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RELATIONSHIP OF GROWTH HORMONE RECEPTOR GENE WITH SOME OF PHYSIOLOGICAL CHARACTERISTICS OF COMMON CARP *CYPRINUS CARPIO L.*

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ABSTRACT
This study was conducted to determine the relationship of polymorphism in the growth hormone receptor gene with some of physiological characteristics (white and red blood cells, hemoglobin levels, volume of compacted blood cells, and total protein) in 45 samples of common carp (*Cyprinus carpio*). Below are the most important results obtained: The results of DNA sequencing, and single nucleotide polymorphism (SNP) showed there are three mutations at the following sites: C24074T, A24375G, and G24485A.The results of the blood parameters at site C24074T showed that the number of white blood cells was 136.27 $\times 10^3$ cells/mm^3 in the CC genotype, 131.87 $\times 10^3$ cells/mm^3 in the TT genotype, while it was 116.77 $\times 10^3$ cells/mm^3 in the CT genotype. The levels of hemoglobin were 9.23, 8.90, and 8.13 mg/100 ml in the CC, TT, and CT genotypes, respectively, and no significant differences were recorded in the average number of red blood cells, the volume of packed blood cells, and the total protein rate of the mentioned genotypes. As for the site A24375G, significant differences were recorded between the genotypes of the white blood cell count and hemoglobin levels, and no significant differences were recorded in the number of red blood cells, volume of packed blood cells, and the total protein rate, while at the site G24485A, no significant differences were recorded in all the studied characteristics. It is concluded from this study that there is a significant effect of the difference in genotypes of the growth hormone receptor gene on the physiological characteristics of common carp.
Keywords: genotypes, polymorphism, red blood cells, white blood cells, hemoglobin

INTRODUCTION
The growth hormone receptor gene is a single copy with 9 exons in mammals and an extra exon in the double copy of the gene in fish (GHR-I and GHR-II) (Ozaki et al., 2006). Growth hormone (GH) interacts with growth hormone receptors (GHR) on target tissues producing signals on the cell membrane, as well as promoting physical growth (Kopchick and Andry, 2000), in addition to anticipating in other biological functions such as reproduction (Trudeau, 1997), Immunity (Yada et al., 1999), and osmotic regulation in fish (Sakamoto et al., 1997). The growth hormone receptor is a protein belonging to the hematopoietin cytokine receptor family that includes growth hormone receptors, prolactin receptors, and a number of other cytokine receptors (Moutoussam et al., 1998). 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 (Lupchinski et al., 2011). Genetic diversity studies at the level of DNA represent an expanding field in aquaculture that aim to know those differences in DNA associated with productive traits in order to use them to help select individuals at an early stage and early

detection of their productive performance (Al-Azzawy and Al-Khshali, 2018), this method is known as GeneAssisted Selection–GAS (De-Santis and Jerry, 2007). A genetic polymorphism is a variation in the DNA sequence between individuals, groups, or populations includes single nucleotide polymorphisms (SNPs), sequence repeats, recombination, insertions, and deletions, where SNPs reflect a specific locus at which more than one nucleotide, and two alleles are identified at the SNP locus (Al-Alnjawi and Al-Khshali, 2020). Mutations in the primary sequence of the GH receptor gene can affect the expression level of the GH receptor gene, affect the binding ability of GH, affect morphology after GH binding, etc., and ultimately lead to changes in the productive and physiological characteristics of the fish (Zhang et al., 2006). 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 (Al Khshali and Saleh, 2020). Given the scarcity of studies in this regard in Iraq, the current study aimed to determine the genetic morphology or polymorphism in the growth hormone receptor gene, calculate the allelic frequency, locate mutations in the growth hormone receptor gene for samples of common carp and the relationship of genotypes with some of physiological characteristics with that have a direct effect on fish health.

MATERIALA AND METHODS

The experiment was conducted in the fish laboratory/College of Agricultural Engineering Sciences-University of Baghdad, 16 glass tanks where used with dimensions of 30 x 60 x 40 cm, water was supplied from tanks that were filled a day before use, part of the aquarium water was daily changed, and pumps were used to provide oxygen to the tanks.

The water temperature of the tanks was daily measured during the duration of the experiment by an electronic thermometer, and the heaters (capacity 900 watts) were used in each tank for providing the appropriate temperature for the growth of common carp, which is 25 degrees Celsius (Guderley and Blier, 1988). Fish were fed a commercial formula containing 26.47 proteins, fish were numbered by medical ear ring of type Caflon for research purposes (American made), as they were fixed near the pectoral fin, and the information of each fish was recorded according to the colors of each ring.

