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A NOVEL SNP IN EXON 5 OF THE GHR GENE AND ITS ASSOCIATION WITH AVERAGE DAILY GAIN IN BEHEIRY EGYPTIAN WATER BUFFALOES *(BUBALUS BUBALIS)*

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ABSTRACT

The polymorphisms in growth hormone receptor gene (GHR) were determined by advanced molecular techniques as well as their association with growth traits in Beheiry Egyptian water buffalo. Therefore, the objective of this study was to detect GHR gene polymorphisms in Beheiry Egyptian water buffalo. Our results revealed the presence of SNP C742T in Beheiry Egyptian water buffalo by using PCR and SSCP methods followed by nucleotide sequencing to identify its different allelic patterns in the same animals under study. Three genotypes (CC, TC and TT) were identified by SSCP technique. Statistical analysis showed that, genotype TT was significantly higher than CT and CCgenotypes at age from 6 to 12 month ($P < 0.05$). These genotypes exhibited an association with growth traits (body weight, average daily gain) in Beheiry Egyptian water buffaloes. We could concluded that ther were a significant differences in growth traits between Beheiry Egyptian water buffaloes (TT, TC and CC genotypes) and so the breeders must take in consideration the presence of this SNP as well as its association with growth traits in the selection of Egyptian water buffalo for breeding.

Key words: Growth hormone receptor (GHR) gene, PCR, SSCP, SNPs, Beheiry Egyptian water buffalo.

INTRODUCTION

The Egyptian people undergo continuous increase in number and consume large amount of red meat. Adult water buffalo range in weight from 400 to 900 kg with an average increase in live weight of 0.90 kg per day has been recorded but the dressing percentage is always lower than that of cattle ***(Abd El-Aziz and Sadek, 2000)***. In Egypt, buffaloes beef and veal account for more than 41% of total red meat production and buffalo milk account for at least 56% of the total milk production. The greatest asset of the buffaloes as a domestic animal is its ability to subsist on the coarsest fodder and convert it most efficiently into milk with remarkably high butter fat content and meat of exceptional leanness. However, this production is still not enough for its consumption. Therefore, the genotype selection is used to improve the meat production.

The growth hormone receptor (*GHR*) is a single-chain glycoprotein which consists of 620-amino-acids, ~130 kDa, ***(Leung et al., 1987)***. The bovine GHR consists of 10 exons and 9 introns ***(Garrett et al., 2008)*** which is located on chromosome 20 ***(Menon et al., 2001)***.Growth hormone manifested its effect on growth and metabolism by binding with GHR on the surface of the target cells ***(Hradecka et al., 2008)***. Therefore, any change in the functional regions of GHR can alter the activity of GH in the target tissues ***(Oleński et al., 2010)***.

Previous studies have identified the *GHR* gene and have studied their association with growth, food efficiency and carcass quality of beef cattle ***(Maj and Zwierzchowski, 2006)***. They indicated that single nucleotide polymorphism (SNP) (A to G) is placed either in exon 4 or promoter gene and both of them had a significant association

with body weight and carcass quality ***(Sherman et al., 2008)***. In addition, several SNPs have detected in the 5′-regulatory region of the GHR gene (A/T, C/T, A/G and T/C) influence the quality and production of milk and meat in cattle ***(Moisio et al., 1998)***. But another polymorphism was detected in exon 10 that did not show significant effects on growth, size and meat conformation traits ***(Sherman et al., 2008)***. Two SNPs were identified in intron 8 showing changes in the binding capacity of GHR that may affect on the physiological properties of the receptor ***(Maj et al., 2007)***. The aim of the present study was to detect SNPs in the candidate GHR gene and its association with growth traits in Beheiry Egyptian water buffalo.

MATERIALS AND METHODS

Samples and DNA extraction:

All procedures involving animals were maintained at El-Nataff El-Gidid Experimental Stations (Mahalet Mousa in Kafr el-sheikh Governorate, Egypt) belonging to Animal Production Research Institute, Ministry of Agriculture. A total of 200 Beheiry Egyptian water buffalo bulls were examined. All data concerning growth traits were attained from farm records including (body weight and average daily gain) at different ages (3, 9, 12, 18, 24 months). Approximate 5ml of blood samples were collected aseptically from jugular vein into vacutainer tube containing EDTA anticoagulant. All samples were delivered to laboratory in ice box. The genomic DNA was extracted from white blood cells using Gene JET genomic DNA extraction kit following the manufacturer protocol (Fermentas, #K0721, USA). All equipments used in this study were autoclaved and sterilized before collection of samples as well as during all procedures in the laboratory.

