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Original Research

Relationship of the myostatin gene with the chemical body composition of common carp (*Cyprinus carpio*)

Authors:
Naser A. Saleh
Mohammed Sh. Al-Khshali

Institution:
Department of Animal Production, College of Agriculture, University of Baghdad.

Corresponding author:
Naser A. Saleh

ABSTRACT:

This study was undertaken at the college of Agriculture, University of Baghdad, Department of Animal Production/Fish Laboratory from 18.10.2017 to 10.1.2018 to determine the polymorphism of myostatin gene, and its relationship with the chemical body composition in 68 samples of common carp (*Cyprinus carpio).* Results of sequencing and Single Nucleotide Polymorphism (SNP) showed three genotypes at site T2230C in the myostatin gene and the distribution percentage of genotypes were 5.88, 38.24 and 55.88% for the TT, TC and CC genotypes respectively, and the variation among these percentages were highly significant (P<0.01). The allele frequency of T allele was 0.25, and C allele frequency was 0.75, where the effect of myostatin gene genotype was significant (P<0.05). Results of the study showed that most of the body composition ratios were significantly affected by the different genotypes of the myostatin gene, where CC Genotype was higher and the protein ratio was 16.25%, fat was low with 4.12% , ash 1.76% and humidity ratio 79.60% compared to the other genotypes TT and TC. The study summarized that it is possible to adopt the polymorphism of myostatin gene at the site T2230C in developing the genetic strategic improvement in fish to achieve the largest economic return from their breeding projects by selecting and crossing genotypes that have achieved best performance.

Keywords:

Common carp, Myostatin gene, Chemical body composition.

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INTRODUCTION

Myostatin (MSTN), also known as growth differentiation factor GDF-8, has the size of 6.4 Kilobyte (kb) (Cheng *et al*., 2003). This gene is located at the chromosome 11 in common carp and consists of three exons and two introns (Yanhong *et al*., 2012). The gene expression is mainly located in the skeletal muscles, and the act of this gene is in the growth and muscle formation, and plays a crucial role in the negative regulation of muscle mass function (Li *et al*., 2012). As well as much genes regulate the muscles growth in common carp such as insulin-like growth hormone (Aljboory and AL-Khshali, 2018).

Myostatin protein is naturally excreted in the body to prevent muscle hypertrophy through its effect on the fixed receptors of structural cells, as opposed to the normal growth in a process known as Myogenic, it regulates the size and mass of skeletal muscles (Joulia and Cabello, 2007). In the absence of the myostatin gene, muscles hypertrophy is present in various mammalian species, including rodents, humans and other farm animals. This confirms the function of the gene in regulating muscle growth by inhibiting the generation and differentiation of myoblast by reducing the gene expression in the process of muscle formation and myogenic (Langley *et al*., 2002). In addition myostatin gene enhance the growth and differentiation of muscle cells, therefore increasing the protein content in the muscle fibers, decreasing the fat ratio in the body structure and also the distribution of fat within the muscle fibers, with the decrease in total moisture in the mutant genotype (Tseng *et al*., 2011). The myostatin gene was discovered in 1997 by Si-jin Lee and Alexandra through their experiments on mice, they controlled the myostain inhibition and obtained mice with significantly larger muscles compared to the untreated ones (McPherron *et al*., 1997). The gene acts as a negative regulator of skeletal muscle development and growth by inhibiting tissue construction and peripheral end-response of various muscle cells. Several studies of the gene have been performed in other animal species such as chickens, cows, fish, etc. (Kambadur *et al*., 1997). Due to the lack of studies on this subject in Iraq, the present study aimed to investigate the relationship between the myostatin gene with the chemical body composition of common carp.

