The effect of the genotypes of the growth hormone gene and its relationship with indicators of blood biochemical characteristics of common carp *Cyprinus carpio L.*

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ABSTRACT

This study was conducted in the central of health laboratory/ Animal and Fish Resource Research Center / Agricultural Research Department / Ministry of Science and Technology, for the period from 15/8/2021 to 15/11/2021 with the aim of investigating in polymorphism the *Growth Hormone-1 Gene GH-1* and its relationship to a number of physiological traits (total protein, albumin and cholesterol) of *Cyprinus carpio L.* In 90 fish representing three different ecosystems (30 fish for each site), including floating cages, ponds, and the Tigris River south of Baghdad. The polymorphisms of the *growth hormone* gene were investigated using Single-Stranded Conformation Polymorphism (SSCP) and polymerase chain reaction (PCR).blood was taken from the caudal vein of each fish, and to extract DNA in addition to measuring , albumin and cholesterol.genotyping of samples by SSCP analysis showed six different banding patterns including A,B,C,E, F, and H with a frequency of 24.4, 5.6, 15.6, 15.6, 16.4, and 22.4, respectively, for a population of fish in the studied sites. The H Pattern was associated with the highest rate of protein concentration (4.05±0.11), and the same Pattern recorded the lowest rate of albumin and cholesterol (0.68±0.07) and (146.7±21.3), respectively.

Keywords. Common carp, *GH-1*, total protein, albumin, and cholesterol

INTRODUCTION

Fish is one of the most commercially traded foods in the world, as its breeding and trade expanded with the passage of time. The history of fishing in Iraq extends to nearly 4,000 years of history. More than 66 species of fish live in the environment of the internal Iraqi waters [3]. The most important economically are the common carp, *Cyprinus carpio*, and *Barbus xanthopterus*, *Barbus sharpeyi*, *Barbus esocinus*, and other species, and fish of the Cyprinidae family constitute the largest proportion of freshwater fish species [5].

In view of the commercial importance of common carp fish, many local studies have been conducted on them in many environments of Iraqi waters, including rivers, ponds, and cages. Common carp *Cyprinus carpio* has a long history in aquaculture in ponds, cages, channels, and other breeding systems. The Common carp is one of the economically important species due to its high nutritional value. therefore, maintaining the integrity of the genetic material of these fish requires methods based on accurate knowledge of their genetic map. as a result, many genetic studies on it have been conducted [13]. In addition to studying the effects of geographical area and adaptation, the growth process in vertebrates is under the control of a large number of hormones, as growth hormone leads the most important axis in growth among these hormones. It has been shown that there is a significant increase in the growth rate that can be achieved through high levels of growth hormone in the body and different genotypes. The growth hormone gene is associated with a number of productive performance traits such as growth, egg

production, and disease resistance in poultry [19].

In addition to its role in the physical growth of fish, growth hormone participates in a number of metabolic functions, including reproduction, osmotic regulation, and food digestion [4]. As a result of technological advances in molecular biology and genetic engineering that achieve high accuracy, low cost, and time reduction in the detection of genetic polymorphisms [10]**,** a number of genetic parameters (genetic markers) such as polymorphism were used in association with performance characteristics because they represent evidence of variation in the genome or genetic material [28].

Blood characteristics are an effective and sensitive indicator for understanding physiological and pathological changes in fish. Previous studies revealed that the difference in blood characteristics is caused by internal and external factors, including the method of blood sampling, laboratory techniques, seasonal variations, body size, genetic characteristics, sex, population density of fish, nutrition, environmental stress and transportation can affect blood traits [23]. Also, the physiological differences in blood values are an indicator of the influence of various environmental and chemical factors surrounding the fish [6].

MATERIALS AND METHODS

Fish samples

The common carp were collected from three different environments: floating cages in the city of Kut, ponds in the Mada'in area, and the Tigris River south of Baghdad. The fish were transferred to the central of health laboratory/ Animal and Fish Resource Research Center, by plastic containers, and when the fish arrived at the laboratory, after bringing the fish, it was left for two hours to get rid of the stress of fishing and transport the blood parameters were calculated to make the required measurements.

