

RELATIONSHIP OF MYOSTATIN GENE POLYMORPHISM WITH SOME GROWTH TRAITS OF COMMON CARP *CYPRINUS CARPIO* L.

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ABSTRACT

The present study was aimed to investigate the polymorphism of the myostatin gene and its relation with some growth traits, included feed conversion rate and efficiency, protein intake and protein efficiency ratio in 68 specimens of common carp *Cyprinus carpio*. Sequencing of single nucleotide polymorphism (SNP) showed three genotypes at site T2230C in the myostatin gene. The distribution rates were 5.88, 38.24 and 55.88% for TT, TC and CC respectively, and the variation among them was high significant, the allelic frequency was 0.25 for allele T, while it was 0.75 for C. Effect of the genotype of the myostatin gene was significantly in the feed conversion rate and efficiency, the protein intake and the protein efficiency ratio of the common carp with the variation of the genotypes of myostatin gene, whereas the feed conversion rate was 6.18, 6.00 and 4.50 at TC, TT and CC respectively. Based on the mentioned results, the positive relation between myostatin gene polymorphism with some growth traits observed in this study may be a useful biomarker in the selection and crossing the genotypes that have achieved the best performance in common carp.

Keywords: single nucleotide polymorphism, sequencing, feed conversion rate, protein intake.

الخشالي وصالح

مجلة العلوم الزراعية العراقية - 2020: 51(1): 317-322

علاقة تعدد المظاهر الوراثية لجين المايوستاتين ببعض صفات النمو لأسماك الكارب الشائع *Cyprinus carpio* L.

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المستخلص

أجريت الدراسة الحالية بهدف تحديد تعدد المظاهر الوراثية (Polymorphism) لجين المايوستاتين وعلاقته ببعض صفات النمو وهي معدل وكفاءة التحويل الغذائي والبروتين المتناول ونسبة كفاءة البروتين في 68 عينة من أسماك الكارب الشائع. أظهرت نتائج تحديد التتابع (Sequencing)، وتعدد المظاهر للنوكليوتيدة المفردة (Single Nucleotide Polymorphism-SNP) ثلاثة تراكيب وراثية في الموقع T2230C في جين المايوستاتين، وبلغت نسب توزيع التراكيب الوراثية 5.88 و 38.24 و 55.88% للتراكيب TT و TC و CC على التوالي، وكان التباين بين هذه النسب عالي المعنوية ($P < 0.01$)، بلغ التكرار الاليلي للـ T 0.25 في حين كان تكرار الاليل C 0.75. وكان تأثير التركيب الوراثي لجين المايوستاتين معنوياً ($P < 0.05$) في معدل وكفاءة التحويل الغذائي والبروتين المتناول ونسبة كفاءة البروتين لأسماك الكارب الشائع باختلاف التراكيب الوراثية لجين المايوستاتين، إذ بلغ معدل التحويل الغذائي 6.18 و 6.00 و 4.50 عند التركيب الوراثي الهجين TC والبري TT على التوالي، بينما بلغ 4.50 في التركيب الطافر CC الذي اختلف معنوياً عن بقية التراكيب الوراثية TT و TC. بناءً على النتائج أعلاه، فإن العلاقة الإيجابية بين تعدد المظاهر لجين المايوستاتين مع بعض صفات النمو التي لوحظت في الدراسة الحالية ربما تعد مؤشراً حيوياً مفيداً بانتخاب وتضريب التراكيب الوراثية التي حققت أفضل أداء في أسماك الكارب الشائع.

الكلمات المفتاحية: تعدد المظاهر للنوكليوتيدة المفردة، التتابع، معدل التحويل الغذائي، البروتين المتناول.