MATERIALS AND METHODS

The study was undertaken, for the period from 18.10.2017 to 10.1.2018, on a sample of 68 common carp. Genetic analysis was carried out by separating the genetic material, to identify the genotypes of the myostatin gene and to study its relationship with the biochemical composition of the body. DNA isolation was carried out using the protocol given by Promega Corporation (2010). 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. PCR amplification protocol was carried out using the protocol given by Promega Corporation (2016). The prefixes are selected (Entron 2, sequence number GQ214770.1 and the genetic diversity of the gene resulting from the phenotype of MSTN mutations (Yanhong *et al*., 2012). The gene fragments and their locations have been confirmed and verified from the National Center for Biotechnology Information (NCBI) for vertebrate genome (NCBI, 1988). The primers used were

Forward: 5'-AGCCTACCATAAAAGGTGTGTG-3'

Reverse: 5' TCAATAGTGTCCATTCCCAAGT-3'

After the amplification of DNA, 5 µl of PCR product was taken and loaded in the agarose gel electrophoresis. The concentration of agarose was 1.5%, voltage was adjusted at 60 volts and 40 Amp for 80 min. The size of DNA was 100-1500bp. Genetic analysis were carried out in a third party laboratory, Promega Corporation, 2800 Woods Hollow Road Madison, WI

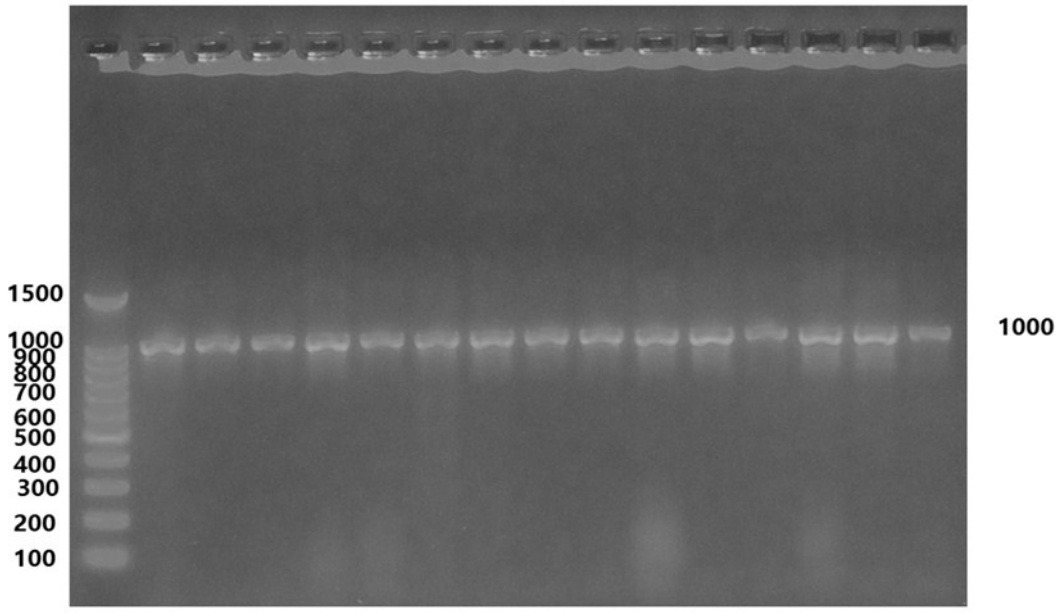

Figure 1. Extraction fragment (1000bp) of the MSTN gene by PCR technique

53711-5399 USA. Sequencing was done through a third party Macrogen, Korea. Single Nucleotide Polymorphism was done through Macrogen, Korea.

Analysis body components

The body composition was analyzed after the growth experiment, according to the method of work mentioned in AOAC (1980) as follows:

Humidity: It was estimated by the oven-dry test by drying the samples at 105°C until the sample weight become constant.

Protein: It was estimated using Kjeldahl method, which comprise a known weight of the sample digested by concentrated sulfuric acid and distilled with boric acid. The titration was done with hydrochloric acid (0.1 N).

Fat: Soxhlet method was used to extract the fat from the body samples of fish by using hexane as an organic solvent for 16h.

