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# Genotyping and Nucleotide Sequences of Growth Hormone Releasing Hormone and Its Receptor Genes in Egyptian Buffalo

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author OEO designed the study, followed up the practical work and wrote the final version of the manuscript. Author MFAS managed the analyses of the study, managed the literature searches and wrote the first draft of the manuscript. Author NAAEM performed the practical work. Author KMS followed up the steps of the search. All authors read and approved the final manuscript*

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## ABSTRACT

**Aim:** The hypothalamic hormone, growth hormone-releasing hormone, is the principal stimulator of pituitary growth hormone (*GH*) synthesis and secretion. *GHRH* and its receptor (*GHRHR*) provide important functions in the regulation of the *GH* axis and in the development and proliferation of pituitary somatotropic axis. This study aimed to identify the genotypes and nucleotide sequences of two multifunctional genes; growth hormone-releasing hormone (*GHRH*) and its receptor (*GHRHR*) in Egyptian buffalo.
**Methodology:** Genomic DNA was extracted from blood samples of 100 healthy buffaloes maintained at the Mahlet Mussa and El-Gmeasa herds from 2010 to 2012. PCR was performed using primers flanking a 296-bp fragment from *GHRH* gene and a 425-bp fragment from *GHRHR* gene of Egyptian buffalo. The PCR-amplified fragments were digested with *Hae*III (*GHRH*) and

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*Eco*57I (*GHRHR*), electrophoresed and analyzed on agarose gels stained with ethidium bromide. The two amplified fragments were also sequenced and aligned with published sequences.
**Results:** Depending on the presence of the restriction site at 241^242 position (GG^CC) in 296-bp amplified fragments of *GHRH,* we genotyped all tested buffalo animals as AA. Due to the absence of the restriction site at position 300^301 ([CTGAAG$(N)_{16}$^] in the amplified fragment of *GHRHR* (425-bp), we genotyped the tested animals as AA. The Egyptian buffalo *GHRH* and *GHRHR* nucleotide sequences were submitted to NCBI/Bankit/GenBank and have the accession numbers JN967799 and KC295414, respectively.
**Conclusion:** The Egyptian buffaloes are characterized by best production traits like high milk fat content as well as higher average daily gain and body weight where they are possess with fixed $GHRH^{AA}$ and $GHRHR^{AA}$ genotypes which were reported as desired genotypes for milk and growth production traits in different cattle breeds and the cattle are genetically homologous with buffaloes. To the best of our knowledge, these polymorphic sites are not identified in other buffalo populations. The identification of genotypes and nucleotide sequences of these two multifunctional genes may be useful in future marker-assisted selection (MAS) for more efficient breeding and genetic conservation programs of Egyptian buffalo.

*Keywords: Buffalo; GHRH; GHRHR; PCR; RFLP.*

## 1. INTRODUCTION

The great adaptive capacity of Egyptian buffaloes (*Bubalus bubalis*) to tropical climates and excellent nutritional efficiency, resistance to diseases, together with the good productive and reproductive potential make these animals one of the main sources for milk and meat in Egypt. The improvement of livestock productivity has been dependent on genetic markers that are associated with economically important productivity traits to promote more efficient selection through marker-assisted selection. Among the putative candidate markers, the genes which are related to the somatotropic axis [1,2,3,4].

The hypothalamic hormone, growth hormone-releasing hormone (*GHRH*), is the principal stimulator of pituitary growth hormone (*GH*) synthesis and secretion. Its pituitary receptor is well characterized as a member of the superfamily of G protein-coupled receptors [5]. *GHRH* and its receptor provide important functions in the regulation of the *GH* axis and in the development and proliferation of pituitary somatotropes [6].

The association between *GHRH* and an increased milk yield was confirmed by Hashizume et al. [7]. Baile and Buonomo [8] found that administering this hormone increased the metabolic activity of mammary gland cells. Furthermore, Zhao et al. [9] reported that administering *GHRH* had a significant effect on glucose transporter gene expression in the mammary gland, resulting in an increased milk yield. Studies of Lappiera et al. [10] proved that administering recombined human *GHRH* to cow resulted in an increased milk yield as well as protein and fat content in milk.

Other studies showed that somatotropes with their synthetic equivalents increased milk production in dairy cows [11] and meat cows [12] as well as improved cattle growth rate. Moreover, Ciampani et al. [13] confirmed the role of *GHRH* in the *FSH* secretion process and thus indirectly stimulates steroidogenesis in Leydig cells and the activity of *FSH* in Sertoli cells in males.

