010) and Othman *et al*., (2012), while Some studies showed the substitution at position 256 in cattle (Genbank accession number AY053546), from A to G. These changes caused a loss of enzyme recognition sites of *Alu*I, so that produced fragment of the length 298 bp, known as the AG genotype (Ge *et al*., 2000; Di Stasio *et al*., 2005). Genotype found in buffalo in this research was GG genotype (Figure4).

Genetic diversity based on molecular marker GH *Alu*I and GHR *Alu*I loci in buffalo were very low.

Dybus and Grzesiak (2006) showed that the GHRH *Hae*III and GH *Msp*I genes in buffalo from Banten Province were Polymorphic. Low diversity in buffalo can be caused by a 100-limited number of males in the population, and the high inbreeding frequency.

In conclusion, it can be concluded that the diversity of GH *Alu*I and GHR *Alu*I genes 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 and GG genotype for locus GHR *Alu*I.

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