*Received:11/9/2019, Accepted:8/12/2019

INTRODUCTION

Myostatin gene is called the Growth Differentiation Factor GDF-8 (MSTN), with size of 6.4 kilo base (Kb) (4), it is located on chromosome 11 in common carp and consists of three exons and two introns (20). Myostatin gene primarily acts on regulation and expression in the skeletal muscles through its important role in growth (12). Myostatin gene is associated with some of traits related to general animal performance such as feed conversion ratio, chest muscle depth, carcass weight without intestines, blood oxygen level, antibody system in poultry (3), weight gain and meat production in cattle (10), and is associated with most traits including body weight, total length and body height in fish (15), body weight and condition factor in common carp *Cyprinus carpio* L. (1,20), body weight and the fillets weight of Atlantic salmon *Salmo salar* (13), as well as the body weight in tilapia *Oreochromis niloticus* (6). Myostatin gene was discovered in 1997 by Si-jin Lee and Alexandra through their experiments on rats, they controlled the myostatin inhibition and got mice with larger muscles, compared to untreated ones. The gene acts as a negative regulator of skeletal muscles development and growth by inhibiting tissue construction and peripheral end-response of various muscle cells (4). Numerous studies have been conducted on the myostatin gene in chickens, cattle and fish (3,6,9). Many genetic markers are currently being used, such as single nucleotide polymorphism (SNP) and Quantitative Loci (QTL), which have helped detect the structure of the myostatin gene and the relationship between its polymorphism and some growth traits in some commercial fish species such as tilapia and some genetically improved salmon species (19). Due to the lack of such studies in Iraq, the present study aimed to investigate the relationship between myostatin gene polymorphism with some of growth traits of common carp using PCR technique.

MATERIALS AND METHODS

The study was carried out in fish laboratory in the College of Agricultural Engineering Sciences, University of Baghdad. 150 specimens of common carp with weight ranged from 100 to 150 g were collected from

a private fish farm southern of Baghdad and reared for the period from 18/10/2017 to 10/1/2018. DNA isolation from the blood of 68 specimens of fish trial (at average weight of 110 ± 5 g) was carried out using the protocol given by Promega Corporation (2016). Polymerase Chain Reaction (PCR) technique was used to amplify the part required to complete the molecular detection and polymorphism of the MSTN gene according to the size of the gene piece and the type of primers used. Primers are selected at intron 2, sequence number is GQ214770.1, and the appearance diversity of the gene resulting from the MSTN mutations (20). The parts of the genes studied have been verified by electronic genome browsers: (NCBI) National Centre for Biotechnology Information

Forward: 5'-

AGCCTACCATAAAAGGTGTGTG-3',

Reverse: 5'

TCAATAGTGTCCATTCCCAAGT-3'

Growth traits studied for a period of 82 days were :

1- Feed Conversion Rate (F.C.R)

Which is one of the criteria for measuring the efficiency of the diet, and represents the ratio between feed provided to fish, to the wet increase in fish weight (16), and was calculated by the formula mentioned by Alazzawy and Alkhshali (1):

$F.C.R. = R / WG$

As:

(R) Ration = Weight of feed provided to fish (g)

W. G. (Weight gain) = wet weight increase of fish (g)

2. Feed Conversion Efficiency (F.C.E)

It is a measure of the utility extent of feed provided to fish, used as a percentage of the weight increase of fish and feed provided (18):

$F.C.E = (WG / R) * 100$

As

WG = Wet weight increase of fish (g)

R = Feed weight submitted to fish (g)

3. Protein Intake (P.I.)

It is calculated according to the equation mentioned by Gerking (8).

Protein intake = feed intake \times Protein ratio in the diet.

4. Protein Efficiency Ratio (P.E.R) - Protein Efficiency Ratio

Which is one of the criteria used to estimate the weight gain of each unit of protein consumed in the diet, and was estimated according to the equation mentioned by Gerking (8).

$$\text{P.E.R}\% = (\text{T.W.G./PI.}) * 100$$

As:

(T.W.G.) Total weight gain = Gross increase (g)

(P.I.) Protein Intake = Protein (g)

Table 1. Water parameters during the experimental period

water temperature (°C)	dissolved oxygen (mg / L)	pH
20-24	5.0 - 7.3	7.5-8.1

The oxygen level should be at least 5 mg / L for the growth of common carp(20) , pH should not be less than 6.4 and not more than 8.6 (7), while the proper temperature for growth of common carp is 25 °C, so the ranges mentioned are within the safe levels for the survival and growth of common carp (2, 5).