By now, genotypes of Egyptian buffalo *GHRH* and *GHRHR* were not reported, so this study aimed to identify the genotypes and nucleotide sequences of these two multifunctional genes in Egyptian buffalo.

## 2. MATERIALS AND METHODS

### 2.1 Genomic DNA Extraction

Genomic DNA was extracted from the whole blood of 100 unrelated Egyptian buffaloes - maintained at the Mahlet Mussa and El-Gmeasa herds from 2010 to 2012- according to established protocol [14] with minor modifications. Briefly, 10 ml of blood taken on EDTA were mixed with 25 ml of cold 2X Sucrose-Triton and 15 ml double distilled water. The tubes were placed on ice for 10 min and mixed by inversion several times. After centrifugation, at 5000 rpm for 15 min at 4ºC, the pellet was re-suspended by 3 ml of nucleic lysis buffer. The content was mixed with 108 µl of 20% SDS and

150 µl of Proteinase K. The tubes were placed in a water bath at 37ºC overnight.

After the incubation, the tube contents were transferred to a 15-ml polypropylene tube and 2 ml of saturated NaCl was added and shaken vigorously for 15 sec. After centrifuging at 3500 rpm for 15 min at 4ºC, the supernatant was transferred to a clean 15-ml polypropylene tube and mixed with absolute ethanol. The tubes were agitated gently to mix the liquids and a fluffy white ball of DNA was formed. The precipitated DNA was picked up using the heat sealed pasture pipette, then washed twice in 70% ethanol and exposed to air to dry completely.

The DNA was dissolved in 200 µl TE buffer in 1.5-ml Microfuge tube and kept overnight in an incubator at 37ºC. DNA concentration was determined and diluted to the working concentration of 50 ng/µl, which is suitable for polymerase chain reaction using NanoDrop1000 Thermo Scientific spectrophotometer.

## 2.2 Polymerase Chain Reaction (PCR)

A PCR cocktail consisted of 1.0 µM upper and lower primers (specific for each tested gene (Table 1), 0.2 mM dNTPs and 1.25 units of Taq polymerase. The cocktail was aliquot into PCR tubes with 100 ng of buffalo DNA. The reaction was cycled for 1 min. at 94℃, 1 min at an optimized annealing temperature that was determined for each primer (Table 1) and 1 min. at 72℃ for 30 cycles. The PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide to test the amplification success.

## 2.3 Restriction Fragment Length Polymorphism (RFLP)

The PCR products for the two tested gene were digested with specific restriction enzyme for each gene (Table 1). The restriction mixture for each sample was prepared by adding 2.5 µl of 10×restriction buffer to 10 units of the appropriate restriction enzyme and the volume was completed to 5 µl by sterile water. This restriction mixture was mixed with PCR product (~25 µl) and incubated overnight at the optimum temperature of the maximum activity for each restriction enzyme. The digested PCR products were electrophoresed on a 3% agarose gel staining with ethidium bromide to detect the different genotypes of the two tested genes.

## 2.4 Sequence Analysis

The PCR products of each tested gene were purified and sequenced by Macrogen Incorporation (Seoul, Korea). Sequence analysis and alignment were carried out using NCBI/BLAST/blastn suite. Results of endouclease restriction were carried out using FastPCR [15]. The nucleotide sequences of the two tested genes in Egyptian buffalo were submitted to GenBank (NCBI, BankIt).

# 3. RESULTS AND DISCUSSSION

## 3.1 *GHRH* Gene

Growth hormone releasing hormone (*GHRH*) is a hypothalamic hormone which stimulates both synthesis and secretion of pituitary growth hormone (*GH*) binds to specific receptors on somatotrophs [17]. Bovine *GHRH* increased the serum concentration of endogenous *GH* [18] and increased milk production [10].

**Table 1. The sequences and information of primers used in this study**

| Gene | Primer sequence 5′ -------- 3′ | PCR conditions (30 cycles) | PCR product size | Restriction enzyme used | References |
|---|---|---|---|---|---|
| *GHRH* | TTC CCA AGC CTC TCA GGT AA<br>GCG TAC CGT GGA ATC CTA GT | 94°C 1 min<br>60°C 1 min<br>72°C 1 min | 296 bp | *Hae*III | [3] |
| *GHRHR* | ACG CCA CCC TCT TTC ACC AG<br>CAT CCT GGG TGC TTC TTG AAG | 94°C 1 min<br>55°C 1 min<br>72°C 1 min | 425 bp | *Eco*57I | [16] |

*GHRH* gene was linked to CSSM30 on bovine chromosome 13 [19] and consists of five exons separated by four introns [20].