Polymerase chain reaction (PCR):

According to Bovine growth hormone (*bGH*) gene (Gene Bank accession no. JF894306) the primers (inserted below) were designed by using oligonucleotide design tool primer 3.0 software . The PCR is performed in a 25μl of reaction mixture containing (2.5 µL 10X DreamTaq™ Green Buffer , 0.5µL (0.20mM) dNTP, 2.0 µL DNA template (approximately 50 ng), 0.5 µL 10µmol/L forward primer, 0.5 µL,10µmol/L reverse primer, 0.5 µL10× Taq DNA polymerase (5 U/µL, Fermentas, #K1071,USA) and17.5µL nuclease free water. The cycling protocol was 94°C for 4min ,30 cycles of denaturing 94°C for 1min, annealing at 56°C for 40sec.,extending at 72°C for 1min,with a final extension at 72°C for10 min. The PCR products were electrophoretically separated on 1% agarose gel stained with ethidium bromide.

Forward and reverse primers of GHR, annealing temperatures (Ta), size of PCR production (bp) and its localization.

Name	sequences	Ta(°C)	Product size (bp)	localization
GHR 2	F:5-AGGAGCTGGCACCTTATATGCAGT-3 R:5- CCCCGCTTATGTAATCTAAAGCCATGT-3	56	472	I4,E5,I5

Single stranded conformational polymorphism(SSCP):

Five µL of PCR products were mixed with 5µL of denaturating dye (95% formamide, 25mM EDTA, 0.025% xylene–cyanole and 0.025%bromophenol blue),heated at 95°C for 10min and chilled in ice then subjected to 12% PAGE(37.5:1 acrylamide: bis-acrylamide) in 1x TBE buffer at 200V for 6h at 4°C . The gel was stained with ethidium bromide. After polymorphisms were detected, the DNA bands of SSCP pattern were extracted and the PCR products were sent to MacroGen Company (South Korea) for sequencing in both directions using ABI 3730XL DNA sequencer (Applied Biosystem, USA). The sequences were analyzed with Geneious 4.8.4software.

RESULTS

We first extract genomic DNA from the blood samples of two hundreds Egyptian Beheiry Egyptian water buffalo bulls for detection of the GHR. One locus containing a partial sequence of part of intron4, exon5 and part of intron 5 of *GHR2* gene was chosen to search for any prospective polymorphisms. The extracted DNA was used as a template for PCR to amplify a partial sequence of *GHR2* gene **(Fig.1)**. We detected trimorphic SSCP patterns of *GHR2* by SSCP technique (TT, TC and CC). Insurance, nucleotide sequences of *GHR2* among Beheiry Egyptian water buffaloes exhibited a novel C742T SNP in exon 5 of *GHR* gene **(Fig.2).** This sequence was submitted to genebank (Accession number KC107765).

