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

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ABSTRACT

This study was aimed of determining the relationship of polymorphism of the growth hormone receptor gene with some of growth traits (daily growth rate, relative and specific growth, ratio and efficiency of feed conversion) in 45 samples of common carp (*Cyprinus carpio*). The following are the most important results obtained: The results of DNA sequencing and single nucleotide polymorphism (SNP) showed there are of three mutations in the following sites C24074T, A24375G and G24485A. No genetic makeup was significantly associated with the studied growth characteristics. It is concluded from this study that there is no significant effect of the difference in the genotypes of the growth hormone receptor gene on the growth characteristics of common carp fish.

Key words: genotypes, polymorphism, daily growth rate, relative and specific growth rate, ratio and efficiency of feed conversion.

علاقة جين مستقبل هرمون النمو ببعض الصفات الانتاجية في أسماك الكارب الشائع *Cyprinus carpio*

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المستخلص

أُجريت هذه الدراسة بهدف تحديد علاقة تعدد المظاهر الوراثية(Polymorphism) لجين مستقبل هرمون النمو بعدد من الصفات الانتاجية (معدل النمو اليومي والنمو النسبي والنوعي ونسبة وكفاءة التحويل الغذائي) في 45 عينة من أسماك الكارب الشائع *(Cyprinus carpio)*. فيما يأتي اهم النتائج المتحصل عليها: اظهرت نتائج تحديد تسلسل الدنا (DNA Sequencing)، وتعدد المظاهر للنيوكلوتيدة المفردة (Single nucleotide polymorphism–SNP) وجود ثلاث طفرات في المواقع الآتية T24074C وA24375G وG24485A. ولم يرتبط اي تركيب وراثي معنوياً مع صفات النمو المدروسة. يستنتج من هذه الدراسة عدم وجود تأثير معنوي لاختلاف التراكيب الوراثية لجين مُستقبل هرمون النمو في الصفات الإنتاجية لأسماك الكارب الشائع.

الكلمات المفتاحية: التراكيب الوراثية، تعدد المظاهر الوراثية، معدل النمو اليومي، معدل النمو النسبي والنوعي، نسبة وكفاءة التحويل الغذائي.

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INTRODUCTION

Aquaculture in general and fish farming in particular are among the fastest growing agricultural sectors in the world (24). The data of the Food and Agriculture Organization (FAO) confirms that the production of fish and fishery products follows a continuous upward trend and is expected to reach approximately 8,000,000 tons total in 2050 (8). The fish production of the carp family (Cyprinidae) in particular reached to 4, 080,045 tons until 2013 (9). The high nutritional value of fish meat and the fact that it is an important source of animal protein show how fish and its products are important to the economy (3). The growth rate of farmed fish is one of the most important factors for the success of aquaculture, also the body mass of fish depends on skeletal muscles because they represent 70% of the body weight, in which the growth of the skeletal muscles of fish is controlled by a group of genes including as GH, GHR, IGF1, MRF4, MSTN and others (19). Identification of genetic variability is an essential step for implementing genetic improvement programs that focus on selecting fish that are characterized by fast growth, high feed conversion rates, and disease resistance (14). Genetic diversity studies at the level of DNA represent the field of expansion in aquaculture that aims to know those differences in DNA are associated with growth traits in order to use them to help select individuals at an early stage with high productive performance (1), this method is called Gene Assisted Selection (GAS) (7). Growth traits in farm animals and fish could be tested based on polymorphisms in growth-related genes using several techniques, where a genetic polymorphism represents a DNA sequence difference among individuals, groups, or populations and includes single nucleotide polymorphisms (SNPs), repeat sequencing, recombination, insertion, and deletion, as SNPs reflect a specific locus in which more than one nucleotide and only two alleles are identified in the SNP locus (22). SNPs for other growth-related genes were analyzed in Atlantic salmon (*Salmo salar*) (21), common bromone fish (*Lates calcarifer*) (11), and common carp (2). Single nucleotide polymorphisms (SNPs) may lead to a change in an individual's phenotypic traits, and the variance can be used as a marker for good traits, and to exclude individuals with poor traits (4). The current study aimed to determine the genetic morphology or polymorphism in the growth hormone receptor gene, calculate the allelic frequency, and locate mutations in the growth hormone receptor gene for samples of common carp fish, as well as the relationship of genotypes to a number of growth traits related to direct effects on fish growth.