The primers used in this study flanked a 296-bp fragment consisting of 14 base pairs from exon 2, 265 base pairs from intron 2 and 17 base pairs from exon 3 of Egyptian buffalo *GHRH* gene. The amplified fragments obtained from all tested buffalo DNA (100 animals) gave the expected fragment at 296-bp (Fig. 1)

Two-way sequence analysis of the *GHRH* amplified PCR product of buffalo DNA was conducted, using both the forward and reverse primers. The buffalo amplicon obtained was found to be 296-bp (Fig. 2). The Egyptian buffalo *GHRH* nucleotide sequence was submitted to nucleotide sequences database NCBI/ Bankit/GenBank and has the accession number JN967799.

The sequence alignment of Egyptian buffalo *GHRH* with published sequence (accession number: DQ064594; *Bubalus bubalis*) was carried out using BLAST and showed that the Egyptian buffalo possess identities at 99% with only one gap between positions 259 and 260 and one SNP (T/C) at position 285 of our sequence (Fig. 3).

These PCR amplified fragments (296-bp) were digested with *Hae*III endonuclease. Depending on the presence or absence of the restriction site at 241^242 position (GG^CC) in these amplified fragments, we can easily differentiate between 3 different genotypes: AA with two digested fragments at 241-and 55-bp, BB with three digested fragments at 193-, 55-and 48-bp and AB with four digested fragments at 241-, 193-, 55- and 48-bp.

All buffalo animals investigated in this study are genotyped as **AA** where all tested buffalo DNA amplified fragments were digested with *Hae*III endonuclease and gave two digested fragments at 241- and 55-bp (Fig. 4) due to the presence of the restriction site at position 241^242 (GG^CC) (Fig. 5).

Dybus and Grzesiak [21] evaluated the relationship between the polymorphism of the *GHRH* and milk production traits of Polish Black-and-White. A PCR-RFLP method was used for its genotyping. The frequencies of the genotypes and alleles were as follows: 0.0545 for AA, 0.3133 for AB and 0.6322 for BB, and 0.2111 for $GHRH^A$ and 0.7889 for $GHRH^B$. There were no significant associations between *GHRH/Hae*III polymorphism and milk production traits of the analyzed cows.

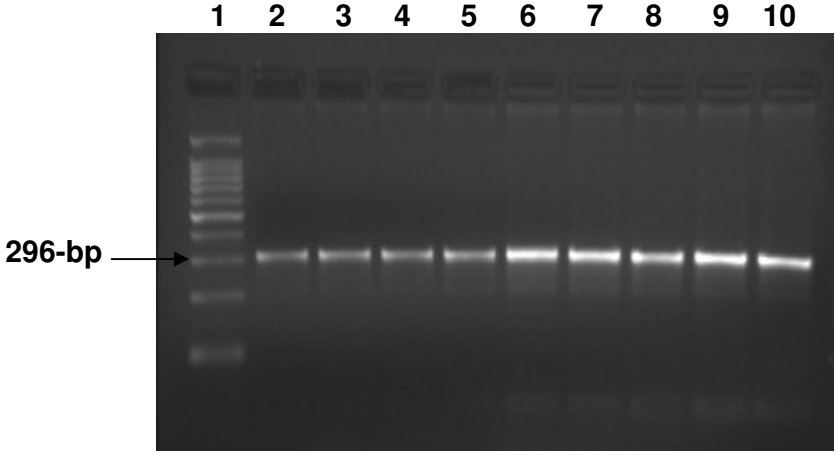


**Fig. 1. Ethidium bromide-stained gel of PCR products representing amplification of *GHRH* gene in Egyptian buffalo**

*Lane 1: 100-bp ladder marker*

*Lanes 2-10: 296-bp PCR products amplified from Egyptian buffalo DNA*

**TCCCAGCCTCTCAGGTAA**GCAGTTCTGACAAGAGAAGCAAGCGAGGCACTTTGAGGATG
CAGACTCGAGCTGGTCCCCAGCTGGCTCCTCAGGCAGCCTCCCTTGCTCATCTCTGGGA
GGGAGGCAGACTAAGCCCCAGAGAGGTCACCACCCAGCCCTGGTTCCAGCCCTCTCTGG
GGACGAGCAGGGCAAGAGGCGACAGAAAGACCTCACAGAGACCAAGTGAGCACAGTCCC
CTGGGCCTCCCACCCCACCCTTTGACCTCTGACTCCTTCT**ACTAGGATTCCACGGTACGC**