Blood properties

Blood samples were taken from the caudal vein of the fish using a syringe of 3 ml. The blood sample was divided into two parts. The first part was placed in a tube 5 ml free from anticoagulant (EDTA) and was used to obtain plasma through centrifugation at 3000 rpm for 10 minutes to separate the serum and put it in special sterile tubes for biochemical analysis and kept by freezing at -20°C. Plasma analyses included total protein, albumin and cholesterol. The second part was used to extract deoxyribonucleic acid (DNA) for molecular measurements.

DNA extraction and polymerase chain reaction

The DNA was extracted from blood samples of common carp according to the *EasePure*®Blood Genomic DNA Kit (TRAN), The concentration of DNA purity was measured by Nano Drop, the primer was prepared according to the manufacturer's instructions by dissolving the powder in water free of nucleic acid enzymes (DNase & RNase) at a final concentration (100 pM/µl) as a working solution and stored at a temperature of -23 °C until use. To prepare a working solution with a final concentration of 10 µl by diluting 10 pmol/µl in 90 µl of water free of nucleic acid enzymes and stored at a temperature of (-23 °C) until use, and its sequence appears in Table (1) according to what was mentioned in [20], and it was verified through the program primeer3 EU333984 plus (Gene bank). Amplified DNA by PCR and the primer works according to its conditions and stages for the polymerase chain reaction, which are as follows: Primary denaturation stage 94°C, one cycle for 5 minutes; denaturation stage 94°C, 35 cycles, 30 seconds per cycle; coalescence stage 58°C, 35 cycles, 30 seconds per cycle; elongation stage 72°C, 35° cycles, 1 minute per cycle; final elongation stage 72°C, one cycle for 5 minutes. Then the Gel electrophoresis to the PCR products, electrical power has been turned on at 50 volt for 40 minutes afterwards the DNA moved from cathode (-) to anode (+) poles. The ethidium bromide stained bands in the gel has been visualized using UV transiluminator at wave-length 350 nm and photographed and the method used by [24].

Table 1. Primers of the interaction of the growth hormone (*GH-1*) gene used in the study.

Primers of *GH-1* (459 pb)	
Forward primer	5`-CACCTCATTGAGTCCTGGGA-3`
Reverse primer	5`-TACACCGGTGCCATCTACAG-3`

Detection of Single-Stranded Conformation Polymorphism

The Single-Stranded Conformation Polymorphism (SSCP) technique is a simplified and sensitive method for detecting mutations and genotyping. The principle of this technique is based on the fact that single-stranded DNA has a specific Mass and size. A change in composition due to a change of one or more bases in its sequence can cause a change in the electrical relay pattern differently. Therefore, dominant and mutated DNA samples show different patterns of bands during their electrophoresis.

This method includes four basic steps:

1. PCR-based amplification of the required gene
2. Denaturation of DNA strands
3. Single-stranded DNA quenching
4. Detection of the difference in the movement of DNA strands in the gel during electrophoresis with the presence of a standard reagent ladder (which contains different sizes and sequences of DNA for the purpose of comparison) [7].

Statistical analysis

The statistical program (Statistical Analysis System-SAS) was used in data analysis [25] according to the completely randomized design (CRD) for more than one factor. The significant differences between the means were compared with the Duncan test [15] by applying the method of least square means to find the significant differences among the different treatments at the probability level (0.05) and the frequency of genotypes was calculated using a program. (software Popgege, version 1.32) [22] according to the following mathematical model equation:

$YijK = \mu+Ai+Bj+ Eijk$

YijK = Observation value k of treatment i and genotype j

μ = the general average of the studied trait

Ai= treatment effect

Bj = genotype effect

Eijk = random error value

RESULTS

The results of Genotyping of samples by SSCP technique for the *GH-1* in common carp yielded 6 different banding Patterns including A, B, C, E, F, and H (fig. 1). The polymorphisms were had a significant impact on the studied traits. The pattern H was superior to the other polymorphisms, and recorded the highest values for the of albumin and cholesterol. In addition recorded the lowest values for age and total protein. While the pattern B was superior to the rest of the polymorphisms in the rate of total protein .