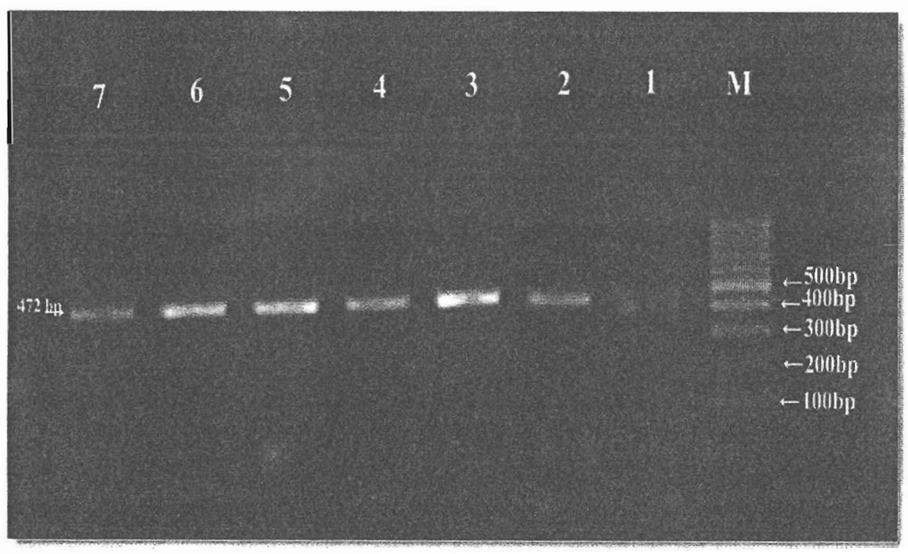

Fig. (1): Ethidium bromide stained agarose gel of PCR products representing amplification of *GHR2* gene in Egyptian buffaloes with size of 472bp , M represents 100 bp ladder.

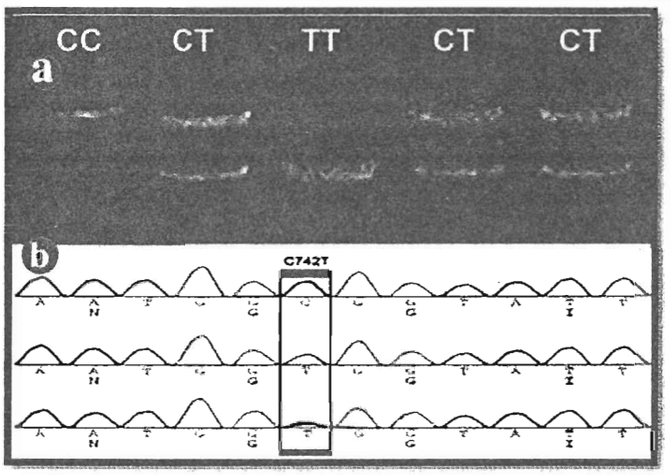

Fig. (2): **(a)** Polymorphism was detected in *GHR*2 exon 5 as there were trimorphic SSCP patterns; genotype CC, TC and TT. (b) Synonymous C742T SNP was detected in *GHR2* locus. The box indicates the position of the polymorphism.

Statistical analysis:

There is no difference between genotype frequency TT and CC are 0.46 but the genotype frequency CT is 0.08. The allelic frequency of C, T is equal 0.5. Three genotypes in Hardy–Weinberg equilibrium ($P<0.05$), ***(Snedecor and Cochran 1976)*** as shown in Table 1.

Correlation of TT, TC and CC genotypes at C742T SNP of *GHR* exon 5 with growth traits (body height, and average daily gain) were analyzed in Egyptian buffaloes at birth, 3, 6, 9, 12, 18, and 24 months old. The average daily gain (ADG) of TT was significantly higher than CT and CC at age from 6 to 12 month ($P < 0.05$) (Table 3) and so there was a correlation between ADG and C742T at age from 6 to 12 month with the homozygous TT genotypes being more favorable than heterozygous CT and CC genotype.

Table (1): Genotypic and allelic frequencies at C742T SNP of Beheiry Egyptian water buffalo GHR exon5 and the estimated Chi-Square (χ^2), HWE = Hardy-Weinberg equilibrium.

SNP	Genotype frequencies			Allele frequencies		χ^2 (HWE)	*P* value
	TT	TC	CC	T	C		
T742C	0.46 (92)	0.08 (16)	0.46 (92)	0.5	0.5	149.03	<0.05

Table (2): Correlation of genotypes at exon5 of the *GHR* gene with growth traits in beheiry Egyptian water buffaloes using LSM (Least Square Means) ± SE (Standard Errors).