**Fig. 2. The nucleotide sequence of Egyptian buffalo *GHRH* amplified fragment. Forward and reverse primers with bold**

```
Query 1   TCCCAGCCTCTCAGGTAAGCAGTTCTGACAAGAGAAGCAAGCGAGGCACTTTGAGGATGC  60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 73  TCCCAGCCTCTCAGGTAAGCAGTTCTGACAAGAGAAGCAAGCGAGGCACTTTGAGGATGC  132

Query 61   AGACTCGAGCTGGTCCCCAGCTGGCTCCTCAGGCAGCCTCCCTTGCTCATCTCTGGGAGG  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 133  AGACTCGAGCTGGTCCCCAGCTGGCTCCTCAGGCAGCCTCCCTTGCTCATCTCTGGGAGG  192

Query 121  GAGGCAGACTAAGCCCCAGAGAGGTCACCACCCAGCCCTGGTTCCAGCCCTCTCTGGGGA  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 193  GAGGCAGACTAAGCCCCAGAGAGGTCACCACCCAGCCCTGGTTCCAGCCCTCTCTGGGGA  252

Query 181  CGAGCAGGGCAAGAGGCGACAGAAAGACCTCACAGAGACCAAGTGAGCACAGTCCCCTGG  240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 253  CGAGCAGGGCAAGAGGCGACAGAAAGACCTCACAGAGACCAAGTGAGCACAGTCCCCTGG  312

Query 241  GCCTCCCACCCCACCCTTT-GACCTCTGACTCCTTCTACTAGGATTCCACGGTACGC  296
           |||||||||||||||||| |||||||||||||||||||||| ||||||||||||
Sbjct 313  GCCTCCCACCCCACCCTTTTGACCTCTGACTCCTTCTACTAGGATCCCACGGTACGC  369
```

**Fig. 3. Sequence alignment of Egyptian buffalo *GHRH* with published sequence. (-/T) gap and (T/C) single nucleotide polymorphism with bold**

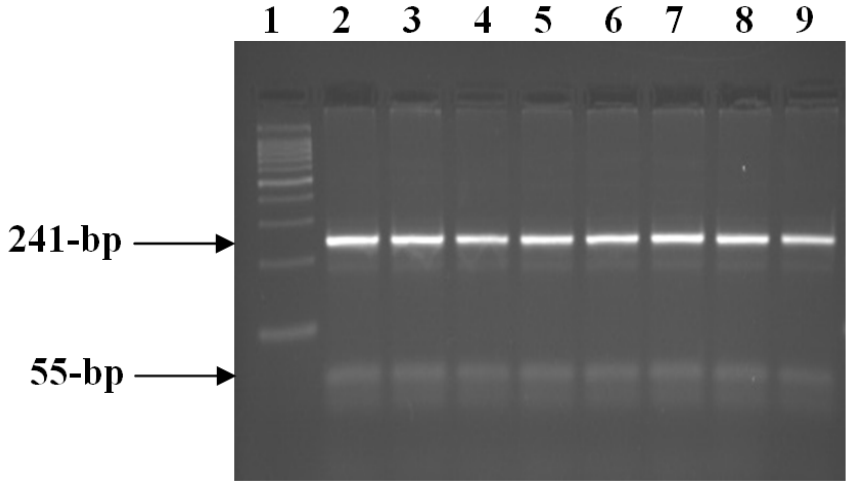


**Fig. 4. The electrophoretic pattern obtained after digestion of PCR amplified buffalo *GHRH* with *Hae*III restriction enzyme**
*Lane 1: 100-bp ladder marker*
*Lanes 2-9: Homozygous AA genotypes showed two restricted fragments at 241- and 55-bp*