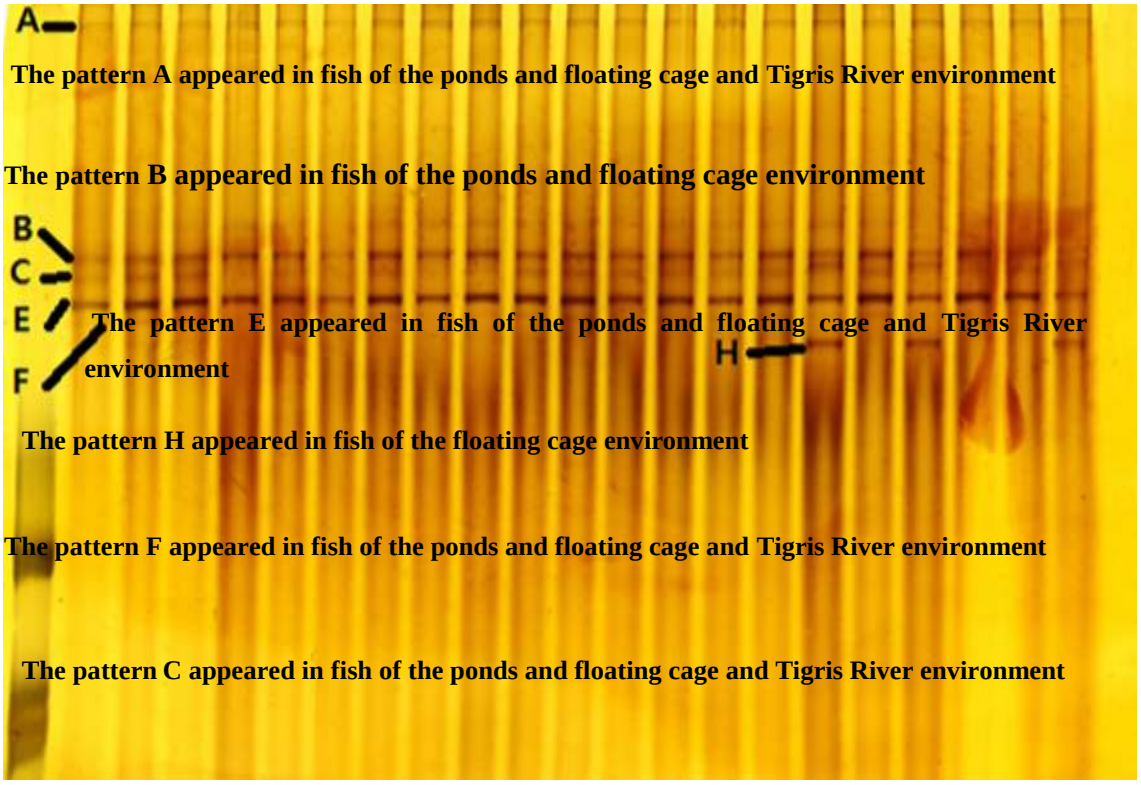

Figure 1. The diverse polymorphisms of *Cyprinus carpio L.* in cages, ponds, and river environments

The pattern H appeared in fish of the floating cage environment
The pattern B appeared in fish of the ponds and floating cage and Tigris River environment
The pattern (F,E,C,A) appeared in fish of the Tigris River environment
The pattern (F,E,C,B,A,H) appeared in fish of the floating cage environment
The pattern (F,E,C,A,B) appeared in fish of the ponds environment

The pattern (F,E,C,A) appeared in fish of the Tigris River environment

Polymorphisms

The results of the current study showed that the size of a piece (459 pb) containing exon 4, intron 4 and exon 5 of the growth hormone *GH-1*, which was amplified from the blood of common carp fish. The gene was investigated and it was noted that it is consistent with most previous studies published by the National Center for Biotechnology Information (NCBI). The analysis of the SSCP technique revealed the presence of six different banding patterns of this hormone, including H, F, E, C, B, and A, with a frequency of 24.4, 5.6, 15.6, 15.6, 16.4, and 22.4, respectively, for the fish community in the studied sites (Table 2).

Table 2. The polymorphisms frequents of the growth hormone *GH-1* in samples of common carp *C. carpio*

Pattern	Frequency %	Location
A	24.4	cages, ponds, river
B	5.6	cages, ponds
C	15.6	cages, ponds, river
E	15.6	cages, ponds, river
F	16.4	cages, ponds, river
H	22.4	Cages

Table 3. Relationship of genotypes with blood enzymes (total protein, albumin and cholesterol) of common carp C. carpio raised in different ecosystems (mean ± standard error).

