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RELATEDNESS BETWEEN GROWTH HORMONE GENE POLYMORPHISMS AND BODY WEIGHT IN FRIESIAN CATTLE IN EGYPT

BY

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ABSTRACT

Association was analyzed between GH gene polymorphisms and body weight in fifty Friesian bull calves reared under Egyptian conditions. DNA from blood was extracted to amplify 223-bp of the gene encoding GH. PCR-RFLP-AluI of 223-bp revealed digested (171, 52-bp) fragment for genotype AluI+/ AluI+, three fragments (223, 171, 52-b.p) for genotype AluI+/ AluI-, and undigested fragment (223 b.p) for genotype AluI-/ AluI-. Using PCR-RFLP method, the incidence of growth hormone genotypes and frequencies of alleles were calculated. Results indicated that the frequencies of AluI+/ AluI+, AluI+/ AluI- and AluI-/ AluI- genotypes in 50 animals of Friesian bull calves were 0.4, 0.56 and 0.04, respectively and frequencies of the AluI+ and AluI-alleles were 0.68 and 0.32. Statistical analysis indicated that there was no significant association between body weight and growth hormone genotypes. However, AluI+/ AluI- genotype was higher in body weight than both AluI+/ AluI+ and AluI-/ AluI-genotypes.

Keywords: Friesian cattle, GH gene, Body weight, PCR-RFLP.

INTRODUCTION

Genetic polymorphisms of candidate genes affecting economic traits have stimulated substantial research interest, because of their impending utilization as an aid to genetic selection and to demarcate evolutionary relationships in different livestock breeds. So far, genetic polymorphism at candidate genes has been extensively explored in cattle breeds

(Sodhi *et al.*, 2007). The primary thrust of research in animal genetics is the identification of genes, which affect the expression of quantitative traits markedly. One of the potential major genes is that of growth hormone gene (*GH*). Growth hormone gene is 2,206 base pair (bp) and consists of five exons separated by four intervening sequences **(Hediger *et al.*, 1990)**. There is extensive literature on the possible relationship(s) between genetic polymorphism of *GH* and production and reproduction traits in cattle. The growth hormone (*GH*) gene is a candidate gene for body weight and weight gain in cattle since it plays a fundamental role in growth regulation. The GH protein is a single-chain polypeptide consisting of 191 amino acids and is synthesized and secreted by the anterior pituitary gland under the hypothalamic control of two hormones, GH-releasing hormone (GHRH), which increases the secretion of GH, and somatotropin release-inhibiting factor (SRIF, also called somatostatin) which inhibits its secretion **(Nicoll *et al.*, 1986)**. It is known that GH is the main regulator of postnatal somatic growth, stimulating anabolic processes such as cell division, skeletal growth and protein synthesis and is involved in nutrient partition by way of regulating the oxidation rate of fats (lipolytic activity), inhibition of glucose transport to peripheral tissues and the regulation of ribosomal activity involved in translation, which, in turn, influences protein synthesis **(Goodman, 1993)**.

The objectives of the present study to reveal GH gene polymorphisms in Friesian bull calves reared under Egyptian conditions, looking for association between body weight performance and GH Polymorphisms using PCR-RFLPs.

MATERIALS AND METHODS

I- Animals:

In the present study, fifty Friesian bull calves were precisely obtained from a private farm at Gamasa, Dakhlia governorate. Based on the farm record, the obtained animals were weaned at about 90 Kg body weight, have their birth weight ranged from 31-38 Kg and their weaning age ranged from 60-100 days.

II. Adjustment or correction of non genetic factors:

The Calf weaning weight was adjusted to 205 days of age by linear interpolation from birth weight, weaning weight and age. The calculation of 205-day using equations described by **Szabo et al., 2012**

$$A = \frac{(B-C)}{D} \times 205 + C$$

Where *A* is 205 days weight (kg), *B* is the weaning weight (kg), *C* is the birth-weight (kg), *D* is the weaning age (days).

