RELATIONSHIP OF GROWTH HORMONE GENE WITH SOME OF PRODUCTIVE TRAITS OF COMMON CARP *Cyprinus carpio*.L

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ABSTRACT

This study was carried out at Al-Radhwaniyah fish Reservoir (Baghdad) to investigate the polymorphism of GH gene and relationship with some of productive characteristics (total weight gain (T.W.G), daily growth rate (D.G.R), relative growth rate (R.G.R) and specific growth rate (S.G.R) in common carp. single nucleotide polymorphism (SNPs) in GH gene was analyzed by direct sequencing. Two SNP were identified in the third intron of GH1, the first SNP at site A1132T was negative correlated with growth traits, AA Genotype (wild) was significant($p<0.05$) correlated with growth traits. The second SNP was happened at site of G1217T, any genotype significantly does not correlated with growth traits. the study summarized that identification of SNPs associated with growth performance can be candidate as genetics markers in marker-assisted selection (MAS) programs for improving growth traits in common carp.

Key Words: growth hormone gene, polymorphisms, productive traits, common carp.
***Part of M.Sc. thesis of the first author.**

علاقة جين هرمون النمو ببعض الصفات الانتاجية في أسماك الكارب الشائع *Cprinus carpio*.L

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المستخلص

أجريت هذه الدراسة في محمية اسماك الرضوانية (بغداد) بهدف تحديد تعدد المظاهر الوراثية في جين هرمون النمو وعلاقتها بعدد من الصفات الانتاجية (الزيادة الوزنية الكلية واليومية والنمو النسبي والنوعي) في اسماك الكارب الشائع، تم تحليل تعدد المظاهر الوراثية للنيوكلوتيدة المفردة في جين هرمون النمو عن طريق التسلسل المباشر وتم تحديد اثنين منها في الانترون الثالث لجين هرمون النمو الاول، الاولى في الموقع A1132T وارتبطت سلبا مع صفات النمو، اذ ارتبط التركيب الوراثي البري (AA) معنويا($p<0.05$) مع صفات النمو المدروسة. وحدث التغير الثاني في الموقع G1217T ولم يرتبط اي تركيب وراثي معنويا مع صفات النمو. خلصت الدراسة الى امكانية اعتماد تعدد المظاهر الوراثية للنيوكلوتيدات المفردة ذات العلاقة بالاداء كعلامات وراثية في برامج "الانتخاب بمساعدة الواسمات الوراثية" بهدف تحسين صفات النمو في اسماك الكارب الشائع.

كلمات مفتاحية: جين هرمون النمو، تعدد المظاهر الوراثية، الصفات الانتاجية، اسماك الكارب الشائع.
***البحث مستل من رسالة الماجستير للباحث الاول.**

***Received:27/7/2017, Accepted:23/11/2017**

INTRODUCTION

Studies of genetic diversity at DNA level represented an expansion field in aquaculture, and aimed to find out those DNA variations associated with productive phenotypes, in order to use them as tools for assisting the offspring selection at any early stage and predict their productive performance (14). Growth hormone or somatotropin is a single-chain polypeptide has a weight of 20 to 22 kilo Dalton (kDa) produced by the pituitary gland (8), along with prolactin (PRL) and somatolactin (SL) the GH/ PRL/SL gene family, which share similar structure and overlapping biological function may be it was common in ancestral genes (12). Growth hormone plays a major role in stimulating somatic growth in Teleosts (21), it is also involved in linear growth, food conversion (1) and many metabolic functions, including reproduction (2), and also plays a role in osmoregulation (18), Ttherefore GH gene is a potential target for genetic studies on variation related to growth traits and a polymorphisms in GH gene that is associated with the growth rate of farmed fish which were the target of many breeding programs (6). In recent years, polymorphisms of GH gene have been reported in several fish, such as Tench *Tinca tinca* (9)**,** Large yellow croaker *Larimichthys crocea* (16), rainbow trout *Oncorhynchus mykiss* (17), tilapia *Oreochromis niloticus* (4) and yellow catfish *Pelteobagrus fulvidraco* (11) and certain polymorphisms have been revealed to be associated with growth traits. Common carp is one of the most important cultured fish species in the world and historically the longest in the fields of fish culture, it bears the high temperature, Oxygen depletion, high stock density and fast growth, therefore it was the first fish reared in Iraq. Murakaeva (13) mentioned that the common carp has very large distribution area (from Central Europe, through Central Asia to East/South-East of Asia) with very different ecological conditions and variable growth rates, so that probably genetic varieties of the GH gene might be of adaptive importance. Both GH genes of common carp are very similar with each other where both genes consist of 5 exon and 4 introns with the 3rd intron in the GHII gene is the largest, therefore it is difficult to construct specific primer pairs for each of the two growth hormone genes to screen the polymorphisms. Due to the lack of ongoing studies in this regard in Iraq, the present study aimed to know the relationship of polymorphism in the growth hormone gene with a some of productive traits in common carps.