Age / month	Growth traits (Kg)	Genotypes		
		CC	TT	TC
Birth	BW	34.228±0.265	34.256±0.621	33.916±0.261
3 mo	BW	79.433±0.558	78.590±1.346	79.242±0.544
Birth – 3mo	ADG	0.502±0.005	0.511±0.017	0.505±0.004
6 mo	BW	114.562±0.414	113.888±1.088	114.477±0.409
3 – 6 mo	ADG	0.398±0.003	0.397±0.006	0.402±0.003
9 mo	BW	147.943±0.523	147.434±1.306	147.900±0.507
6 – 9 mo	ADG	0.374±0.004*	0.378±0.004*	0.369±0.003*
12 mo	BW	180.235±0.317	180.094±0.772	180.215±0.335
9 – 12 mo	ADG	0.361±0.003*	0.366±0.008*	0.361±0.003*
18 mo	BW	258.946±1.869	254.250±4.602	256.565±1.882
12 – 18 mo	ADG	0.439±0.009	0.428±0.021	0.422±0.009
24 mo	BW	484.359±7.195	467.625±18.486	477.315±7.564
18 – 24 mo	ADG	1.254±0.030	1.187±0.079	1.228±0.033

BW = bodyweight; **ADG** = average daily gain. * Significance at $P < 0.05$.

DISCUSSION

As demonstrated from the previously mentioned results by many authors, gene structure and relationship to function is still under study especially the causal mutations effects and their association with growth traits. To throw light on this point identification of polymorphisms in

growth hormone receptor in Egyptian water buffalo is very important to improve its productivity. GHR share with GH by its binding on the cell surface through the growth and metabolism processes so any change in the functional region will effect on the signal pathway and activity of GH on the target cell. Many previous studies have identified that GHR gene is a candidate gene for growth traits. In this study, we identified three genotypes (CC, CT and TT) at C742T in exon 5 and studied their association with growth traits in Beheiry Egyptian water buffalo. These studies recorded that, there were four SNPs in exon 10 at nucleotides number T76C, G200A, T229C, and A257G bp from the 5′ end of the fragment in cattle where two SNPs at nucleotide number 200 and 257 bp changed amino acid encoding from (Ala) to (Thr) and from (Ser) to (Gly), respectively ***(Ge et al., 2000)***. In rabbits, there were no variation was detected in exon 1 and exon 6. But in exon 3 one non-synonymous mutation of C106G was detected, leading to the change of amino acid valine to leucine (V36L) ***(Andersson, 2001)***. Two SNPs were identified at the C863+32T in intron 8 and the A836T in exon 8 where the last one leading to Phe/Tyr amino acid substitution in the receptor protein and a significant differences in the GHR binding capacity ***(Maj et al., 2007)***.

CONCLUSION

It could be concluded from the present study that, there were a significant association between this SNP and meat production and quality traits in Beheiry Egyptian water buffalo bulls. We advice the buffalo breeders to select the animals carrying genotype TT which exhibited higher average daily gain than genotypes CT and CC. Moreover, there were an association with meat production and quality traits in genotype TT.

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تحديد طفرة جديدة في الاكسون الخامس من جين مستقبلات هرمون النمو واتباطه مع متوسط الزيادة اليومية في بحيري جاموس المياه المصرية

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تم تحديد الأشكال في الجين مستقبلات هرمون النمو (GHR) من خلال التقنيات الجزيئية المتقدمة وكذلك ارتباطهم بصفات النمو في بحيرى جاموس الماء المصرية لذا، كان الهدف من هذه الدراسة الكشف عن تعدد الأشكال الجيني GHR في بحيرى جاموس الماء المصرية . كشفت نتائجنا وجود طفرة C742T في بحيرى جاموس الماء المصرية باستخدام PCR و SSCP التي يتبعها تسلسل النوكليوتيدات لتحديد أنماط مختلفة في أليلية في نفس الحيوانات تحت الدراسة .تم تحديد ثلاثة أنماط جينية (CC, TC and TT) بواسطة تقنية SSCP. بالتحليل الإحصائي وجد أن النمط الجيني TT كان أعلى بكثير من النمط الجيني CT و CC في عمر من 6 الي 12 شهر($P < 0.05$). وقد اوضحت هذه الانماط الجينية عن وجود علاقة مع الصفات النمو (وزن الجسم ، متوسط الزيادة اليومي) في بحيرى الجاموس المياه المصرية. وقد استنتجنا من هذه الدراسة الي وجود اختلافات كبيرة في النمو بين الصفات بحيرى الجاموس المياه المصرية والأنماط الجينية (TC, CC and TT) لذا يجب علي المربين أن تأخذوا في الاعتبار وجود هذه طفرة فضلا عن ارتباطه مع صفات النمو في اختيار جاموس المياه المصرية للتكاثر.