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RELATIONSHIP OF THE LEPTIN HORMONE GENE WITH SOME OF THE GROWTH CHARACTERISTICS OF COMMON CARP *CYPRINUS CARPIO* L.

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Abstract

This study was conducted with the aim of determining the genotypes of the leptin gene and its relationship with total weight gain rates and the daily, relative and specific growth rates in 66 samples of common carp (*Cyprinus carpio* L). Results of Sequencing and Single Nucleotide Polymorphism-SNP showed three genotypes at site of 2092 CC, CT and TT, and distribution ratios of genotypes were 4.55, 12.12 and 83.33%, respectively, and the differences between these percentages were highly significant ($P < 0.01$). The allelic frequency of the allele was 0.11 in C genotype, while the allele frequency of T was 0.89. Results showed that there were no significant differences between the genotypes of the studied growth traits, the total weight gain rates in the genotype CC reached 45.66 g/fish, and in the genotype CT 42.25 g/fish, while in the genotype TT was 36.30 g/fish. The daily growth rates were 0.65, 0.60 and 0.51 g/day in the genotypes CC, CT and CT, respectively, and the relative growth rate was 47.29% in the genotype CC, and 44.58% in the genotype CT, while it was 39.87% in the genotype TT. The specific growth rates were 0.55, 0.52 and 0.46% /day in the genotypes CC, CT and TT, respectively.

Key words: leptin hormone gene 1, Single Nucleotide Polymorphism-SNP, growth traits, common carp.

Introduction

World fisheries are experiencing a sharp decline in fish stocks due to overfishing and increased demand for fish and their products, and further increases in the amount of fish produced under current conditions are not expected (Dunham *et al*., 2001) . Fish farming is the last hope for a sufficient amount of fish in the world, as it is a cheap source of animal protein (Ayoola and Idowu, 2008). Relatively recent developments in molecular biology have led to new technologies that have subsequently been applied to screening, characterizing, and assessing genetic diversity in a variety of aquatic organisms, including common carp. The use of genetic markers allows for the careful analysis of fish populations, which is often not possible with morphological signs alone, and knowledge of genetic diversity and the structure of fish populations is absolutely essential for any effective fish management or conservation program (Feng *et al*., 2016). The traditional genetic improvement processes of farm animals in general, whose reliance on statistical methods and their focus on electing individuals distinguished in terms of external appearance have provided not few gains in the field of genetic improvement, on the other hand, scientific progress and the availability of sufficient information on the work of the genome has facilitated Whoever develops election programs with greater accuracy, less time and cost, as the economic characteristics are located under the control of a number of genetic sites, which are called the quantitative traits loci (QTL) sites , and by identifying these sites and determining the associated features. The phenotypic variation of the traits that are to be improved early can not be predicted and election programs based on them. These markers are functional mutations in the genes that affect the traits (Williams, 2005). Obesity in fish is subject to many factors, the most important of which are the hormones that are under the control of genes. Among these hormones is the leptin hormone that is encoded by the leptin gene and controlled by many glands, the most important of which is the hypothalamus (Liu *et al*., 2010). The leptin hormone consists of 167 amino acids, and is excreted from the adipose tissue, hypothalamus, stomach and skeletal muscles (Gale *et al*., 2004). The leptin hormone is among the most important

hormones that work to regulate appetite and the amount of food and controls the weight gain and the spread and burning of fats in the body. It also plays an important role in the production of Lipogenesis, and leptin has an important function, which is the reference to the brain and other tissues from the state of the body and the bond of the body The concentration of leptin in the blood is positive with the amount of fatty deposits deposited (Agarwal *et al*., 2009).

Meterials and Methods

This study was conducted for the period 10/12/2018 to 10/3/2019, fish were placed in glass tanks with dimensions of 60cm × 40cm × 30cm, then the tanks were filled with water with a volume of 60 liters, and were equipped with air pumps to provide constant oxygen and 100-watt hydrates to obtain a suitable temperature for common carp growth of 25°C. The diet feed was purchased from the local market with a diameter of 4 mm, the ratio of dry matter 95.43, the protein 28.82%, and the fat 7.30%. All fish were placed under the same environmental conditions as oxygen, heat and feeding. Fish were tagged by a Chinese-origin numbering device, which is a pistol that contains a needle with plastic numbers, using a DYMO type tray printer to print the plastic numbers, as the exact number was placed near the dorsal fin through the hole in the place by the numbering pistol, and information was recorded for each A fish According to the numbers, the fish were then sterilized with a 0.5% saline solution for two minutes.