Pattern	Total protein g/dL	Albumin g/dL	cholesterol mg/dL	Environmental location
A	2.68±0.12 b	1.05±0.09 a	153.7±10.20 b	cages, ponds, river
B	1.87±0.17 c	1.02±0.10 a	236.3±14.10 a	Ponds
C	2.14±0.12 c	0.84±0.06 ab	150.4±22.90 c	cages, ponds, river
E	2.01±0.10 c	0.90±0.04 ab	149.4±22.80 c	cages, ponds, river
F	2.76±0.13 c	0.76±0.08 ab	242.4±17.90 a	cages, ponds, river
H	4.05±0.11 a	0.68±0.07 b	146.7±21.30 c	Cages

The averages with different letters within the same column differ significantly between them. At the level of significance ($P<0.05$)

Table (3) shows the relationship of genotypes with rates of total protein concentration, albumin concentration and cholesterol in the blood of fish for the studied ecosystems. The H genotype was associated with the highest mean protein concentration and reached (4.05±0.11), and the same type recorded the lowest rate for albumin and cholesterol concentration (0.68±0.07).) and (146.7±21.3), respectively. The values remained between the different genotypes, and this indicates the association of these genotypes with the indicators of the physiological status of the fish and the nature of the environment in which they were raised. Blood plasma is affected by several factors such as fish farms [26], diseases [11] and age and stress [12]. Serum enzyme concentration values are often used to assess health status and stress indicators in fish.

Blood measurement is an important indicator of conditions such as stress, pollutants, nutrition, as well as environmental and physiological conditions. Significant changes occur in the blood composition of fish such as levels of hormones, proteins, sugar, albumin, cholesterol and other essential components. Measurements of fish blood are closely related to the response of fish to environmental and biological factors [16]. The significant increase in the concentration of total protein and the decrease in the concentration of cholesterol and albumin, which were shown by the results of the current study in cage fish, is due to the direct effect of growth hormone on the gene expression centers in the target tissues, and this effect led to the control of lipid synthesis and metabolism in the blood serum, and this is a case Healthy fish by increasing protein synthesis and reducing inflammatory factors represented by albumin and cholesterol [8].

Growth hormone affects fat metabolism by activating the acetate group in hepatic fat, which affects the lipid synthesis pathway [27]. Growth hormone concentration in serum The results of the current study showed significant effects of the genotypes of the growth hormone gene on the average concentration of total protein, albumin and cholesterol.

The relationship of the growth hormone gene with cholesterol, total protein and albumin

The results in figures (2, 3, 4) showed that a significant effects of polymorphisms on the rates of total protein, albumin, and cholesterol of common carp in different ecosystems, where the pattern H (4.05 g) outperformed in total protein on the rest of the polymorphisms (A), (B), (C), (E), and (F) 2.68, 1.87, 2.14,

2.01 and 2.76 g/dL, respectively, while the pattern A (1.05 g) outperformed in the albumin on the rest of the polymo-rphisms (B), (C), (E), (F) and (H) 1.02, 0.84, 0.90, 0.76, and 0.68 g/dL, respectively, whereas the pattern F (242.4 mg) outperformed in cholesterol on the rest of the polymorphisms (A), (B), (C), (E) and (H) 153.7, 236.3, 153.4, 149.4, and 146.7 mg/dL, respectively