TCCCAGCCTCTCAGGTAAGCAGTTCTGACAAGAGAAGCAAGCGAGGCACTTTGAGGATGC
AGACTCGAGCTGGTCCCCAGCTGGCTCCTCAGGCAGCCTCCCTTGCTCATCTCTGGGAG
GGAGGCAGACTAAGCCCCAGAGAGGTCACCACCCAGCCCTGGTTCCAGCCCTCTCTGGG
GACGAGCAGGGCAAGAGGCGACAGAAAGACCTCACAGAGACCAAGTGAGCACAGTCCCC
TG**GG^CC**TCCCACCCCACCCTTTGACCTCTGACTCCTTCTACTAGGATTCCACGGTACGC

**Fig. 5. Endonuclease restriction of Egyptian buffalo *GHRH* using FastPCR GG^CC restriction site with bold**

By direct DNA sequencing in 24 unrelated Korean cattle, Cheong et al. [22] identified 12 single nucleotide polymorphisms within *GHRH* gene. Among them, six polymorphic sites were selected for genotyping in beef cattle and five marker haplotypes were identified. Statistical analysis revealed that -4241A>T showed significant associations with cold carcass weight (CW) and longissimus muscle area (EMA).

Also the polymorphism of cattle *GHRH* gene using PCR-RFLP technique with *Hae*III restriction enzyme was studied by Kmiec et al. [23]. They detected two alleles $GHRH^A$ with frequency of 28.1% and $GHRH^B$ with frequency of 71.9%. This study proved the existence of *GHRH*/*Hae*III polymorphism in the selected gene sequence and revealed statistically higher values for the analyzed milk production traits in cows with $GHRH^A/GHRH^A$ genotype.

Szatkowska et al. [24] analyzed the association between the *GHRH/HaeIII* gene polymorphism with milk production traits of Polish Holstein and Jersey cows. The frequencies of genotypes and alleles for the Polish Holstein cows were 0.078 for AA, 0.339 for AB and 0.583 for BB. In all lactations, the Jersey cows with AA genotype exhibited the highest milk fat content. In the 2nd and 3rd lactations the AA Jersey cows had lower milk yields compared with the AB or BB cows.

The association of the *GHRH* gene with growth traits in Chinese native cattle was investigated by Zhang et al. [25]. PCR-SSCP and sequencing were used to detect mutations of the *GHRH* gene. One novel mutation 4251nt (C > T) was found and the frequencies of C allele were 0.8778 and 0.8476 for Qinchuan and Nanyang cattle, respectively. Body weight with the CT genotype was significantly higher than those with CC genotype in Nanyang cattle.

## 3.2 GHRHR Gene

The hypothalamic hormone, growth hormone-releasing hormone (*GHRH*), is the principal stimulator of pituitary growth hormone (*GH*) synthesis and secretion. Its pituitary receptor is well characterized as a member of the superfamily of G protein-coupled receptors [5]. *GHRH* and its receptor provide important functions in the regulation of the *GH* axis and in the development and proliferation of pituitary somatotropes [6].

The primers used in this study flanked a 425-bp fragment consisting of 50-bp from exon 6 and 375-bp from intron 6 of Egyptian buffalo *GHRHR* gene. The amplified fragments obtained from all tested buffalo DNA (100 animals) at 425-bp (Fig. 6).

Two-way sequence analysis of the *GHRHR* amplified PCR product of buffalo DNA was conducted, using both the forward and reverse primers. The buffalo amplicon obtained was found to be 425-bp (Fig. 7). The Egyptian buffalo *GHRHR* nucleotide sequence was submitted to nucleotide sequences database NCBI/ Bankit/GenBank and has the accession number KC295414.

The sequence alignment of Egyptian buffalo *GHRHR* with published sequence (accession number: EF600712.1; *Bubalus bubalis*) was carried out using BLAST and showed that the Egyptian buffalo possess identities at 100% with published sequence without any SNP in this shared fragment (Fig. 8).

These PCR amplified fragments (425-bp) were digested with *Eco*57I endonuclease. Depending on the presence or absence of the restriction site at position 300^301 ([CTGAAG$(N)_{16}$^], we can easily differentiate between 3 different genotypes: AA with undigested one fragment at 425-bp, BB with two digested fragments at 300- and 125-bp and AB with three digested fragments at 425-, 300- and 125-bp.

All buffalo animals investigated in this study are genotyped as **AA** where all tested buffalo DNA amplified fragments were treated with *Eco*57I endonuclease and gave one undigested fragment at 425-bp (Fig. 9) due to the absence of the restriction site at position 300^301 ([CTGAAG$(N)_{16}$^].