Studied productive traits

1) Total Weight Gain (T.W.G)g

It is the difference between the final weight and the Initial weight of the fish and it is calculated as follows (Uten, 1979):

T.W.G. = F.W. – I.W.

As: (F.W) Final weight (g), (I.W) Initial weight (g)

2) Daily Growth Rate ((D.G.R) g/day

It is defined as the calculated weight gain of the fish per day (g/day) and was calculated from the formula mentioned by Jobling (1993):

D.G.R. = (W2 - W1)/(T2 - T1)

As: W1 = Initial weight (g), W2 = final weight (g)

T2 - T1 = the time period between the two weights.

3) Relative Growth Rate (R.G.R)%

It is defined as the amount of weight gain attributed to the Initial weight and was calculated according to the formula mentioned by Uten (1979):

R.G.R. = {(W2 - W1) / W1} × 100

As: W1 = Initial weight (g), W2 = final weight (g)

4) Growth Rate Specific (S.G.R.) (%/Fish)

The daily weight gain is expressed as a percentage of the natural logarithm of the final and Initial weights, estimated in days Brown (1957):

S.G.R. = {(L n W2 - L n W1)/(T2 - T1)} × 100

As:

LnW2= natural logarithm of final weight at time T2.

LnW1= natural logarithm of Initial weight at time T1.

T2 - T1 = the time period between the two weights.

DNA Extraction

DNA was extracted from fish blood samples for the purpose of molecular examination of the leptin gene 1, according to the method of ABIO pure. Primers were selected and as shown in Table 1 for the purpose of performing molecular detection and phenotypic multiplication of genes and mutations present in the leptin gene 1 (Tang *et al*., 2016). The initiator of the Korean company Macrogen was prepared as a powder.

Statistical Analysis

Statistical Analysis System (SAS, 2012) was used to analyze the data according to the Complete Randomized Design (CRD), using the method General linear model (GLM) for the lost data and compared the significant differences between the mean of the genotypes according to the Dunkin test Multiple range test Duncan at the probability level (0.05) and the following law was applied to calculate the allelic frequency according to the Hardy Weinberg's equilibrium rule.

Results and Discussion

Results of the sequence of nitrogenous bases included all the required information, and included the sequence of the nitrogenous bases of the gene segment of 343 bp, and also included the genetic mutations and the genotypes, and the results were aligned (Alignment) on the global website of the Gene bank (www.ncbi.nim.nih.gov) and showed a match With the leptin gene in common carp fish, the results also showed that the mutation site was in the

Table 1: Primers Sequence.

Primer Name	Seq.	Annealing Temp.^{0}C	Product size
C. carpio_leptin-F	5'- CTGCACTGGTGCCAAGTTTAA-3'	60	334bp
C. carpio_leptin-R	5'-ACCTCAGGGTAAAGTTCTGGATC-3'		

second Exon, specifically at the location of 2092 bp, and the multiplicity of the genetic manifestations of the gene will be discussed at the said site.

Genotypes of the leptin gene (Site C2092T)

Results of sequencing determination of the leptin gene showed a change in Single Nucleotide Polymorphism-SNP in the second exon, specifically in the location of 2092 bp from the leptin gene 1, as the base changed from T to C and the genotypes were extracted by Geneious Software Version 10.1.3 The fish sequences were compared with the genotype of the common carp found in the World Gene bank.

Number: LOC109110334 ,The Alignment of the fish sequences showed that three genotypes were found at the studied site of the leptin gene 1 (Fig. 1). The presence of one green curve indicates the TT-Mutational genotype. Not getting a boom in Both strips, and the presence of green and blue curves indicates the CT-Heterozygous mutation, that is, a mutation in one of the strips, and the presence of one blue curve and the change of the base at the bottom of the curve indicates the CC-Wild genotype of a change in both strips.