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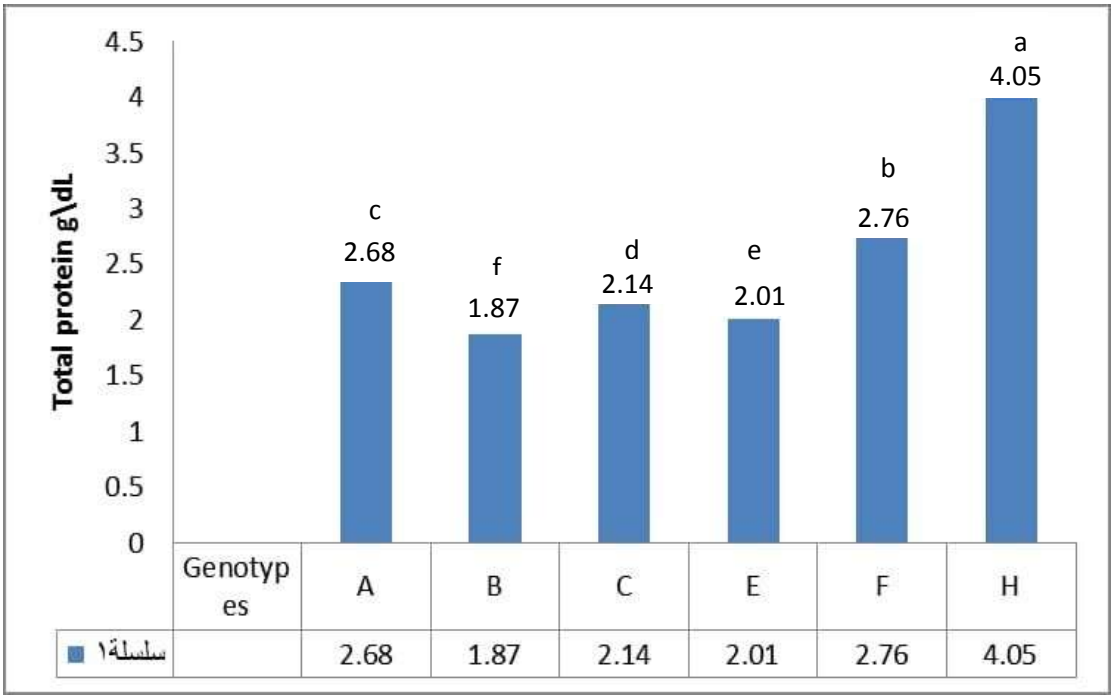

Figure 2. Effect of polymorphisms on total protein (g/dL) of *Cyprinus carpio L.* in three ecosystems: floating cages, ponds and the Tigris River

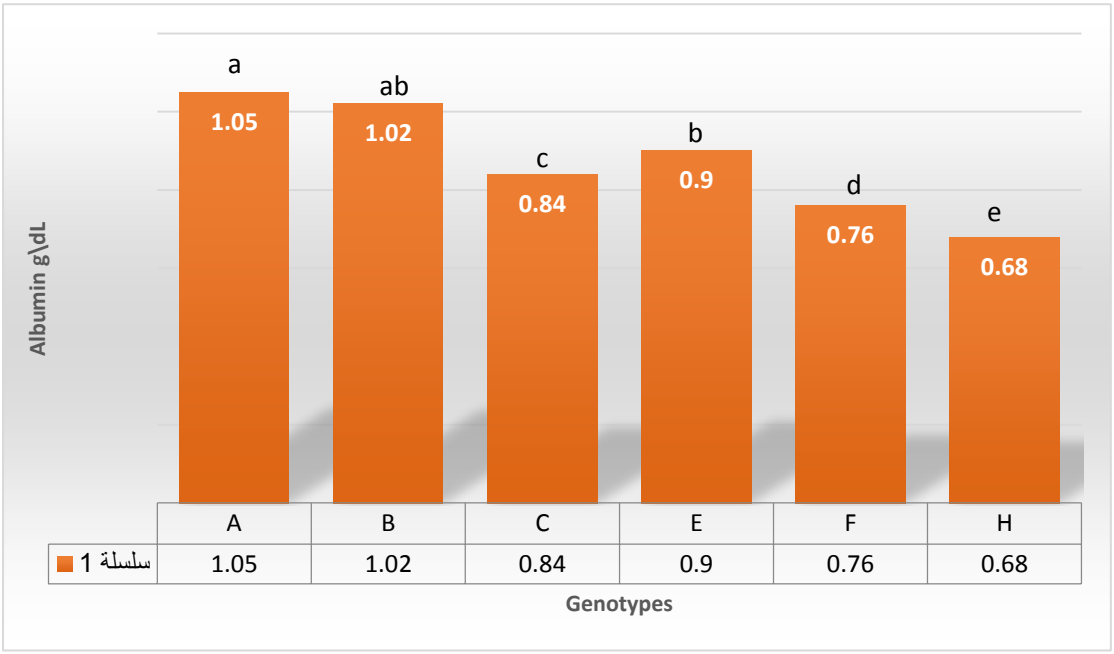

Figure 3. Effect of polymorphisms on albumin (g/dL) of *Cyprinus carpio L.* in three ecosystems: floating cages, ponds and the Tigris River