MATREIALS AND METHODS

This study was conducted at Al-Radhwaniyah fish Reservoir in Baghdad, 40 of common carp were collected from a private fish farm and reared for 110 day in ponds measuring 7 * 3 * 1.2 meter and were fed with commercial pelleted food with crude protein of 26.8%, crude fat 1.5% and energy 3165 kilo calories (kcl). All the experimental fish were reared under similar environmental conditions. Fish were marked up by a device from a Hallprint Fish Tags (Australia), where they were numbered by a hole near the dorsal fin. Some parameters describing the growth traits of common carp such as initial weight (IW), final weight (FW), TWG, DGR, RGR and SGR were studied.

Genomic DNA Extraction

one ml of blood were collected from the heart muscle of all trial fish. These samples were collected in EDTA tubes and kept in freezer (-18 ºC). for DNA extraction by using DNA extraction kit (Geneaid,korea) before DNA extraction blood volume was reduced to 20 microliters (µl) and phosphate-buffered saline (PBS) increased to 200 µl because all the blood cells of fish are nucleated and contained DNA and proteins levels in fish blood are higher than in mammals blood.

PCR amplification

The primers was supplied from BIONEER (korea) as lyophilized powder of different picomols concentrations. The sequences of primers are shown in Table. 1 (Gene bank EU333984). Thermal cycle with the following profile: Initial denaturation at 94 C º for 3 minutes, then 35 cycles of 94 C º for 30 seconds, 54 C º for 30 seconds ,72 C º for 1 minutes.

Table 1. Sequence of primer and the region covered of the growth hormone gene according to murakaeva (13)

Primer pair	Gene	Part of the Gene	Length of PCR fragments base pare (bp)
GH-c: 5´-AGG AAC GCA GAC AGC TGA GTAA - 3´	GH I	third to fourth exon	About 430 and 770 (both are allele variants)
GH-d: 5´-TAC GGT CAG GCT GTT TGA GA - 3´	GH II		About 650 or 900 (both are allele variants)

PCR reaction was performed in 0.2ml tubes by mixing master mix reagents in final volume of 20 µl.The amplification was performed in a TECHNE (T-C 5000) thermal cycler and the reaction mixture was prepared according to the procedure that suggested by the manufacture company (BIONEER, Korea) using 2 µl of DNA and 1µl of each primer and then complete the PCR reaction volume to 20 µl by distilled water finally reaction mixture vortexes thoroughly. PCR products visualized in a 1.5% agarose gel electrophoresis stained with Ethidium bromide.

SNP Detection and Genotyping

Fragments in 40 individuals were sequenced by direct sequencing, Macrogene Company (USA). SNPs were identified and genotyped with Geneious (version 10.3.1) and blasted with the published common carp GH1 sequence (LOC 109081196) in National Center for Biotechnology Information (NCBI). According to Vignal *et al*. (23) to be considered a SNP, the less frequent allele must exist at a frequency of 1% or more in the population. Because the number of samples we have is few, therefore we set up minimum frequency of 25% for a SNP to be selected to achieve reliable outcomes.

Statistical analysis: The Statistical Analysis System (SAS) was used in data analysis (20) according to complete randomized design (CRD) using the general linear model (GLM) and Duncan multiple range test was used to compare the average means at a significant level ($p<0.05$) (5). The genotype frequencies were calculated and HWE was tested using a chi-square test of PopGene32 .

RESULTS &DISCUSSION

Polymerase Chain Reaction (PCR) amplified regions, which showed only one band with molecular weight of 770 bp (figure 1). To detect the PCR product, DNA ladder (100-3000bp) was used and the gel was visualized by photo documentation system. The same PCR product size was obtained in 770 bp Which represents the GH1 gene but we did not show the second piece on the site either 650 or 900 bp Which represents the GH2 gene in Common carp according to Designer primer by Murakaeva (13). Thats may be due to the variability of our common carp strain or that the fish we used in this experiment a combination were represented more than one strains, especially in Iraqi waters there were several strains of common carp coming from several countries and uncontrolled mating between these fish.