MATERIALS AND METHODS

Experimental setting: The experiment was conducted the fish laboratory/College of Agricultural Engineering Sciences-University of Baghdad, The experiment was conducted for three months, 16 glass tanks where used with dimensions of 30 x 60 x 40 cm, eight fish/tank are placed. Water was supplied from tanks that were filled a day before use, part of the aquarium water was changed daily, and pumps were used to provide oxygen to the tanks. The water temperature of the tanks was measured daily during the duration of the experiment by an electronic thermometer, and the heaters (capacity 900 watts) were used in each tank to provide the appropriate temperature for the growth of common carp, which is 25 degrees Celsius (10). The fish were fed a commercial formula containing 26.47% proteins. The fishs were numbered by medical ear ring of type Caflon for research purposes (Caflon /USA), as they were fixed near the pectoral fin, and the information of each fish was recorded according to the color of each ring.

Blood sampling DNA extraction polymerase chain reaction: Blood samples of experimental fish were collected from the caudal vein, as 2 ml of blood was obtained in a 5 ml sterile syringe and placed in a 5 ml tube containing EDTA anticoagulant (0.5 ml). The tubes containing blood were numbered according to the number of each fish.The DNA was extracted from blood samples using the ready-made kit according to the protocol provided by the company (Promega/USA) which included to the following steps: DNA extraction, PCR amplification, sequencing, and assembly. After the polymerase chain reaction and the process of amplifying the

pieces of the genetic material, the electrophoresis process begins, the samples were transported on electrical energy of 100 volts and a current of 50 milliamps for an hour, the size and number of packages in the DNA was determined by a UV device, the primer for the growth hormone receptor gene was provided by Macrogene /USA, intron are targeed 2-4, and the primer was in dried powder form, and its sequence shown Table (1) as reported by Tao et al. (18). The primer works according to its specific conditions, which are described as follows: denaturation phase 65 °C, 30 cycles of 30 seconds for one cycle, stage C extension 72°C, 30 cycles of 30 seconds per cycle, and final extension phase 72°C, 1 cycle of 7 minutes.

Table 1. Primer sequence and region covered by the growth hormone receptor gene

Primer pair	Part of the Gene
Forward	**Intron 2-4**
5´-ACAGCAGTATTCCTTTTACATAGTAGCAG – 3´	**The size of the studied piece of the gene**
Reverse	
5´-CACAATACTGTTACTTTAATAGCTGCCTAG – 3´	**(645 pb)**

Tao et al (2011)

The samples of 20 µl of PCR product of 45 samples were sent by scientific office to Macrogene Company to obtain the real sequences of the nitrogenous bases of the required segment of the gene using the AB DNA sequencing system, as the sequencing process was performed for one strand of DNA was forward, as requested by the company, for determining the polymorphism of the growth hormone receptor gene for common carp, after which an alignment was made between the samples and the reference gene and the differences between them were determined using the Geneious program.

Statistical analysis

The data was statistically analyzed using the program Statistical Analysis System–SAS (2012) (17), to study the effects of the genotypes of the growth hormone receptor gene and each SNP on the traits studied, and the significant differences between the means were compared using the test Duncan(2004). Polynomial according to the application of the method of least squares means (LSD).

Mathematical model: $Y_{ij} = \mu + G_i + e_{ij}$

RESULTS AND DISCUSSION

The results showed there were three SNPs for the growth hormone receptor gene, the first SNP was at site T24074C (T→C), and revealed three genotypes (TT, TC, and CC). The second SNP was at locus A24375G (A→G) and revealed three genotypes (AA,GA, and GG). The third SNP at locus G24485A (G→A) revealed three genotypes (GG, GA, and AA). Table 2 shows the proportions of the distribution of genotypes and proportions of alleles according to the Hardy-Weinberg Law (HWE).