Ash: It was estimated by burning the samples in a waf-

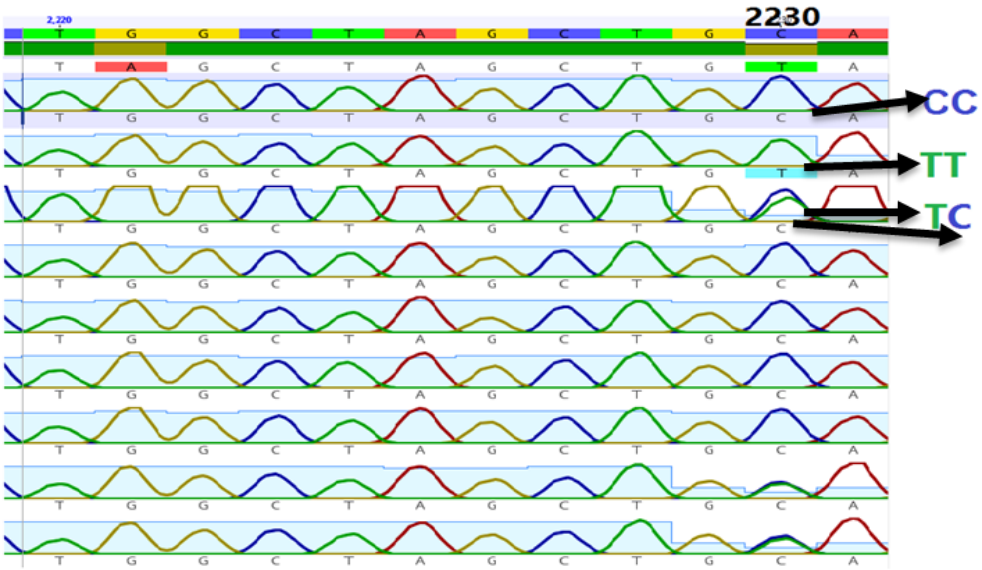

Figure 2. Sequencing of myostatin gene of each sample identified with combination of alleles

Figure 3. Novel (SNP) in the 2nd intron, at 2230bp the base changed from T to C of the MSTN gene

Table 1. Number and percentages of genotypes and allele frequency myostatin (mutation) T2230C

S. No	Genotype	Numbers	Percentages %
1	TT	4	5.88
2	TC	26	38.24
3	CC	38	55.88
4	Total	68	100
5	Chi-square(χ^2)	----	** 37.761
	Allele		**Frequency**
	T		0.25
	C		0.75
	**(P<0.01)		

fle furnace at 550°C for 5h.

Statistical analysis

Data were statistically analyzed using the Statistical Analysis System (SAS, 2012) to study the effect of polymorphism of the MSTN gene according to the mathematical model below, and the differences between the averages were compared with the application of the least square means method SAS (2012).

According to Completely Randomized Design (CRD) (Steel and Torrie, 1990)

$Yij = \mu + Gi + eij$

where, Yij: is the value of the genotype I; μ: the overall mean of the characteristic; Gi: the effect of polymorphism of MSTN gene T2230C (TT, CT, CC); eij: random error which is naturally distributed at an average of zero and a variation of $\sigma^2 e$. Duncan (1995) multiple range test was used to significantly compare between the means. The chi-square (χ^2), test was applied to calculate the initial redundancy in the mutation according to the Hardy-Weinberg law.

RESULTS AND DISCUSSION

Myostatin gene fragment was extracted by PCR technique using PCR kit, primers, total DNA samples and by adjusting the thermal cycles (denaturation at 95° C for 5 min, annealing at 65°C for 30 sec/ 35 cycle and final extension at 72°C for 5 min) (Gábor *et al*., 2014).

Table 2. Relationship of the myostatin gene genotypes T2230C mutation with body components in common carp (mean ± standard error)

S. No	Trait (%)	Genotype			Significant
		CC	TC	TT	
1	Humidity	79.60±0.01^b	79.13±0.26^b	80.72±0.02^a	*
2	Protein	16.25±0.09^a	15.06±0.13^b	13.64±0.00^c	*
3	Fat	3.15±0.02^b	4.41±0.02^a	4.12±0.02^a	*
4	Ash	1.22±0.09^b	1.68±0.02^a	1.76±0.02^a	*

The different letters within a row indicate a significant difference; *(P<0.05).