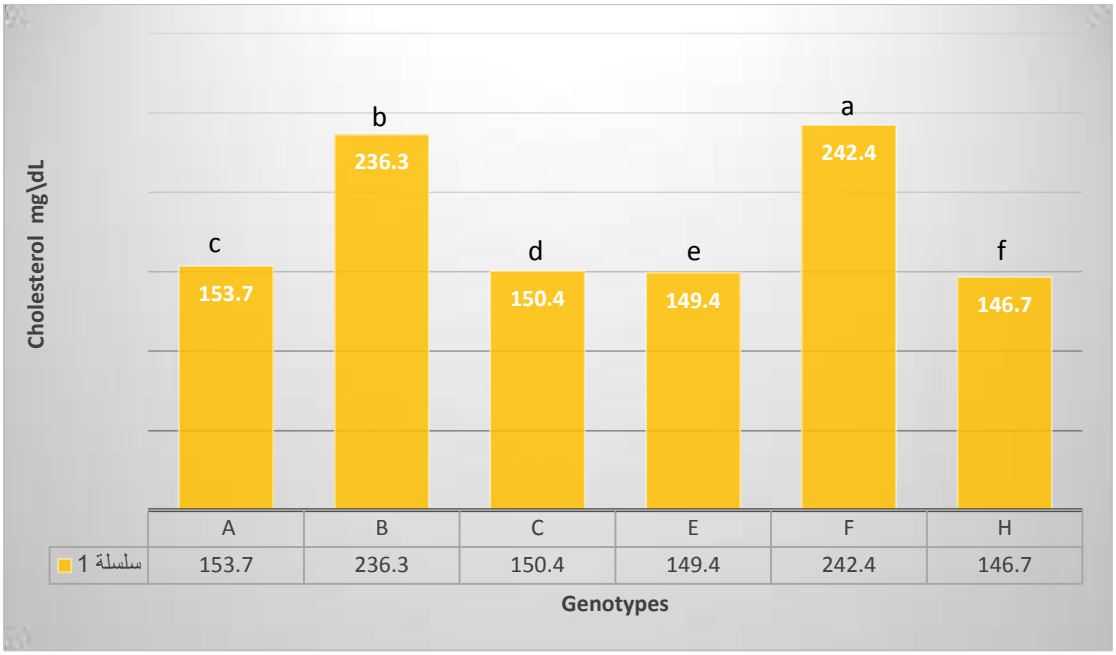

Figure 4. Effect of polymorphisms on cholesterol (g/dL) of *Cyprinus carpio L.* in three ecosystems: floating cages, ponds and the Tigris River

DISCUSSION

The alleles of *GH-1* contain different non-coding regions (introns), while the coding regions (exons) are highly similar due to the specific function of the hormone [18]. In this study, several polymorphisms of the *growth hormone* gene *(GH-1)* were found in different ecosystems. It is known that the difference in introns does not lead to a change in the amino acid sequence of the gene, where it is removed by splicing when mRNA in transcribed, but it plays a role in regulating the gene expression of growth hormone inside the body.

Measuring biochemical indices in fish blood are a valuable and important biological indicator in the processes of disease diagnosis and health prediction, and changes in these values can be used as indicators of disease, malnutrition, or environmental deficiencies [21]. The levels of total protein, cholesterol, and triglycerides are key indicators of health in fish. Increased protein concentration is

accompanied by decreased activity of the enzyme aminotransferase, which indicates low deamination capacity and lack of protein catabolism as well as lack of fluid balance control [14]. High cholesterol levels indicate disorders of lipid and lipoprotein metabolism, especially in liver disease [2]. The results showed that these indicators were significantly higher in cage fish as shown in Table 3., which is consistent with the results of [14] on sea fish and a study by [17] on grass carp. [1] mentioned that adding one medicinal plant, aniseed, to the diets of common carp led to an improvement in the growth and health of the fish, and the blood characteristics were not affected by the addition of this plant.