Statistical analysis

Data were statistically analyzed according to SAS (14) to study the effect of polymorphism on the MSTN gene. Significant differences were compared between means at probability level of 0.05 with the application of minimum squares mean method. Chi-square-22 test was used to compare the percentage distribution of

Water temperature (°C) was measured using thermometer (mn21) , dissolved oxygen (mg / L) by oxygen meter (Hana 208), and pH by pH meter (Hana 196). These parameters measured during the period of the experiment are shown in table (1) , the temperature ranged between 20 - 24 °C, dissolved oxygen concentrations varied between 5-7.3 mg / L throughout the experimental period, and the pH values ranged between 7.5 - 8.1

the gene polymorphism with each mutation in the sample of the studied fish. The following law was applied to calculate the allele frequency in each mutation according to Hardy Weinberg's equilibrium.

RESULTS AND DISCUSSION

The target segment was extracted from the myostatin gene (Fig.1), results showed a single bundle with size of 1000 bp after amplified by polymerase chain reaction (PCR). A sample of 5 µl of PCR product was carried in 1.5% Adjust the voltages at 60 volts and current at 40 mA for 80 min using known sizes of DNA parts (Marker) bp1500-100 to determine the sizes of the extracted segments.

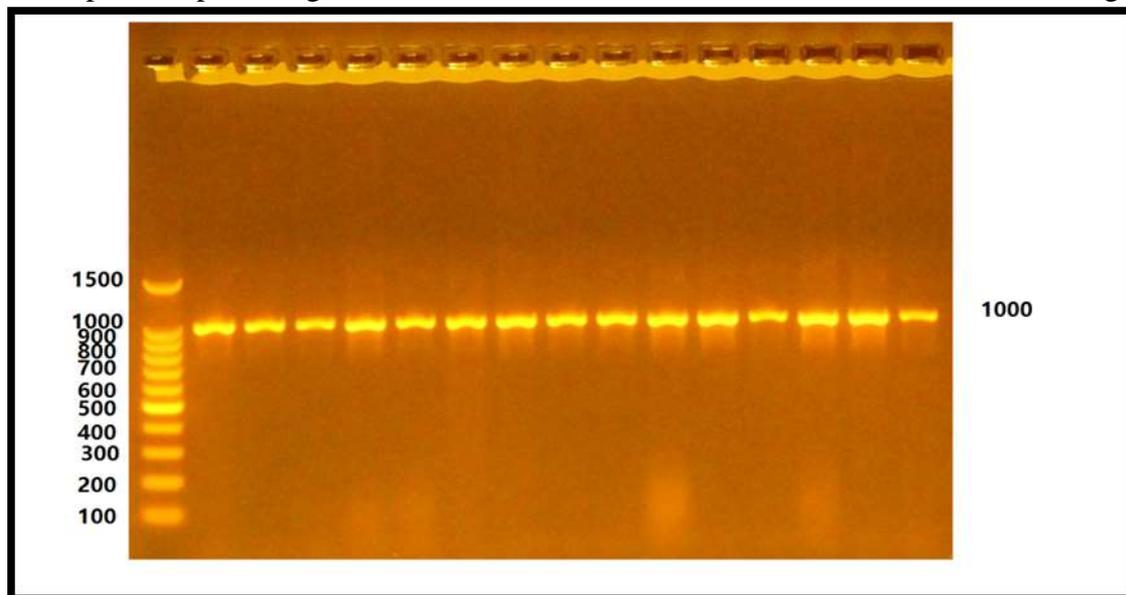


Figure 1. Extraction of Myostatin gene segment (1000 bp) using PCR Polymorphisms of the myostatin gene by the sequence of nitrogen bases