A RFLP was identified within a PCR amplification product of the bovine growth hormone releasing hormone receptor (*GHRHR*) gene using the restriction endonuclease *Eco57*I [16]. Digestion of the 425-bp product with *Eco*57I revealed a polymorphism with two alleles characterized by an uncut band of 425 bp (Allele A) and two cut bands of 125 and 300 bp (Allele B). Frequency of the A allele was 0.15 in the MARC (Meat Animal Research Center) reference families.

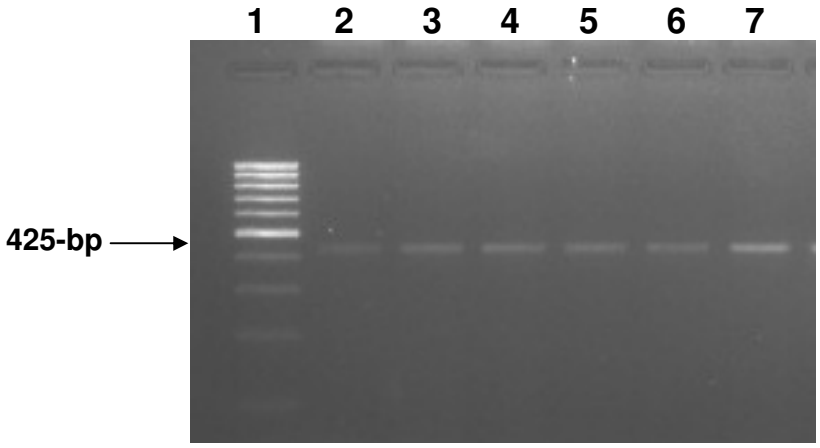


**Fig. 6. Ethidium bromide-stained gel of PCR products representing amplification of *GHRHR* gene in Egyptian buffalo**

*Lane 1: 100-bp ladder marker*

*Lanes 2-7: 425-bp PCR products amplified from Egyptian buffalo DNA*

**ACGCCACCCTCTTTCACCGGGAGAACACGGA**CCACTGCAGCTTCTCCACTGTAACAGTCA
TGGGTGGGGGTGCTGGTGCGGGCGAGGAGGTTGGATTAGAGATGTCAGCCTGTCCAGTCC
AGTGGGCTGACCCCGGGGCTCTGGCTTTGCCAAGGACAGAGCTGGAAAGCCCCCCTCCCC
CTTCCCGCCCCTCCTTGGGGTCAAGTCCTAAATCCTCCTGTGCCCAGCCCCGTCATTCCCT
GACTCCACTCTCTGCTCCATGTTCTGTATTCTGGTTTCATTCCCAGCCTGTAGCCCAGCCCA
GAGCACACTTCACTCCACTCTTGCTTCCATCTCAAACTTCCTCTGGGCTCTGTCTCTGCTGG
GTGTGGGTGTACCAGGCACTGGACAAAGCCAGGTCT**CTTCTTCAAGAAGCACCCAGGATG**