The biochemical blood characteristics are an important tool that can be used as effective and sensitive indicators for monitoring physiological and pathological changes in fish. Normal ranges for indicators of blood values for fish have been established by specialists in fish physiology and pathology [29]. Studies indicated that measuring blood parameters is important to know the health status of fish, metabolic disorders, pollutants, stress, environmental and physiological conditions, and all of these factors mentioned above lead to significant changes in fish blood indicators such as levels of hormones, proteins, sugar, albumin, cholesterol and other blood values. Indicators of fish blood values are closely related to the response of fish to environmental and biological factors [16], and

serum biochemical measurements are often used to assess health status and stress indicators in fish [9]. The results of the current study also showed significant effects in the percentage of total protein, albumin and cholesterol in the blood in different fish breeding environments, where a significant difference was recorded in pond fish at 4.05 g/dL in total protein concentration compared to cages and river fish at 2.68 and 2.14 g/dL, respectively as shown in Table 3.

CONCLUSION

Based on the obtained results, it is very likely that the polymorphisms has a significant effect on the growth of common carp and that most of the polymorphisms were significantly associated with total protein, albumin, and blood cholesterol by the different polymorphisms in all environmental regions. Therefore, further studies are required on other sites of the *GH-1* and genes directly related to growth traits, food conversion, and disease resistance, and to find the best genetic combination through which maximum

REFERENCES

[1] Ahmed, Muhammad Ali, Muhannad Habas Al-Ashaab and Raed Sami Atti. (2017). Effect of adding anise and cinnamon on growth indicators and some physiological characteristics of the diets of young common carp fish, Cyprinus carpio. Diyala Journal of Agricultural Sciences. 9(2) 16-28.

[2] Allen, F.M, Patrick JW, Roger TH (2005). Blood biochemistry of the oyster toadfish. Journal of Aquat Anim Health 17:170–176.

[3] Al-Faisal, A. J. Updating checklist of freshwater fishes of Iraq. Mesopotamia Environmental Journal . 2020, Vol.5,No.4:1-7.

[4] Almuly, R. ; Poleg-Danin, Y. ; Gorshkov, S. ; Gorshkova, G. ; Rapoport,B. ;Soller,M. ; Kashi, Y. and Funkenstein, B.(2005). Characterization of the 5′flanking region of the growth hormone gene of the marine microsatellite in the proximal promoter. *Fish Sci*., 71: 479–490.

[5] Al-Tamimi, Louay Muhammad Abbas. (2004).Ecology,life and assessment of the fish community in the Euphrates River near Musayyib power station .Doctorate thesis, College of Agriculture, University of Basra, 147 pages.

[6] Anthony, O., kechukwu, I. and Obinnaya, C., 2010.Haematological profile of Nigeria. Journal *American Science*, 6, 123-30.

[7] Badi, M. A., Al-Shuhaib, M. B. S., Aljubouri, T. R., Al-Thuwaini, T. M., Dawud, H .H ., Hussein, T. H ., . & Mohammed , A . K . (2021) . Rapid and optimized protocol for efficient PCR-SSCP genotyping for wide ranges of species. *Biologia*, *76*(8), 2413-2420.

[8] Berenjkar N.S; Khalesi M.K.; Rahimi M. G.; Farhadi A .O.2015.Association between growth hormone gene polymorphisms and growth traits in wild common carp, Cyprinus carpio from the Caspian Sea. Iranian Journal of Fisheries Sciences. 17:3 (2018) 533-541.

[9] Brown, J. A. ; Moore, W. M. and Quabius, E. S..(2001). Physiological effects of saline waters on zander. J. Fish Biol., 59: 1544-1555.

[10] Caetano, A. R. (2009).Marcadores SNP: conceitos básicos, aplicações no manejo eno melhoramento animal e perspectivas para o futuro.Rev. Bras. Zootec., 38: 64-71.

[11] Chen, Y.E., Jin, S. and Wang, G.L., 2005. Study on blood physiological and biochemical indices of Vibrio alginilyticus computer-assisted image analysis. *Acta Veterinary Brno*, 66, 101-10.