Table 3. Chemical body composition for 68 samples

S. No	Genotype	Humidity (%)	Protein (%)	Fat (%)	Ash (%)
1	CC	78.12	15.21	3.12	1.02
2	CC	77.86	16.11	3.25	1.11
3	CC	80.02	14.83	3.35	0.92
4	CC	82.12	15.95	3.13	0.84
5	CC	80.23	16.05	3.15	1.45
6	CC	79.22	16.25	2.89	1.87
7	CC	79.74	17.35	2.85	0.68
8	CC	76.96	15.93	3.19	1.32
9	CC	77.45	16.01	4.15	1.87
10	CC	78.89	15.25	3.05	0.92
11	CC	82.27	16.68	2.98	0.83
12	CC	80.12	16.85	2.48	0.91
13	CC	85.19	16.91	3.45	1.58
14	CC	79.06	16.25	4.21	0.85
15	CC	77.19	15.75	3.23	0.72
16	CC	79.89	16.13	3.01	1.56
17	CC	83.11	14.15	2.85	1.82
18	CC	82.22	14.85	2.15	1.95
19	CC	79.66	17.15	3.11	1.97
20	CC	81.11	16.34	2.97	0.82
21	CC	80.03	16.88	3.32	1.11
22	CC	79.22	17.05	4.01	1.53
23	CC	78.23	17.75	3.66	1.19
24	CC	77.22	16.35	2.65	1.08
25	CC	79.37	16.82	2.05	1.15
26	CC	79.43	15.97	3.65	1.21
27	CC	79.55	16.45	3.05	2.01
28	CC	79.01	18.01	3.34	1.03
29	CC	79.11	16.76	2.55	1.12
30	CC	79.22	15.85	3.47	1.01
31	CC	79.42	16.04	3.89	1.16
32	CC	79.31	15.23	2.95	1.12
33	CC	79.22	16.11	3.11	1.02
34	CC	79.25	16.45	3.45	1.33
35	CC	79.19	16.55	2.85	1.11
36	CC	79.22	16.95	2.95	1.17
37	CC	79.03	16.12	3.55	1.03
38	CC	79.16	16.31	2.75	1.14
Average	**----**	**79.60**	**16.25**	**3.15**	**1.22**
39	TC	78.98	15.01	4.22	1.82
40	TC	80.86	16.22	3.85	1.11
41	TC	80.52	14.73	4.35	1.92
42	TC	81.12	15.08	4.13	1.84
43	TC	78.23	16.13	4.95	1.45
45	TC	79.22	15.25	3.89	1.87
46	TC	79.74	16.35	4.85	1.68
47	TC	76.96	15.33	4.19	1.32
48	TC	77.45	14.01	4.15	1.87
49	TC	78.89	15.15	4.05	1.92
50	TC	78.27	15.68	4.98	1.83
51	TC	77.12	14.85	4.48	1.91
52	TC	76.19	15.91	4.45	1.58

Continue…

53	TC	79.06	14.25	4.89	1.85
54	TC	77.19	15.25	4.23	1.72
55	TC	79.89	15.13	3.91	1.56
56	TC	83.11	14.15	4.85	1.82
57	TC	79.22	14.85	4.15	1.95
58	TC	79.66	15.15	4.11	1.97
59	TC	78.11	15.34	3.97	1.82
60	TC	79.03	14.48	4.32	1.61
61	TC	79.22	14.05	4.86	1.53
62	TC	78.23	14.15	4.66	1.59
63	TC	77.22	15.05	4.65	1.38
64	TC	82.37	15.12	4.85	1.35
Average	**----**	**79.13**	**15.06**	**4.41**	**1.68**
65	TT	79.98	14.51	4.12	1.81
66	TT	80.71	13.22	3.95	1.71
67	TT	80.48	12.73	4.31	1.79
68	TT	81.71	14.08	4.11	1.74
Average	**----**	**80.72**	**13.64**	**4.12**	**1.76**

Legend: CC, TC, TT corresponds to their respective base pairs.

The samples were then migrated from each sample model and imaging the output of the migration to make sure the extraction process is successful and obtained the required fragment size (1000 bp) for myostatin gene. The size of the DNA fragments were used as a marker (100-1500 bp) DNA ladder (Figure 1).

Polymorphisms through the sequencing of the nitrogen bases

Results of the nitrogen sequence were obtained for 1000bp gene segment and extracted the genetic mutations, as well as the curves or peaks file to segregate the genotypes, the peak blue refers to genotype CC, the green refers to genotype TT while the double peaks blue and green refers to genotype TC (Figure 2).