III. Calculation of breeding value:

Breeding value was calculated to rank the used animals according to their excellence in body weight and daily gain using the equations described by **Falconer and Mackay 1996.** $BV = X^{-} + h^2 (X - X^{-})$ where BV= breeding value, X= average of weaning weight, h^2 = heritability for weaning weight (0 .4)**,** X^{-} = corrected 205 day weight for animal.

IV- Experimental samples:

Fifty blood samples from Friesian bull calves reared under Egyptian conditions were collected by Jugular vein puncture into tubes containing anticoagulant disodium EDTA. The samples were stored at -20°C until needed for DNA extraction.

V- Experimental protocol:

I-Extraction of genomic DNA (John et al., 1991):

The genomic DNA was extracted using Gene JET genomic DNA extraction kit following the manufacturer protocol **(Fermentas, #K0721/USA).**

2- Polymerase chain reaction (PCR):

PCR was done for amplification of fragment of growth hormone gene spanning over fourth quarter of 4th intron (49- bp) and almost whole of the fifth exon except last triplet codon (174-bp) with expected amplicon size of 223-bp using the following primers described by **(Biswas et al., 2004).**

Forward: 5′-GCTGCTCCTGAGGGCCCTTCG-3′

Reverse: 5-′GCGGCGGCACTTCATGACCCT-3

The polymerase chain reaction mixture was carried out in a 50 µl consisted of: 3µl DNA, 21 µl H2o (d.d water), 25 µl PCR master mix (**Jena Bioscience, Germany**), 0.5 µl Primer forward and 0.5 µl Primer reverse. The final reaction mixture was placed in a thermal cycler and the PCR program was carried out by initial denaturation at 94 $C^0$for 5 min followed by 34 cycles of 94 C^0 for 1 min for DNA denaturation, annealing temperature at 67

C^0 for 1 min, extension at 72 C^0 for 1 min and final extension at 72 C^0 for 10 min. The samples were held at 4 C^0. Representative results of PCR analysis detected by agarose gel electrophoresis then the fragment patterns were visualized under U.V using gel documentation system.

*3-**Restriction fragment length polymorphism (RFLP):***

The amplified DNA fragment of GH gene were digested with restriction enzyme AluI (**Fermentas, England**) at 37 C^0 for 5 min. The cleaved fragments were detected by agarose gel electrophoresis then the fragment patterns were visualized under U.V in gel documentation system.

4- Statistical analysis:

Data analyzed by using (**SPSS PC$^+$ 1994**) to determine association between body weight and GH polymorphisms (genotypes).

Results and Discussion

1- PCR amplification using specific primers for GH gene:

The genomic DNA from fifty Friesian bull calves (High and low body weight was extracted to amplify GH gene. In all samples, PCR amplification of GH gene yielded a fragment of 223-bp as shown in Figure (1).

2- Genotyping of GH gene (223-bp) using RFLP technique:

Restriction analysis of 223-bp PCR products digested with AluI revealed that, two fragments (171, 52 b.p) fragment for genotype AluI+/ AluI+, three fragments (223, 171, 52 b.p) for genotype AluI+/ AluI-, and undigested fragment (223 b.p) for genotype AluI-/ AluI- as shown in figure (2). Using PCR-RFLP method, the incidence of growth hormone genotypes and frequencies of alleles were calculated. Results indicated that the frequencies of AluI+/ AluI+, AluI+/ AluI- and AluI-/ AluI- genotypes in 50 animals of Friesian bull calves were 0.4, 0.56 and 0.04, respectively and frequencies of the AluI+ and AluI-alleles were 0.68 and 0.32 (Table 1). Statistical analysis indicated that there was no significant association between body weight and growth hormone genotypes. However, AluI+/ AluI- genotype was higher in body weight than both AluI+/ AluI+ and AluI-/ AluI-genotypes (Table 2).