[12] Cnaani, A., Tinman, S., Avidar, Y., Ron, M. and Hulata, G., 2004.Comparative study of biochemical parameters in response to stress in O.aureus, O. mossambicus and two strains of O. niloticus. Aquaculture Research, 35, 1434-1444.

[13] Chistiakov, A. and Voronova ,V.(2009).Genetic evolution and diversity of common carp Cyprinus carpio L.Cent. Eur. J. Biol. 4(3) . 304–312.

[14] Coz -R -Rakovac R, Strunjak- perovic I, Hacmanjek M, Topic PN, Lipej Z, Sostaric B (2005). Blood chemistry and histologica properties of wild and cultured sea bass Dicentrarchus labrax) in the north Adriatic Sea. Vet Res Commun 29:677–687.

[15] Duncan, D.B. (1955). Multiple Rang and Multiple F-test. Biometrics. 11: 1 – 42.

[16] Fernandes , M . N. and Mazon , A . F .(2003). Environmental pollution and fish gill morphology. In: Val AL, Kapoor BG (eds) Fish adaptation. Science, Enfield, pp 203– 231.

[17] Hrubec ,T.C, Smith S.A . Weiss D. J, Wardrop K. J .(2010). Hematology of fishes. In:Schalm veterinary hematology. Ames (LA): John Wiley and Sons; p. 994-1003.

[18] Ma , Q. ; Liu , S. ; Zhuang , Z . ; Lin , L . ; Sun , Z . ; Liu , C . and Tang , Q. (2012).Genomic structure, polymorphism and expression analysis of the growth hormone (GH) gene in female and male Half-smooth tong sole (*Cynoglossus semilaevis*). *Gene*, 493: 92–104.

[19] Marques, D. S.(2009). Polimorfismos no gene do Hormônio do Crescimento em linhagens de codorna (Couturnix japonica) e sua associação com características de desempenho. 73 f. Dissertação (Mestrado em Zootecnia) –Universidade Estadual de Maringá, Maringá , Paraná.

[20] Murakaeva, B.A. (2008). Structure, evolution and expression of the duplicated growth hormone genes of common carp (*Cyprinus carpio L*.). Ph.D. thesis, Humboldt University, Berlin, Germany.

[21] Peres, H. Costas, B. Perez-Jimenez ,A. Guerreiro, I. Oliva-Tele , A . values for selected haematological and serum biochemical parameters of Senegalese sole (Solea senegalensis Kaup, 1858) juveniles under intensive aquaculture conditions. J Appl Ichthyol 2015; 31: 65 – 71.

[22] Quinn, G.P. and Keough, M.J., (2002). Experimental design and data analysis for biologists. Cambridge University Press. Cambridge,537 P.

[23] Rey Vazquez, G.; Guerrero, G.A. (2007). Characterization of blood cells and hematological parameters in Cichlasoma dimerus. J Tissue Cell 39:151– 160.

[24] Sambrook , J . ; Fritschi , E .F. and Maniatis, T .(1989). Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, New York.

[25] SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1thed. SAS. Inst. Inc. Cary. N.C. USA.

[26] Svobodova Z, Kroupova H, Modra H, Flajshans M, Randak T, Savina .LV, Gela D (2008). Haematological profile of common carp spawners of various breeds. Journal of Applied Ichthyology 24, 55 –59.

[27] ThidarMyint, H.; Yoshida, H.; Ito, T.; He, M.; Inoue, H. and Kuwayama, H. (2008). Combined administration of ghrelin and GHRH synergistically stimulates GH release in in Arctic charr (Salvelinus alpinus L.). Heredity, 34:118-123 .

[28] Wang, S.; Sha, Z., Sonstegard, T. S.; Liu, H., Xu, P.; Somridhivej, B., Peatman,E., Kucuktas, H. and Liu, Z.(2008). Quality assessment parameters for EST- derives SNPs from catfish. BMC Genomics, 9:1-11.

[29] Xiaoyun, Z. ; Mingyun, L. Khalid, A. and Weinmin, W. (2009).Comparative of haematology and serum biochemistry of cultured and wild Dojo loach *Misgurnus anguillicadatus*. Fish Physiol Biochem 35:435– 441.