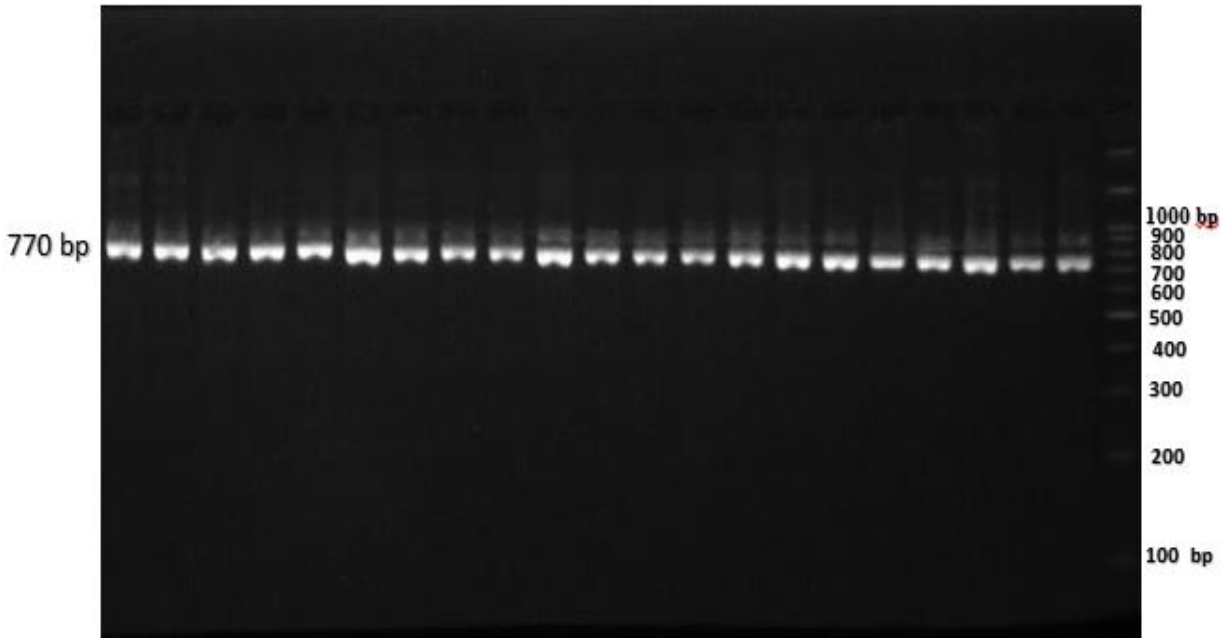

Figure 1. The PCR products were agarose gel 1.5% and 40 volt for 1 hour. Visualized under U.V light after stain with Ethidium Bromide

Genotyping and Allele Frequencies

Results of sequence shown correspond with the GH gene (GH1) in common carp. Results showed two SNPs in the third intron of GH1 gene (figure.2), First SNP was at site 1132 bp (A → T) and revealed three genotypes (AA, AT and TT). Second SNP at site 1217 bp (G → T) and revealed two genotypes (GG and GT). Table.2 shows the distribution ratios of genotypes and allele frequency according to Hardy Weinberg equilibrium (HWE).

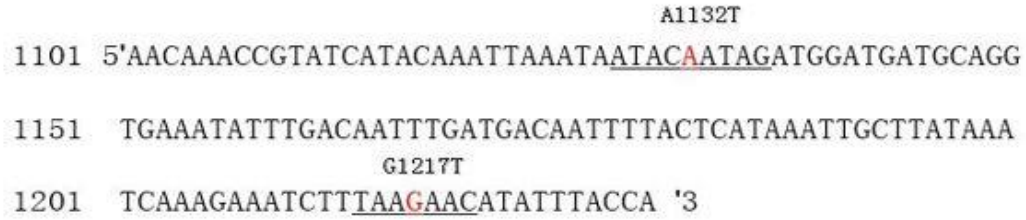

Figure 2. Location of SNPs in GH1 gene of common carp

Table 2. Frequencies of genotypes and alleles of the common carp GH1 gene

SNP	Genotype	No. (%)	Frequencies of alleles (%)	Chi-square value ($\chi 2$)
A1132T	AA	17 (42.5)	A 68	11.369 **
	AT	20 (50)	T 32	
	TT	3 (7.5)		
	GG	22(55)	G78	4.287 *
G1217T	GT	18(45)	T 22	
	Total 40 (100%)	*(p<0.05)	**(p<0.01)	

Effect of GH gene polymorphism in growth traits

Table.3 shows the growth traits of common carp. Results showed significant difference (P <0.05) at site of A1132T,it was found in the average of final weight among the different genotypes as above in the AA genotype and reached 303.62 g / fish and reached 240.6 g / fish in the AT genotype and 229 g / fish in the TT genotype, while the results did not differ significantly between the second and third genotypes. Results showed significant differences(P <0.05) in the T.W.G of fish as reached 109.37 g / fish in AA, 63.78 g / fish in AT and 58.33 g / fish in TT genotype , as for average D.G.R was the highest in AA (0.99 g /