Results of the nitrogen bases sequence of the 1000p gene segment were resulted in Genetic mutations, Curves or peaks to find the genotypes. Alingment of the results has been

done on the Global Genebank website (www.ncbi.nlm.nih), and showed compatibility with the myostatin gene in common carp. The sequencing results of the

myostatin gene showed a change in single nucleotide polymorphism (SNP) in the second intron, specifically at the site of 2230 bp of the myostatin gene. The nitrogenous base changed from T to C, and the genotypes were extracted by Geneious Software program. The sequence of fish with genotype of wild common carp found in the global gene bank Number: LOC109091639. Alignment showed three genotypes at the studied site of the myostatin gene, wild- TT, Heterozygous-TC and mutant genotype CC, where the change occurred in the both bands (CC). Table (2) shows the numbers and percentages of the distribution of fish genotypes, with 5.88% for fish carrying TT genotype, 38.24% for fish with TC and 55.88 for fish carrying CC. The law of the allele frequency count was applied according to the Hardy and Weinreb equilibrium rule, with the frequency of the allele T 0.25% and the C 0.75% allele, while in previous studies, the distribution ratios of genotypes AA, AG and GG in common carp were 20.37, 59.88 and 19.75% respectively, and the allelic frequency of both A and G in that study were 0.51 and 0.49% respectively (19). In another study, Atlantic salmon *Salmo salar* showed three genotypes TT, TC and CC from MSTN-1b analysis with 39.11, 27.49 and 33.40% respectively, while the allelic frequency of T and C were 0.29 and 0.71%, respectively (13).

Table 2. Number and percentages of genotypes and allele frequency of myostatin gene (mutation T2230C)

Genotype	Number	percentages (%)
TT	4	5.88
TC	26	38.24
CC	38	55.88
Total	68	% 100
Qi square (χ^2)	---	** 37.761
Allele	Frequency	
T	0.25	
C	0.75	

Relationship of the myostatin gene with the feed conversion ratio and efficiency, protein efficiency and protein intake

Results in Table (3) show significant differences ($P < 0.05$) in the feed conversion ratio and efficiency, protein intake and protein efficiency ratio of common carp according to the different genotypes of the myostatin gene. Feed conversion rate was 6.18 in the hybrid

genotype TC during the experiment, and it was 6.00 in the wild genotype TT, while it was 4.50 in the mutant genotype CC, which significantly differed from the rest of the genotypes. The change in the sequence of the myostatin gene seems to have improved the metabolic rate (10). Feed conversion efficiency is different among genotypes, it was 21.80% significantly higher ($P < 0.05$) than the rest genotypes, while there is no significant difference between hybrid and wild genotype. The mutant genotype was superior to the rest genotypes by the protein efficiency and the protein intake (82.61% and 100.54 respectively). Terova *et al.* (17) isolated the DNA sequence of the myostatin gene in sea bass *Dicentrarchus labrax*, which encodes to 376 amino acids and is associated with regulating the growth during fasting periods, this gene sequencing has been increased the feed consumption, normal refeeding, achieve optimal growth and increase muscle mass. The polymorphism of the myostatin gene was detected in this study by definition of single nucleotide polymorphism (SNPs) using direct sequencing. The target segment was isolated from the gene by PCR, comparing the sequencing of all the fish, and extraction of the genotypes, where the differences in the alleles were found in the second intron of the myostatin gene. Results showed the superiority of the individuals carrying the mutant genotype (CC) in most of the studied characteristics of growth, that is due to the important role of the myostatin gene in the increase of expression and muscle growth. According to this results, individuals carrying this genotype can be elected, as the growth traits in fish regard as important economic characteristics on which the selection is based, and then using the gene expression in the improvement of these traits. The T2230C mutation has altered the nucleotide sequence, and may have inhibited the function of the gene, thus increased muscle growth. (20) revealed that the individuals of common carp achieved an increase in weight, length and gave the best condition factor (K). Results of the present study were agreed with the results of (11), which showed the correlation of the myostatin gene with the growth traits of body weight and total length in zebrafish.

Table 3. Effect of myostatin gene polymorphism on growth traits of common carp in the mutation of T2230

Parameter	CC	Genotype TC	TT	Signification level
Feed conversion ratio	4.50 ±0.04 b	6.18 ± 0.18 a	6.00±0.00 a	*
Feed conversion efficiency	21.80 ±0.19 a	16.57±0.55 b	16.66 ±0.00 b	*
Protein intake	100.54 ±68.37 a	89.53±0.43 b	81.08±0.05 c	*
Protein efficiency ratio	82.61 ±0.74 a	62.76±2.08 b	63.13 ±0.01 b	*

Means with different letters within one row are significantly differed

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