DNA extraction and polymerase chain reaction

The DNA was extracted from blood samples using the ready-made kit according to the protocol used by the company (Promega) according to the following steps: 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 prepared by Macrogene Company, and the primer was in dried powder form, and its sequence is shown in table (1) as reported by Tao et al. (2011).

The primer works according to its specific conditions, which are described as follows: denaturation phase 95 °C, 30 cycles for 30 seconds for one cycle, stage C extension 72, 30 cycles for 30 seconds per cycle, and final extension phase 72, 1 cycle for 7 minutes.

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

Primer pair	Part of the Gene
5′-ACAGCAGTATTCCTTTTACATAGTAGCAG – 3′ Forward 5′-CACAATACTGTTACTTTAATAGCTGCCTAG – 3′ Reverse	Intron 2-4 (645pb)

Detection of single nucleotides and genotypes

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 forward strand of DNA, 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.

BLOODS TESTS:

Blood samples of the experimental fish were collected from the caudal vein. 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 blood-containing tubes were numbered according to the number of each fish, and they were sent to a private laboratory, the tubes were placed in the SPINCELL 3 device (Japanese origin) to determine the blood picture of the experimental fish, the number of white and red blood cells, the level of hemoglobin and the volume of the packed blood cells. Anticoagulant-free ml (EDTAA), and serum was obtained after centrifugation at 3000 rpm for 15 minutes and placed in sterile tubes for total protein determination, the tubes were placed in a Japanese Fujifilm apparatus, and the data were recorded.

STATISTICAL ANALYSIS

The data was analyzed statistically using the program Statistical Analysis System–SAS (2012) 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 A). 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

SNP	Genotype	NO.(&)	Frequencies of alleles (%)	Chi-square value (χ^2)
	TT	18(40)	T 50	**16.20
T24074C	TC	9(20)		
	CC	18(40)	C 50	
A24375G	AA	12(26.67)	G 41	**10.86
	GA	13(28.89)		
	GG	20(44.44)	A 59	

Table 3 includes the studied blood parameters for site T24074C, including white blood cell count (WBC, red blood cell count (RBC), hemoglobin concentration (Hb),packed blood cells PCV, total protein (TP). The results showed significant differences (P<0.05) in the number of white blood cells between the genotypes CC and TT on the one hand, and CT genotype on the other hand, which amounted to 136.27 × 10^3 cells/mm^3 in the genotype CC and 131.87 × 10^3 cells/mm^3 In the TT genotype, while it was 116.77 × 10^3 cells/mm^3 in the CT genotype, the results showed that no significant differences were recorded between the CC, CT and TT genotypes in the number of red blood cells, which amounted to 1.65, 1.42, 1.47 × 10^6 cells. / mm3, respectively. The results also showed significant differences (P<0.05) between CC and TT genotypes in hemoglobin levels of 9.23 mg/100 ml and 8.90 mg/100 ml, respectively, with a significant difference from the CT genotype, which amounted to 8.13 mg/100 ml. The blood stacked for the CC, CT and TT genotypes, there were no significant differences, which amounted to 33.38, 32.3 and 31.5%, respectively, and it was noted that no significant differences were recorded in the levels of total protein, which amounted to 2.02, 2.01 and 2.03 g/100 ml for the CC and CT genotypes and TT respectively.

As for the site A24375G, the results of white blood cell counts showed significant differences (P<0.05) for the AA genotype, which amounted to 135.94 x 10^3 cells/mm^3 compared to the genotype GA, which amounted to 122.17 x 10^3 cells/mm^3, while the GG genotype did not differ significantly (P>0.05). With both structures of 130.87 x 10^3 cells/mm^3, the results showed that no significant differences were recorded between the genotypes AA, GA and GG for red blood cell count, which amounted to 1.63 x 10^6, 1.45 x 10^6 and 1.46 x 10^6 cells/ mm^3, respectively, and the results showed significant differences (P<0.05) in hemoglobin concentrations for genotype AA which amounted to 9.29 mg/100 ml and genotype GA which amounted to 8.40 mg/100 ml, while GG genotype did not differ significantly with both structures with 8.71 mg / 100ml.