Table 2 shows the numbers and percentages of the distribution of genetics of the fish, as the percentage was 4.55% for fish bearing the CC genotype, 12.12% for fish bearing the genotype CT and 83.33% for fish with the genotype TT, and the results showed a highly significant superiority of fish carrying the genotype TT and the lack of carrier of the genotype CC has been applied the law for the calculation of allelic repetition according to the Hardy and Weinberg equilibrium rule, as the frequency of alleles C 0.11% and alleles T 0.89%. We note the superiority of fish carrying the mutant genotype TT.

It is noted the superiority of the genotype TT on the rest of the genotypes, and the reason for this may be due to an uncontrolled strike and mixing between common

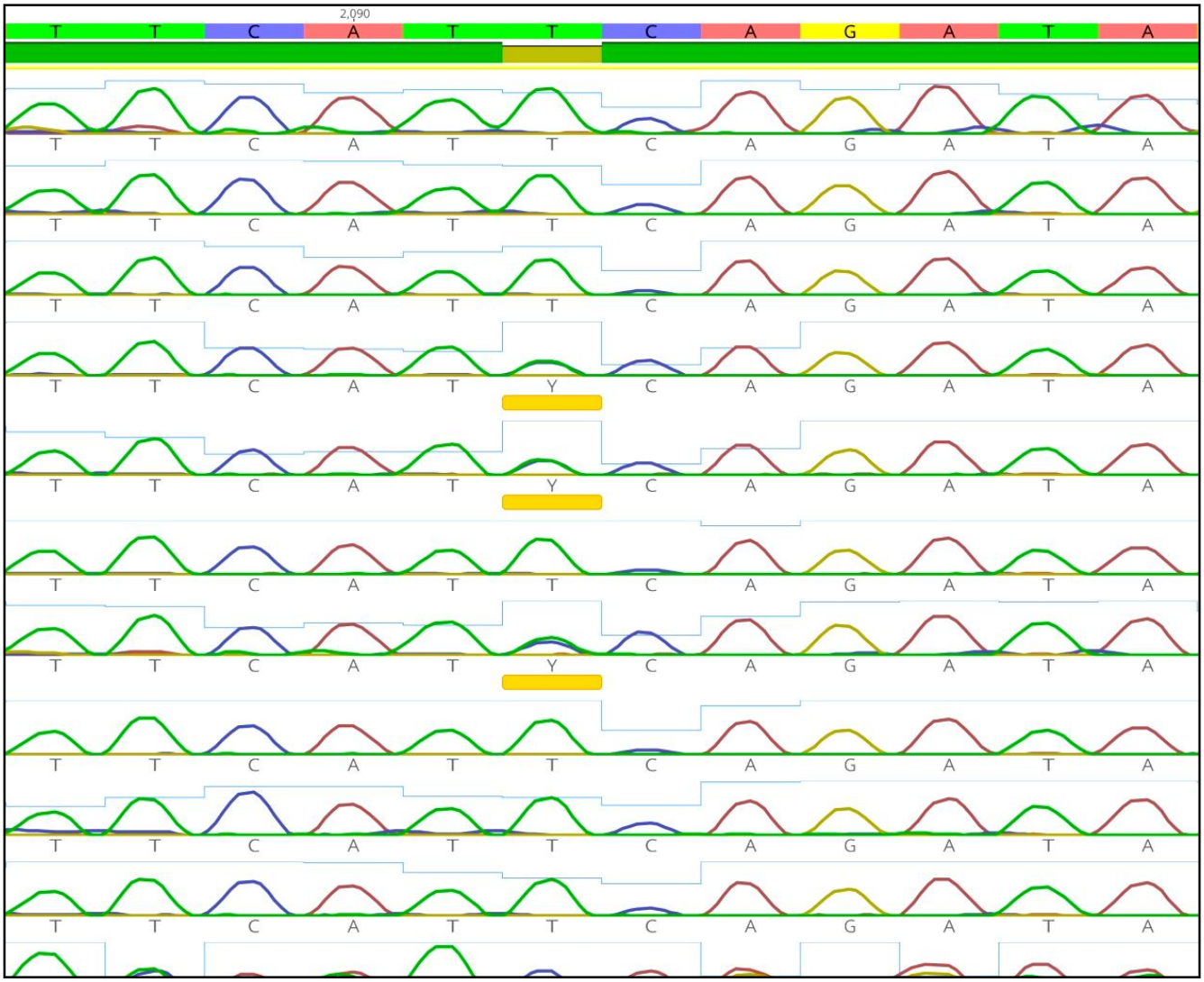

Fig. 1: Genotypes at the 2092 site of the leptin gene

carp strains, especially that Iraqi internal waters contain strains of common carp from different places and sources, and that the intermarriage between these strains may have led to the production of these forms With a greater percentage compared to fish carrying CT and CC genotypes in fish farms, the results of the current study were consistent with the results of Tang *et al.,* (2016) in their study of common carp, as the distribution rates of genotypes at the site T113C were 2.4% for wild genotypes (CC) , 47.5% for heterozygous genotypes (CT) and 50.1% for mutant genotypes (TT), and the frequency of the allele T was 73.85% and the allele C was 26.15%, and the results of the current study did not match the results of the Wu *et al.,* (2016) study when they studied golden pampano (*Trappotos blochii*) as they were extracted Three genotypes are TT, CT, and CC at the site C203T in the leptin hormone gene. The percentages of genotypes were 3.35% for the genotype TT, 39.60% for the genotype CT and 57.05% for the genotype CC, The allele frequency T 23.15% and the allele C 76.85%. In other studies on fish in this area it may be due to the different environmental conditions and the size of the studied sample.

Effect of polymorphism of the leptin gene on some site growth characteristics (site T2092C).