**Fig. 7. The nucleotide sequence of Egyptian buffalo *GHRHR* amplified fragment. Forward and reverse primers with bold**

```
Query  1     ACGCCACCCTCTTTCACCGGGAGAACACGGACCACTGCAGCTTCTCCACTGTAACAGTCA  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7647  ACGCCACCCTCTTTCACCGGGAGAACACGGACCACTGCAGCTTCTCCACTGTAACAGTCA  7706

Query  61    TGGGTGGGGGTGCTGGTGCGGGCGAGGAGGTTGGATTAGAGATGTCAGCCTGTCCAGTCC  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7707  TGGGTGGGGGTGCTGGTGCGGGCGAGGAGGTTGGATTAGAGATGTCAGCCTGTCCAGTCC  7766

Query  121   AGTGGGCTGACCCCGGGGCTCTGGCTTTGCCAAGGACAGAGCTGGAAAGCCCCCCTCCCC  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7767  AGTGGGCTGACCCCGGGGCTCTGGCTTTGCCAAGGACAGAGCTGGAAAGCCCCCCTCCCC  7826

Query  181   CTTCCCGCCCCTCCTTGGGGTCAAGTCCTAAATCCTCCTGTGCCCAGCCCCGTCATTCCC  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7827  CTTCCCGCCCCTCCTTGGGGTCAAGTCCTAAATCCTCCTGTGCCCAGCCCCGTCATTCCC  7886

Query  241   TGACTCCACTCTCTGCTCCATGTTCTGTATTCTGGTTTCATTCCCAGCCTGTAGCCCAGC  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7887  TGACTCCACTCTCTGCTCCATGTTCTGTATTCTGGTTTCATTCCCAGCCTGTAGCCCAGC  7946

Query  301   CCAGAGCACACTTCACTCCACTCTTGCTTCCATCTCAAACTTCCTCTGGGCTCTGTCTCT  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7947  CCAGAGCACACTTCACTCCACTCTTGCTTCCATCTCAAACTTCCTCTGGGCTCTGTCTCT  8006

Query  361   GCTGGGTGTGGGTGTACCAGGCACTGGACAAAGCCAGGTCTCTTCTTCAAGAAGCACCCA  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  8007  GCTGGGTGTGGGTGTACCAGGCACTGGACAAAGCCAGGTCTCTTCTTCAAGAAGCACCCA  8066

Query  421   GGATG  425
             |||||
Sbjct  8067  GGATG  8071
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**Fig. 8. Sequence alignment of Egyptian buffalo *GHRHR* with published sequence**

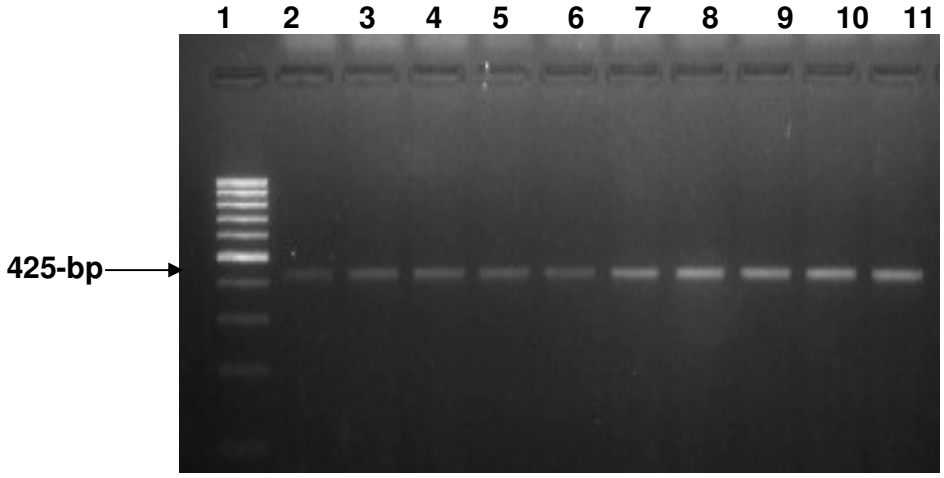


**Fig. 9. The electrophoretic pattern obtained after digestion of PCR amplified buffalo *GHRHR* with *Eco*57I restriction enzyme**

*Lane 1: 100-bp ladder marker*

*Lanes 2-11: Homozygous AA genotypes showed one undigested fragment at 425-bp*

Zhang et al. [26] screened the 5′ flanking region, the coding region and partially introns of *GHRHR* to detect the SNPs in the predominant cattle breeds of China. The genotypes were named AA, AB and BB. Fixed effects of genotype and age were included as independent variables in the linear model. The result indicated that three linked mutations in *GHRHR* gene were significantly associated with body weight of 12 months and average daily gain of 12 months ($P<0.05$). The individuals with genotype AA had higher average daily gain and body weight than individuals with genotype AB. While the differences between the individuals with genotype BB and the individuals with genotype AA and AB were not significant. So, three linked mutations in *GHRHR* gene have effect on growth traits in bovine. This result proved the *GHRHR* gene as an important candidate gene controlling growth performance and carcass traits in farm animals.

## 4. CONCLUSION

It is concluded that the Egyptian buffaloes are characterized by best production traits like high milk fat content as well as higher average daily gain and body weight where they are possess with fixed $GHRH^{AA}$ and $GHRHR^{AA}$ genotypes which were reported as desired genotypes for milk and growth production traits in different cattle breeds and the cattle are genetically homologous with buffaloes. To the best of our knowledge, these polymorphic sites are not identified in other buffalo populations. The identification of genotypes and nucleotide sequences of these two multifunctional genes may be useful in future marker-assisted selection (MAS) for more efficient breeding and genetic conservation programs of Egyptian buffalo.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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