The results of packed blood cells (PCV) of AA,GA and GG genotypes showed that there were no significant differences (P>0.05) between them, which amounted to 33.30, 32.00 and 31.50%,

respectively, and there were no significant differences for the levels of total protein, which amounted to 2.02, 2.01 and 2.03 g/g. 100 ml for the genotypes AA, GA and GG, respectively. As for the site G24485A, the results showed that no significant differences (P>0.05) were recorded in the numbers of white blood cells between the genotypes, as it reached 131.22 x 10^3 cells/mm^3 in genotype AA and 117.80 × 10^3 cells/mm^3 for GA genotype and 132.07 × 10^3 cells/mm^3 for GG genotype. The results of the red blood cells showed that no significant differences were recorded between the genotypes AA, GA and GG, which amounted to 1.57, 1.44 and 1.44 × 10^6 cells/mm^3, respectively. The results showed that no significant differences were recorded in hemoglobin levels between genotypes AA, GA and GG, they reached 8.69, 8.57 and 8.98 mg/100 ml, respectively. The results showed that no significant differences were recorded for the size of the packef blood cells for the genotypes AA, GA and GG, which amounted to 31.70, 30.25 and 32.96%, respectively. It was also noted that no significant differences were recorded for the levels of total protein, they were 2.04, 2.00 and 2.02 g/100 ml for the genotypes GG, GA and AA, respectively.

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

Loci	Genotype	WBC	RBC	Hb	PCV	TP
	CC	136.27±2.64 A	1.65±0.09 A	9.23±0.22 A	33.38±1.26 A	0.01±2.02 A
T24074C	CT	6.62±116.77 B	0.07±1.42 A	0.17±8.13 B	1.13±32.33 A	2.01±0.01 A
	TT	3.78±131.87 A	0.06±1.47 A	0.22±8.90 A	0.96±31.55 A	2.03±0.01 A
	AA	135.94±2.69 A	1.63±0.08 A	9.29±0.21 A	33.30±1.14 A	0.01±2.02 A
A24375G	GA	5.59±122.17 B	0.05±1.45 A	0.20±8.40 B	32.00±0.96 A	2.01±0.01 A
	GG	4.67±130.87 AB	0.09±1.46 A	0.27±8.71 AB	1.29±31.50 A	2.03±0.01 A
	GG	131.22±4.29A	1.44±0.11 A	8.69±0.19 A	31.70±1.56 A	0.01±2.04 A
G24485A	GA	19.13±117.80 A	1.44±0.10 A	0.82±8.57 A	30.25±1.70 A	2.00±0.00 A
	AA	2.37±132.07 A	0.06±1.57 A	0.17±8.98 A	0.80±32.96 A	2.02±0.01 A

The analysis of blood characteristics is an important indicator of some conditions such as stress, pollutants, nutrition, as well as environmental and physiological conditions, as significant changes occur in the blood compositions in fish such as levels of hormones, proteins, glucose, cholesterol and other essential components, and fish blood parameters are closely related to the response of fish to environmental factors (Abed and Al-Shawi, 2015(. The blood in fish transports a variety of substances such as nutrients, hormones, minerals, and components of immunity, microorganisms, water, gases, toxins and feces (Ciesla, 2007). The most important functions of blood are the supply of oxygen and nutrients (including glucose, amino acids, and fatty acids) to cell tissues, removal of waste products (such as carbon dioxide, urea and lactic acid), immune functions, and clotting, given the important and diverse roles of blood, blood parameters may provide more accurate picture of fish metabolism and health status, as well as a blood picture can provide useful information about fish health, immune system response, short- and long-term effects of aquaculture conditions, water quality, potential disease outbreaks, and nutritional status (Rebl et al., 2021). Fish may face stress factors such as deteriorating water qualities, pollution, diseases, and the state of adaptation to environmental conditions is a reflection on the balance of the physiological activities of the fish such as red and white blood cells, hemoglobin, and packed cells, Red blood cells are the main cells of the blood, and their number is associated with the speed of movement and high activity of the body. **(**Rambhaskar and Srinivasa, 1986). It is concluded from the above results there is polymorphism in the growth hormone receptor gene in common carp in the three detected sites, and the significant effect of the difference in the genotypes of the growth hormone receptor gene on the physiological characteristics of common carp, and the presence of significant differences in some blood parameters (WBC, Hb) which indicates that there is an effect of polymorphism to reveal the effect of the gene to know the genetic differences between fish.