It is clear from Table 3 that there are no significant differences in the daily growth rates, the total weight increase rates and the relative and specific growth rates of common carp according to the different genotypes of the leptin gene. The daily growth rates in the genotypes CC, CT and CT reached 0.65, 0.60 and 0.51 g/day consequently. The total weight gain rates in the genotype CC reached 45.66 g / fish, and in the genotype CT 42.25 g/fish while in the genotype TT the total weight gain rate was 36.30 g/fish, and the relative growth rate in the genotype CC was 47.29%, In the genotype CT 44.58%, while in the genotype TT 39.87%, while the growth rate has reached specific genetic compositions CC and CT and TT0.55, 0.52 and 0.46% /day, respectively.

Table 2: Number and percentages of genotypes and allelic frequency of the leptin gene (mutation C2092T).

Genotype	Number of fishes	Percentage (%)
CC	3	4.55
CT	8	12.12
TT	55	83.33
Total	66	100%
Kai square value (÷2)		119.82 **
Allele		Frequency
C		0.11
T		0.89
** (P<0.01)		

The polymorphism of leptin hormone gene related to performance is the goal of many studies in breeding programs, and the determination of polymorphism in the leptin hormone gene for common carp fish is still unknown or below the required level, and by observing the current study in the results The C2092T site obtained from the hormone leptin gene did not significantly affect the growth traits, either positively or negatively, despite the observation of mathematical increases for the studied traits of the genotypes in the site where the mutation occurred, and that the differences other than total Beer of the data led to the absence of significant differences between the genotypes, as these results indicate that the mutation occurring at the site C2092T did not affect the expression centers in the gene, and therefore there is a difficulty in the election between individuals carrying these structures, as the dependence of the genetic expression (Gene Expression) In improving the characteristics studied, it seems to be ineffective.

The overall weight increase, daily growth rate, and relative and specific growth rate are commercially important criteria for animal husbandry and aquaculture, and these characteristics are influenced by genetic makeup (controlled by a large number of genes) and thus selection is made on the basis of these characteristics (Schwartz *et al*., 2000), and are subject to The nature of management and environmental factors (Pickering, 1993). There is no doubt that studies on the genetic improvements in common carp are few and, if any, they achieve what is required in this aspect. Optional breeding may be the most effective method for improving fish farming and other farm animals (Venter *et al*., 2001).

Table 3: The effect of polymorphism of the leptin gene on some growth characteristics of common carp during the trial period (mutation C2092T).