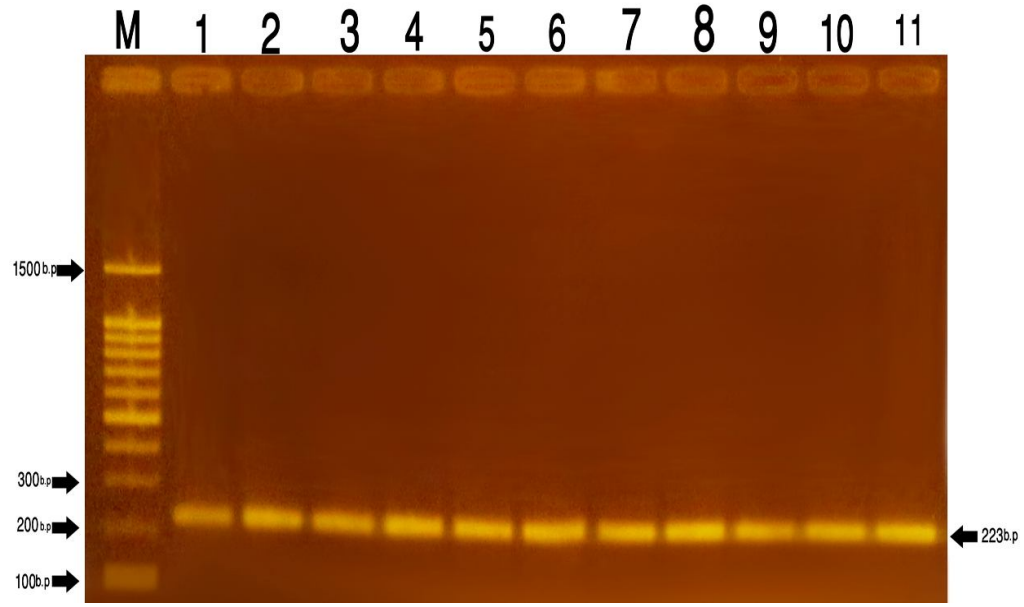

Figure (1): PCR amplification of GH gene (223-bp). Where, lane M is DNA marker and lanes 1----- 11 are Friesian bull calves (as an example).