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

Loci	Genotype	NO.(%)	Frequencies of alleles (%)	Chi-square value (χ^2)
	TT	**18 (40)**	**T 50**	**16.20****
T24074C	**TC**	**9 (20)**		**(p <0.01)**
	CC	**18 (40)**	**C 50**	
Total		**45**		
A24375G	**AA**	**12 (26.67)**	**G 41**	**10.86****
	GA	**13 (28.89)**		**(p <0.01)**
	GG	**20 (44.44)**	**A 59**	
Total		**45**		
	GG	**31 (68.89)**		**50.02****
G24485A	**GA**	**4 (8.89)**		
	AA	**10 (22.22)**		**(p <0.01)**
Total		**45**		

Table 3 shows the daily growth rate (DGR), relative growth rate (RGR), specific growth rate **(**SGR), ratio of feed conversion (FCR), Efficiency of feed conversion (FCE) at site T24074 C. The results showed no significant differences (p>0.05) in the daily growth rate (g/day/fish) between the different genotypes, which averaged to 0.35 g/day/fish in genotype CC, and 0.31 g/day/fish in genotypes CT and TT, and the results showed that there were no

significant differences (p>0.05) between genotypes in the relative growth rate, which averaged to 56.61, 48.76 and 49.44% in CC, CT, and TT genotypes, respectively. Also, no significant differences (p>0.05) were recorded between the gnotypes in the specific growth rates, which were 0.51, 0.45, and 0.47%/day for the CC, CT, and TT genotypes, respectively, and the results showed that no significant differences (p>0.05) were recorded among the CC genotypes , CT and TT in the feed conversion ratio, which averaged to 7.11, 7.75 and 7.09, respectively. As well as, no significant differences (p>0.05) were recorded in the efficiency of feed conversion, which averaged to 14.05, 12.89 and 14.09% in the CC, CT, and TT genotypes, respectively. As for the site, A24375G, the results showed that there were no significant differences in the daily growth rate among the different genotypes, which averaged to 0.36 g/day/fish in genotype AA, 0.25 g/day/fish in genotype GA, and 0.34 g/day/fish in genotype GG. The results showed that there were no significant differences among the different genotypes in the relative growth rate, as it reached 56.62, 38.56, and 54.20% in the genotypes AA, GA, and GG, respectively. The results also showed that there were no significant differences among the genotypes in the specific growth rate. It was 0.52, 0.37, and 0.50%/day in genotypes AA, GA, and GG, respectively, and the results of the feed conversion ratio, which reached 6.9, 10.02, and 7.1 in genotypes AA, GA, and GG, respectively, showed no significant differences were recorded. The results of feed conversion efficiency showed values of 14.43, 9.97, and 13.99% for genotypes AA, GA, and GG, respectively, without significant differences between them. As for the site G24485A, the results showed that there were no significant differences in the daily growth rate between the different genotypes, which averaged to 0.30 g/day/fish in genotype GG, 0.31 g/day/fish in genotype GA, and 0.32 g/day/fish in genotype AA, and the results showed that no significant differences (P>0.05) were recorded among the different genotypes in the relative growth rate, which reached 48.03, 49.09 and 51.25% in the genotypes GG, GA, and AA, respectively, as well as no significant differences were recorded in the specific growth rate, it reached 0.46, 0.46, and 0.48%/day in the genotypes GG, GA, and AA, respectively. The results showed that the feed conversion ratios were 17.4, 7.54, and 6.39 in the genotypes GG, GA, and AA, respectively, with no significant differences were recorded. As for the efficiency of feed conversion, it was 13.49, 13.25, and 15.63% for the genotypes GG, GA, and AA, respectively, without recording any significant differences.

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

Loci	Geno type	daily growth rate (g/day/fish)	relative growth rate (%)	specific growth rate (%/day)	ratio of feed conversion	Efficiency of feed conversion (%)
	CC	0.35±0.03	56.61±5.72	0.51±0.04	7.11±1.19	14.05±1.62
T24074C	CT	0.31±0.05	48.76±8.98	0.45±0.06	7.75±1.21	12.8±2.36
	TT	0.31±0.02	49.40±3.53	0.47±0.03	7.09±0.82	14.09±1.08
		NS	NS	NS	NS	NS
	AA	0.36±0.03	56.62±5.13	0.52±0.04	6.92±1.07	14.43±1.45
A24375G	GA	0.25±0.07	38.56±11.17	0.37±0.09	10.02±3.07	9.97±3.62
	GG	0.34±0.02	54.20±4.19	0.50±0.03	7.14±0.78	13.99±1.17
		NS	NS	NS	NS	NS
	GG	0.30±0.02	48.03±4.40	0.46±0.03	7.41±1.12	13.49±1.31
G24485A	GA	0.31±0.04	49.09±6.67	0.46±0.05	7.54±0.94	13.25±1.81
	AA	0.32±0.02	51.25±4.08	0.48±0.03	6.39±0.59	15.63±1.23
		NS	NS	NS	NS	NS