Characteristics	Genotype		
	TT	CT	CC
Daily growth rates (g/day)	0.03±0.51a	0.04±0.60a	0.05±0.65a
Total weight gain rates (g/fish)	2.44±36.30a	2.85± 42.25a	3.92±45.66a
Relative growth (%) rates	2.71±39.87a	3.83±44.58a	4.28±47.29a
specific growth (%/day) rates	0.02±0.46a	0.03±0.52a	0.04±0.55a

Perhaps the reason for the absence of significant differences due to the lack of a positive relationship between the hormone leptin and obesity has been recorded by Londraville *et al.,* (2014), the role of leptin as an indicator of obesity may not be evident in fish. The levels of leptin in the blood are strongly correlated with the amount of fat accumulation. When the fat stores are high, the leptin levels in the plasma

are high and vice versa. Low fat stores lead to a decrease in the levels of the leptin in the plasma. As a result, it causes a decrease in the feed intake, which in turn negatively affected the growth rates because the leptin plays an anti-saturating role (Neuropeptide Y-NPY), which is also secreted from the hypothalamus gland (Liefers, 2004). Also, the results may be attributed to another reason. Genetic factors do not furnish the degree of digestion of protein, dry matter, and energy is due to the surface area of the digestive tract in fish (Stevens *et al*., 1999). It is possible that there are no significant differences to the age of the fish, so as the fish age, the capacity of the gut, secreted enzymes and everything related Digestion.

Several studies have shown that Introns cover a much larger portion of the Exons in the genome (Venter *et al*., 2001), and Introns are usually significantly more diverse than neighboring Exons (Özlem and Dursum, 2011), as a result of which Introns have more polymorph than exons, In addition, the studied mutation SNPs identified in this study were present in the second Exon region of leptin 1, specifically at the site bp 2092, and the mutation in this site was from the mutation mistakes and there were no statistically significant differences in characterization of manifestations, as the amino acid changed from phenylalanine to Serine (www.ncbi.nim.nih.gov), which means that transformed amino acids were not necessary and did not change the function of leptin, or that transformed amino acids did not perform the normal physiological roles of leptin 1, and this result is consistent with the results of Huang *et al.,* (2014), while studying a samples of 200 orange-spotted grouper fishes (*epinephelus coioides*) and 12 traits, there were six mutations, four of them in exons and two in Introns, one of these mutations was recorded at site c.149G> (Missense mutations in which the amino acid arginine is converted to the amino acid glotamine) results were significant and for only two traits namely body width and the length of the head, the rest of the qualities of body weight, total length, height standard, the trunk, the caudal peduncle, the length of caudal peduncle, snout length, diameter of the eyeball, the distance between the eyes and the coefficient case the results were not the moral. The results of the current study differ with the results of Tang *et al.,* (2016) when they studied a sample of 557 common carp fishes and their study resulted in three mutations and all mutations were present in exons, as well as the absence of significant differences due to the difference of the genotypes among them in terms of size The samples are for each composition. On site 2092, the number of fish bearing the wild genotype (CC) was three fish and in the genotype (CT) eight fish, and in the genotype mutant (TT) fifty-five fish, perhaps the absence of significant differences due to the breeding season lost It was observed that the season may have a role in increasing the season The genetic expression of the leptin hormone, in a study by Trombley *et al.,* (2012) to see the effect of the season on the genetic expression of the leptin hormone in the salmon juveniles of the atlantic salmon (*Salmo salar*) was slow growth during the first four weeks of the experiment (April) which can be explained through The temperature of the water, the experiment started during a period during which the fish came out of the winter with a decrease in the water temperature and this period is characterized by poor appetite and a lack of movement and activity of fish and a state of low metabolism, and one of the main results of this study is that the specific diet for a period of seven weeks led greatly increased in an expression of Lep1 as well as higher levels of leptin in plasma compared to fish in June, this study demonstrated that no association was found between plasma hormone levels in the plasma and weight ratio, although there was a negative relationship between leptin levels in the plasma and fat content ,The body in fish that were fully fed in the month of June. Similar results were found in rainbow trout (*Oncorhynchus mykiss*), as no association was observed between leptin levels and weight gain rate (Kling *et al*., 2009). These results may also be due to experiment conditions, environmental factors, and the source of the studied strains.

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