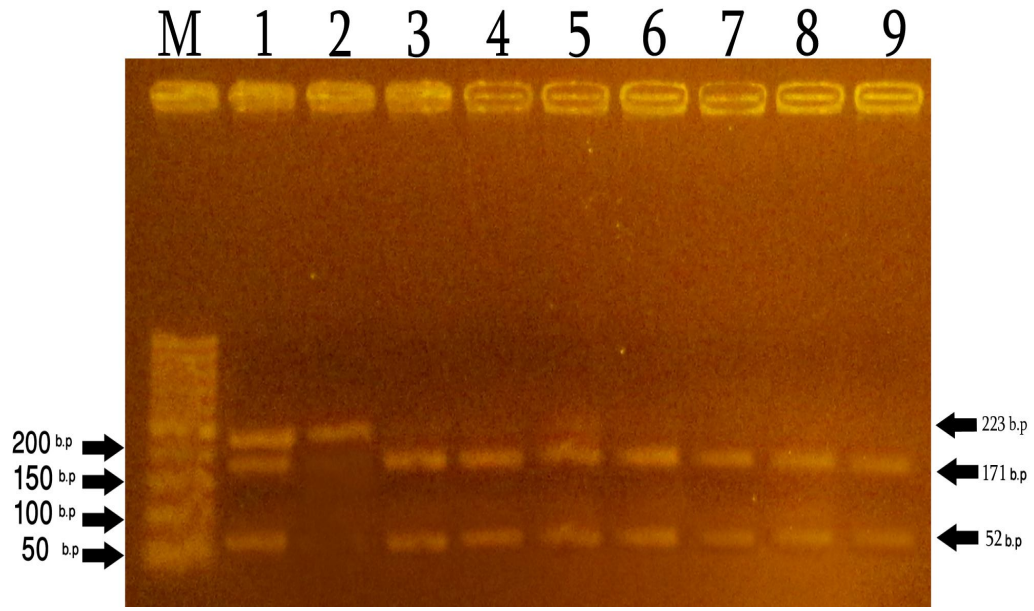

Figure (2): Representative AluI restriction fragment pattern of growth hormone gene (223-bp). Lane (1) = AluI+/ AluI- genotype (223, 171 and 52-bp bands). Lane (2) = AluI-/ AluI- genotype (band of 223-bp). Lane (3, 4, 5, 6, 7, 8, 9) = genotype AluI+/ AluI+ (bands of 171 and 52-bp). Molecular size marker (50-bp ladder was loaded in lane M).

Table (1): Frequency of genotypes and alleles in the growth hormone locus.

Cattle breed	No.	Number/frequency of genotypes			Allele frequency	
		AluI+/ AluI+	AluI+/ AluI-	AluI-/ AluI-	AluI+	AluI-
Friesian	50	20/0.4	28/0.56	2/0.04	0.68	0.32

Table (2): Associations of growth hormone genotypes with corrected body weight.

Genotype	M ± SE of corrected body weight
AluI+/ AluI+	168.01±3.39
AluI+/ AluI-	173.15±1.67
AluI-/ AluI-	165.92±8.81

The fragments obtained in this study were similar to those revealed by **Biswas et al., (2004).**

The frequencies of AluI+/ AluI+, AluI+/ AluI- and AluI-/ AluI- genotypes were 0.4, 0.56 and 0.04, respectively and frequencies of the AluI+ and AluI-alleles were 0.68 and 0.32. Similar frequencies of alleles AluI+ and AluI-at RFLP-AluI in the bovine GH gene were found by **Grochowska et al., (2001)** who studied association between gene polymorphism of growth hormone and carcass traits in dairy bulls, the frequencies of the AluI+ and AluI-alleles were 0·68 and 0·32, respectively, and by **Oprazadek el al., (2005)** who indicated polymorphism of GH gene and growth rates, feed intake and utilization, slaughter indicators and meet quality in cattle. The amplified DNA was digested with *AluI* restriction nuclease. Results indicated that allele frequencies at the studied *loci* were: 0.68/0.32 for GH (AluI+, AluI-) variants. However, as reported by **Reis et al., (2001)** who analyzed growth hormone *AluI* polymorphism in eight Portuguese bovine breeds. The overall gene frequencies for AluI+ and AluI- were 0.78 and 0.22, respectively. The obtained results disagree with results of **MA et al., (2007)** who indicated Polymorphism of the growth hormone gene and its association with growth traits in Ongole grade crossed with Simmental Cattle, the frequencies of AluI+ and AluI-alleles were 0.82 and 0.18, respectively.

The association between RFLP-AluI of the GH gene and growth traits were studied in a group of 50 Friesian bull calves. Statistical analysis indicated that there was no significant association between body weight and growth hormone genotypes. However, AluI+/ AluI-genotype was higher in body weight than both AluI+/ AluI+ and AluI-/ AluI-genotypes.. These results agree with those of **Reis et al., (2001)** who analyzed growth hormone *AluI*

polymorphism in eight Portuguese bovine breeds. According to results, the genotype AluI+/ AluI- is positively associated with higher weights at latter stages of growth and by **Biswas et. al., (2004)** who reported growth hormone gene polymorphism and its effect on birth weight in cattle and buffalo. AluI+/ AluI- genotype had higher birth weight than other genotypes. Hence, AluI+/ AluI- genotype in Holstein Friesian favored higher birth weight. The results of this study agree also with those of **Oprazadek et al., (2005)** who indicated polymorphism of GH gene and growth rates, feed intake and utilization, slaughter indicators and meet quality in cattle. The AluI+/ AluI- heterozygotes were heaviest and consumed most.

In another study on RFLP-AluI of the GH gene and growth traits, **Pereira et al., (2005)** indicated association of GH polymorphisms with growth traits in a synthetic beef cattle breed. Results indicated that two alleles for the *GH* locus, leucine (L) and valine(V). Significant effects were found for GH genotype on YW ($p \leq 0.05$), with positive effects associated with the GH (leucine/valine) genotype.