NS: no significant

The importance of the growth hormone receptor gene manifests as one of the main genes associated with growth traits and genotypes in the breeding programs and genetic improvement. It has been observed that Polymorphism coincides with growth

traits in fish and the single nucleotide polymorphism (snp) that lies with the encoding regions is extensively exploited in the genetic and genomic markers development (20). Growth rate is an important economic characteristic of aquaculture animals, which could be influenced by many different genes and environmental factors (19). The growth hormone receptor is one of the vital factors of the GH/IGF growth axis, so mutations in the GH receptor gene have an important effect on fish growth. It has been observed that polymorphism is associated with growth traits in fish, this was demonstrated by Kang et al. (12) in flounder fish (*Paralichthys olivaceus*), Sánchez-Ramos et al. (16) in gold bream (*Sparus aurata*) and Blanck et al (6) in tilapia (*Oreochromis niloticus*). The expression of the GHR gene in fish is influenced by age, food type, environmental factors, and hormone level, it was found that GHR gene expression is widespread in different organs of fish, and is mainly expressed in the liver (23). GHR gene polymorphisms can affect the normal function of GH and thus growth traits such as body weight and length (5). The polymorphism in the growth hormone receptor gene was detected in this study by determination of a single nucleotide SNP-(Single neoclutide polymorphism) using direct sequencing after the target segment of the gene was amplified by PCR, and comparison the sequencing of all fish and extracting genotypes, the differences were found in the structure of the alleles in intron 2-4 of the growth hormone receptor gene (18). As the results showed that there was no significant impact in all the studied mutations of the growth hormone receptor gene on the studied growth characteristics of the experimental fish, it could be inclusively attributed that this gene which is responsible for protein coding is A receptor for growth hormone, as it works to introduce it into the cell, while the hormone plays its role in stimulating immunity. The experiment fish were taken from a commercial flock, so it is certain that the mothers are of good and selected traits, therefore the source of the eggs of these fish (the mothers) has been carefully selected, and one of the most important characteristics that focus is on is the speed of growth, so there is indirect selection Among the mutations that hinder growth in these fish. As it is known, there are certain mutations in this gene that are significantly associated with growth retardation. These results agree with the results found by Zhao et al. (25) on *Cynoglossus semilaevis* fish, where two SNP sites were identified in the growth hormone receptor and the difference in body weight among the three genotypes of SNP1 was significant (p <0.01), as the pure genotype outperformed the recessive, indicating that SNP1 could be used as a potential genetic marker for selection, while this study did not agree with the findings of Tao et al. (18) on the domesticated carp strain (*Cyprinus carpio* var jian), as five SNPs were detected and all recessive genotypes were significantly ($p < 0.01$) associated with weight gain except for the C12T site, which was associated with a hybrid genotype, and did not agree with the study of Ruan et al. (15) that the examined SNP sites in the growth hormone receptor gene in Nile tilapia were significantly associated with weight gain. The reason for this difference could be due to the difference in the location of the mutation, the size of the sample studied, and the source of the studied strain, perhaps the mutations affected the growth hormone receptor sequence and then affected the GH-IGF1 system, in which the growth hormone receptor is an essential and important factor, as it is known that weight is a quantitative trait as it is affected by a large number of genes (13). It could be concluded from the results that the polymorphism of the growth hormone receptor gene in common carp fish in the seven discovered sites, there is no significant effect of the difference in the genotypes of the growth hormone receptor gene on the growth characteristics of common carp fish, and the mutations that were discovered in all sites did not affect the studied growth characteristics, whether positive or negative.

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