REFERENCES

Abed, R. S., and Al-Shawi, S. A. (2015). Effect of Supplementary Garlic oil and powder (*Allium sativum*) on some Growth characters, and liver enzymes and Blood picture In common carp. *Cyprinus carpio* Linnaeus. Thi-Qar University Journal for Agricultural Researches, *4*(2)

Al Khshali, M. S., and Saleh, N. A. (2020). RELATIONSHIP OF MYOSTATIN GENE POLYMORPHISM WITH SOME GROWTH TRAITS OF COMMON CARP *CYPRINUS CARPIO* L. The Iraqi Journal of Agricultural Science, 51(1), 317-322.

Al-Alnjawi, N. A., and Al-Khshali, M. S. (2020). Polymorphism of MSTN Gene (T2230C and A2232G) and Its Relationship with Some Physiological Traits and Growth of Common Carp (*Cyprinus carpio* L) Year 2108. Chief Editor Dr. Varsha Rani, 103, 15.

Al-Azzawy, M. A. N., & Al-Khshali, M. S. (2018). RELATIONSHIP OF GROWTH HORMONE GENE WITH SOME OF PRODUCTIVE TRAITS OF COMMON CARP *Cyprinus carpio*. L. The Iraqi Journal of Agricultural Science, *49*(6), 1011.

Ciesla, B. (2007). 2 From Hematopoiesis to the Complete Blood Count. In Library of Congress Cataloging-in-Publication Data.

De-Santis, C. and Jerry D. R., (2007). Candidate growth genes in finfish - Where should we be looking?.Aquaculture, 272, 1, 22–38.

Duncan, D. B. (2004). k-ratio t tests, t intervals, and point estimates for multiple comparisons. Encyclopedia of Statistical Sciences.

Guderley, H., and Blier, P. (1988). thermal acclimation in fish: conservative and labile properties of swimming muscle. Can. J. Zool., 66: 1105-1115.

Kopchick, J. J., and Andry, J. M. (2000). Growth hormone (GH), GH receptor, and signal transduction. Molecular genetics and metabolism, 71(1-2), 293-314.

Lupchinski, J.r.; Vargas,E.; Lopera-Barrero,L.; Ribeiro,N.M.; Povh,R.P.; Gasparino,J.A., and Braccini, G. L. (2011). Genetic characterization of three Nile tilapia (*Oreochromis niloticus*) strains. Arch .Zootec., 60:985-995.

Moutoussamy, S., Kelly, P. A., and Finidori, J. (1998). Growth-hormone-receptor and cytokine-receptor-family signaling. European Journal of Biochemistry, 255(1), 1-11.

Ozaki, Y., Fukada, H., Tanaka, H., Kagawa, H., Ohta, H., Adachi, S., and Yamauchi, K. (2006). Expression of growth hormone family and growth hormone receptor during early development in the Japanese eel (*Anguilla japonica*). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 145(1), 27-34.

Rambhaskar, B. and Srinivasa, R. K. (1986). Comparative haematology of the species of marine fish from Visakhapatnam coast. J Fish Biol 30:59–66.

Rebl, H., Bergemann, C., Rakers, S., Nebe, B., and Rebl, A. (2021). Plasma Treatment of Fish Cells: The Importance of Defining Cell Culture Conditions in Comparative Studies. Applied Sciences, 11(6), 2534.

Sakamoto, T., Shepherd, B. S., Madsen, S. S., Nishioka, R. S., Siharath, K., Richman III, N. H., ... and Grau, E. G. (1997). Osmoregulatory actions of growth hormone and prolactin in an advanced teleost. General and comparative endocrinology, 106(1), 95-101.

SAS . (2012). SAS User's Guide : Statistics Version 6th ed., SAS Institute Inc.

Tao, W., Ma, L., Ruan, R., and Yu, J. (2011). SNP loci associated with weight gain on growth hormone receptor genes in *Cyprinus carpio var. Jian*. Acta Hydrobiologica Sinica, 35(4), 622-629.

Trudeau, V. L. (1997). Neuroendocrine regulation of gonadotrophin II release and gonadal growth in the goldfish, *Carassius auratus*. Reviews of reproduction, 2, 55-68.

Yada, T., Nagae, M., Moriyama, S., and Azuma, T. (1999). Effects of prolactin and growth hormone on plasma immunoglobulin M levels of hypophysectomized rainbow trout, *Oncorhynchus mykiss*. General and comparative endocrinology, 115(1), 46-52.

Zhang, F., Zhang, Q., Tengholm, A., and Sjöholm, A. (2006). Involvement of JAK2 and Src kinase tyrosine phosphorylation in human growth hormone-stimulated increases in cytosolic free Ca2+ and insulin secretion. American journal of physiology. Cell physiology, 291(3), C466–C475.