The results of the sequencing of myostatin gene which comparing with data of NCBI, showed novel Single Nucleotide Polymorphism (SNP) in the second intron, specifically at 2230bp of the myostatin gene. The base changed from T to C (Figure 3).

The studied fragment of the common carp myostatin gene in intron 2

Showed the compatibility of the myostatin gene in common carp fish. Results also showed mutations at several sites of the gene, most of them in the second intron, and we found it as a multiple genetic manifestation of the myostatin gene.

The genotypes of the myostatin gene (location T2230C)

The results of the sequencing of myostatin gene showed Single Nucleotide Polymorphism (SNP) in the second intron, specifically at 2230bp of the myostatin gene. The base was changed from T to C, and the Geneious software (10.1.3) (Geneious, 2017) extracted the genotypes. The sequence of fish compared with the wild genotype of the common carp found in the global gene bank Number: LOC109091639. Alignment showed the sequence of fish in finding three genotypes at the studied site of the myostatin gene. TT-Wild genotype and genetic makeup Heterozygous-TC Transient homologous structure change in both bands (CC).

Table 1 showed the numbers and percentages of fish genotypes, with 5.88% of the fish carrying the genotype TT, 38.24% of the fish carrying the genotype TC and 55.88% of the fish carrying the CC genotype, and less value of TT genotype. The law for the initial repeat count was applied according to the Hardy and Weinberg equilibrium rule. The frequency of the allele T was 0.25% and C was 0.75%. In previous studies, the Common carp distribution of AA, AG and GG were 20.37, 59.88 and 19.75% respectively, and the frequency of

allele A and G were 0.51 and 0.49% respectively (Yanhong *et al*., 2012). In another study on commercial Atlantic salmon *Salmo salar*, three significant genotypes of MSTN-1b found to be TT, TC and CC respectively, with 39.11, 27.49 and 33.40% respectively and the frequency of allele T and C as 0.29 and 0.71%, respectively (Penaloza *et al*., 2013).

Relationship of the myostatin gene genotypes with the chemical body composition

Strong relationship was observed between the different genotypes of the myostatin gene and the components of body in common carp, that each type of the genotype was compared for this gene site with component body. Results of the present study showed significant differences in the body components according to the genotypes of the gene (Table 2). Humidity was affected by the different genotypes of myostatin gene, 80.72, 79.13 and 79.60% at the first site of mutation in the T2230C of TT, TC and CC respectively. Significant differences were recorded (P<0.05) for genotype TT-wild with other genotypes. There were also significant differences (P<0.05) in the protein content, where the mutant genotype CC significantly increased to the ratio of 16.25% compared to the TC-hybrid and TT-wild with ratio of 15.06 and 13.64% respectively. This may be due to the effect of gene on growth and increase in muscle size in the mutant genotype CC, as pointed out by Tseng *et al*. (2011). He has also pointed out that the myostatin gene play an important role in promoting the growth and differentiation of muscle cells and thus increasing the protein content in muscle fibers, indicating the preference for genetic mutant composition in the rest of the genotypes.

Results showed that there were differences in fat ratio according to the different genotypes, with the values of 4.12% in the genotype TT at 4.41% in TC at (P<0.05), and for genotype CC is 3.15% (Table 3). The low fat content in the body composition can be attributed to fish with mutant genotype (Zheng *et al*., 2015). Fat move from different regions of the body to the muscular tissue for being use as an energy source in muscle structure, so lipids are low in mutant genetics and these were proposed by Gao *et al*. (2016) in Zebrafish (*Brachydanio rerio*). Fraher *et al*. (2015) found that in zebrafish the process of controlling the pathways of lipid metabolism is important in demolitions and construction especially fat, which is an important source for use in the formation of muscles fiber, in particularly fatty acids, found in the mutant genotype fat distribution between the muscles on the contrary in the genetic wild focused under the skin, while the ratio of ash in both genotype TT and the hybrid TC were significantly different (P <0.05), they were 1.76 and 1.68% with mutant genotype CC (1.22%).

CONCLUSION

The results of the study revealed the polymorphism features in the myostatin gene in common carp fish. The location of the mutation is at T2230C. The effect of valuable moisture, protein, fat and ash were significantly affected (P<0.05). The mutant genotype showed protein superiority, low fat, moisture and ash in the body components on the rest with different genotypes.

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