fish / day) and the lowest value was in TT (0.63 g / fish / day). AA genotype significantly differed from the other Genotypes (P <0.05). Results showed that R.G.R was significantly affected (P <0.05) by different genotypes of GH gene, which was 56.42%, 36.60% and 34.17% for AA, AT and TT genotypes respectively. Direction of S.G.R was also significantly affected (P <0.05). Values were 0.40% g / day, 0.28% g /day and 0.26% g /day sequentially for the same genotypes above. Results of G1217T showed no significant differences in the final weight between the genotypes. Values were 264.19 g for GG and 268.62 g for GT, as for T.W.G differences computations were found in GG 77.57 g and in GT 88.76 g with no significant difference. Also, the characteristics D.G.R, R.G.R and S.G.R were recorded as differences in computations according to different Genotypes with nonsignificant superiority. The importance of growth hormone gene is reflected as one of the candidate genes for the study of genetic variation and its relationship with growth characteristics and a sign to study the evolutionary relations of different fish and study the characteristics of growth and the possibility of application (10). In fish, it was found that polymorphism was associated with growth characteristics. This was demonstrated by Kang *et al*. (7) on olive flounder (*Paralichthys olivaceus*), Sanchez Ramos *et al.* (19) on gilthead seabream (*Sparus aurata*) and Blank *et al.* (3) on Nile tilapia (*Oreochromis nilotcus*). Several evidences of the key role of growth hormone have been reported as GH gene is a major research target in the aquaculture sector and one of the main goals is to achieve the largest increase in weight in shortest time, so the polymorphism of GH gene in the growth-related is the target of many studies in breeding programs, and the identification of SNPs in the GH gene for common carp is still below the level of ambition. In this study, SNPs were identified using direct sequencing and differences in alleles were found in the third intron of GH gene.

Table 3. Effect of growth hormone gene Polymorphism in growth traits of common carp (means ± standard error)

	A1132T			G1217T	
Traits	**AA**	**AT**	**TT**	**GG**	**GT**
I.W (g)	**194.25± 7.33 [a]**	**176.89 ± 6.8 [a]**	**170.66 ± 4.6 [a]**	**186.66± 6.1 [a]**	**180.05 ± 7.60 [a]**
F.W (g)	**303.62 ± 14.69 [a]**	**240.68 ± 8.6 [b]**	**229 ± 10.1 [b]**	**264.19 ± 10.5 [a]**	**268.62 ± 15.9 [a]**
T.W.G (g)	**109.37 ± 9.84 [a]**	**63.78 ± 3.5 [b]**	**58.33 ± 8.0 [b]**	**77.52± 6.7 [a]**	**88.76 ± 10.12 [a]**
D.G.R (g/day)	**0.99± 0.08 [a]**	**0.57± 0.03 [b]**	**0.53± 0.07 [b]**	**0.70± 0.06 [a]**	**0.80± 0.09 [a]**
R.G.R (%)	**56.42 ± 4.31 [a]**	**36.60 ± 2.1 [b]**	**34.17 ± 4.6 [b]**	**41.57 ± 3.1 [a]**	**48.70 ± 4.3 [a]**
S.G.R (% g/day)	**0.40± 0.03 [a]**	**0.29± 0.01 [a b]**	**0.26± 0.01 [b]**	**0.31± 0.01 [a]**	**0.35± 0.01 [a]**

The superiority of performance was observed for the individuals who carry the AA genotype at site A1132T with most of the studied growth traits, so the individuals carried this genotype can be selected. Where the mutation in this site has affected negatively, which led to deterioration in the growth characteristics of individuals who carry them. These results different with (17) in GH2 of rainbow trout he was found three genotypes (AA, AB and BB).The homozygous (BB) achieved significant results compared with heterozygous (AB) (p<0.05), and insignificant results compared with wild (AA). Ni *et al.* (15) investigated polymorphisms within the exon regions of olive flounder *Paralichthys olivaceus* GH and found that exon 4 had two SSCP haplotypes, AA and AB. The AB genotype had one non synonymous mutation at site 1763 (C→T) that was positively correlated with body weight. In site G1217T, the statistical analysis does not indicate significant differences in different genotypes, that is meaning the change in this site does not affect the performance of fish. these results agree with (11) which found three genotypes at site of 2100 bp in yellow catfish (*Pelteobagrus fulvidraco*) and did not observed any significant differences among the genotypes and the body weight. Tian *et al.* (22) found three genotypes in mutation at site of 5045 T> C in Basilwasky (*Siniperca chuatsi*) and no significant differences were observed in body weight among different genotypes.

According to the obtained results it is highly probable that the genotype has direct influence on growth parameters of common carp.

The statistical analysis showed that the genotype at a site of A1132T was better associated with the studied growth characteristics.

The SNP in G1217T did not affect of growth characteristics whether positive or negative.

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