Also **MA et al., (2007)** indicated Polymorphism of the growth hormone gene and its association with growth traits in Ongole grade crossed with Simmental Cattle, the average birth weight, 2 months body weight and daily body weight gains of AluI+/ AluI- genotype were tend to higher than that of AluI+/ AluI+ genotypes. **Silveira et al., (2008)** reported growth hormone 1 gene (GH1) polymorphisms as possible markers for the production potential of beef cattle using the Brazilian Canchim breed as a model. The *Alu*I genotypes showed no significant differences ($p > 0.05$).

The results of this study disagree with the results of **Hartatik et al., (2011)** who studied relatedness between polymorphism of growth hormone gene and growth traits of Limousin Cross Madura cattle**.** Results indicated that, the polymorphism GH gene does not affect the growth traits. **NiuZhiGang (2011)** reported GH Gene Polymorphism with Early Growth Traits in Xinjiang Brown Cattle. The frequencies of AluI+/ AluI-+, AluI+/ AluI- and AluI-/ AluI- genotypes were 0.138, 0.397and 0.465. The differences in the results may be due to differences in breeds and location where this study was done on 50 animals of Friesian bull calves reared under Egyptian condition. The AluI+/ AluI-+ genotype showed largest growth rate in the growth hormone/Alu I sites. It was significantly higher than the AluI+/ AluI- and AluI-/ AluI- genotypes.

In conclusion, PCR-AluI digestion of 223-bp of growth hormone give three genotypes (AluI+/ AluI-+, AluI+/ AluI- and AluI-/ AluI-), there was no significant association between body weight and growth hormone genotypes. However, AluI+/ AluI- genotype was higher in body weight than GG and HH genotypes and can be selected for high body weight.

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الملخص العربى

العلاقة بين الاختلافات فى جين النمو ووزن الجسم فى ابقار الفريزيان فى مصر

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استخدم في هذه التجربة ٥٠ من عجول الفريزيان تم الحصول عليهم من احد المزارع ألخاصة وتم اجراء تفاعل انزيم البلمرة المتسلسل لتكبير ٢٢٣ زوج قاعدى من جين النمو وتم الحصول على حزمه حجمها ٢٢٣ زوج قاعدى فى كل العجول محل الدراسة.

أما بالنسبة للتفريق بين العجول تم استخدام طريقة اختلاف أطوال تقطيع الشريط الوراثي (RFLP)عن طريق استخدام إنزيم القطع (AluI) مما نتج عنه هضم الحزمة(223) زوج قاعدي إلى حزمتين وهما (52ـ171) زوج قاعدي في بعض من هذه العجول وهذه العجول أخـــذت التركيب الوراثي (GG) وفى نوع آخر من العجول ظهرت ثلاثة حزم وهى (52، 171، 223) زوج قاعدي وهذه العجول أخذت التركيب الوراثي (GH)، وعجول أخرى لم يتم هضم الحزمة 223 زوج قاعدي وهذه العجول أخذت التركيب الوراثي (HH) ومن خلال التحليل الاحصائى وجد ليس هناك فروق معنوية بين وزن الجسم والتراكيب الوراثية لهرمون النمووولكن العجول ذات التركيب الوراثى (GH) كان لها وزن عالي عن كل من العجول ذات التركيب الوراثي (GG) ‘(HH).

ونخلص من هذه الدراسة أنها أظهرت قيمة هرمون النمو كمعلم لصفات النمو كما انها اظهرت كفـاءة استخدام المعلمات الوراثية الجزيئية (RFLP) للتعرف على الاختلافات فى جين النمو وعلاقة هذه الاختلافات بوزن الجسم ويمكن استخدام هذا الاختلاف للانتخاب لصفة وزن الجسم وخاصة فى الاعمار الصغيرة مما يساعد على الانتخاب المبكر.