Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(2): 1013-1016

Received: 01-01-2018
Accepted: 02-02-2018

ST Aljuboory
Department of Animal Production, Agricultural Faculty, University of Baghdad, Iraq

MS Al-Khshali
Department of Animal Production, Agricultural Faculty, University of Baghdad, Iraq

Correspondence
ST Aljuboory
Department of Animal Production, Agricultural Faculty, University of Baghdad, Iraq

Relation of insulin like growth factor gene (IGF-1) WHTH chemical analysis common carp (*Cyprinus carpio*)

ST Aljuboory and MS Al-Khshali

Abstract
The present study was conducted to identify genotypes of insulin- like growth factor (IGF-I) and its relation with chemical analysis of the body of common carp of common carp *Cyprinus carpio* in Al-Radhwaniyah Fish reservoir (Baghdad). Genotypes of insulin like growth factor was identified using restriction fragment length polymorphism (RFLP) in specified mutation in the site 3759T > G. Results of statistical analysis on chemical analysis of the body showed significantly effect of moisture and protein ($p< 0.05$) due to variation of genotypes. Heterozygous genotype (TG) achieved the highest rate of moisture (82.50±0.17%) whereas wild genotype (TT) recorded 79.55±0.17%. TT genotype had achieved the highest protein rate 15.95± 0.80% and also excelled significantly ($p< 0.05$) compare with GT genotype which reached 9.65±0.60%. Lipid and ash did not affected significantly by any genotypes. The present study conclude that the possibility of credibility upon polymorphism in insulin like growth factor as assign for chemical composition of fish body and select them to be parents for coming generation.

Keywords: insulin like growth factor gene, chemical composition, common carp

1. Introduction
Molecular genetics has played important role in breeding of the aquatic organisms through application of genetically modified technology to change fish gene in order to promote important production characteristics commercially such as growth rate and disease resistance [1]. Genetic technology has used growth hormones by using genetic modification because production costs in aquaculture projects depended upon time, which will reduce these costs, obtaining healthy animals, reduce the exposing to dangerous diseases and predators whereas the traditional methods of selection were responsible for great improvement in growth rate, but with slow progress relatively [2].
Insulin like growth factor (IGF-I) is known as somatomedin C which is peptide multi-hormone plays focal role in growth and development of vertebrates [3]. IGF-I consisted of 70 essential peptide amino acids, and contains three internal chains of disulfate [4]. Insulin like growth factor (IGF-I) is mainly manufactured in liver by stimulation of growth hormone (GH). It flows through the blood stream and reach all targeted tissues in order to perform its function effectively by manufacturing of growth hormone - releasing hormone (GHRH) from hypothalamus, then flow to the pituitary gland by receptors located on the surface secreting growth hormone which directed towards the receptors in all parts of the body such as hepatic glands that is enriched with GH receptors, and when it is reaching liver it will start manufacturing IGF-I [5]. But there is general agreement that the concentration of IGF-I in serum effects on the releasing of GH from anterior pituitary gland by feedback mechanism for definite inhibition of genetic transcription and secreting of growth hormone (GH) [6].
Insulin like growth factor (IGF-I) played an important role in cells reproduction, differentiation and functional formation of fish immunity system [7]. Most studies of last decade on fish focused on revealing identity of IGF in different species of fish, improving the blood properties or Peptide tissue levels (mRNA or IGF-I), variables measurement of IGF-I in blood and IGF-I tissues, its response to different nutrition conditions, season and control evaluation upon production of IGF-I by other endocrine gland such as thyroid [8] or estrogen [9].
IGF-I in fish did not only relate with growth, but also with metabolic process [10], development [11], reproduction [12] and osmoregulation in marine fish [13].
The objective of the present study was to determine genetic morphology, polymorphism in the

insulin-like growth hormone gene in a common carp fish sample and the allele frequency calculation with a number of physiological traits that have a direct impact on fish health and growth.

2. Materials and Methods

2.1 Study area details

The present study was performed in Al-Radhwaniyah fish reservoir in Baghdad, for the period from 15/10/2016 until 5/2/2017. 95 samples of *C. carpio* were collected and reared in concrete basins of 7*3* 1.2 meter for 110 days. Fish were fed on commercial diet containing 26.8% proteins, 1.5% Fats and energy of 3165 Kilo calorie. The fish were numbered by planting the figure near the pectoral fin of each fish.

2.2 Extraction of DNA and Polymerase Chain Reaction

One ml of blood was collected from the heart muscle of all trial fish. These samples were collected in EDTA tubes and kept in freezer (-18 ºC) for DNA extraction by using DNA extraction kit (Geneaid, korea). They adopted method of Sambrook *et al*. [14] for Electrophoreses, it specimen transferred by electricity power of 70 Volt and current of 40mA for one hour. Results were filmed by photo documentation system.

Primer was selected for molecular detection and identifying polymorphism for genes and mutation in IGF-I gene according to Feng *et al*. [15] Primer was provided by Korean pioneer company and its sequences showed in Table 1. Primer works according to specific conditions as in the following:

Initial denaturation at 95° C°, one cycle for 4 minutes, Denaturation at 94° C°, 35 cycle for 30 seconds for single cycle, Annealing at 56° C°, 35 cycle for 30 second for single cycle, Extension at 72° C°,35 cycle for one minutes, Final Extension at 72° C°, single cycle for 5 minutes.

Table 1: Sequence of the primer used and the area covered by the insulin-like factor growth hormone gene

Gene	Sequence region	
IGF-I	Second Intron	F:5 GCACAATGGCTCAAGGAAGT 3
		R:5 GTTTGTATCTGGGGAATGGG 3

2.3 Chemical Analysis

Chemical analysis was taken after growth experiment in nutrition research institute. Ministry of health depending upon A.O.A.C [16]:

1. **Moisture content:** Moisture content was estimated through drying the samples upon (105) temperature till firmness of weight.
2. **Protein content:** protein content was rated by using microkjeldahl which digest given weight from the sample with the existence of concentrated H2So4 distilling with boric acid, then subjected to mixing process with hydrochloride acid (0.1N) to identify nitrogen amount with will multiplied with the factor 6.25 to rate protein percentage in the sample.
3. **Fat content:** Fat content was estimated by using Soxhlet apparatus device with the existence of hexane as organic solvent in order to heat fish samples for 16 hours.
4. **Ashes content:** ash content was estimated by burning of the samples in muffle furnace 550 c temperature for four hours.

2.4 Statistical analysis

Statistical Analysis System was used in data analysis SAS (2012) [17] according to complete randomized design (CRD) using the general linear model (GLM) and Duncan multiple range test [18] was used to compare the means between genotypes at a significant level ($p < 0.05$).

3. Results and Discussion

3.1 Polymerase chain reaction – PCR

Fig. 1 refers to the extracted fragment from IGF-I gene, results of electrophoresis showed the required fragment which amounted 1061 bp after amplifying by Polymerase Chain Reaction (PCR), The sample used consisted of 5 microliters migrated mergers and the dye was loaded within 1 hour, with a voltage of 70, 40 m. Amber, then the result was filmed by photographic documentation placed with DNA fragments of a specific size marker to ensure successful extraction, and these results are agree with the primer designed according to Feng *et al* [15] in terms of the required bundle appearance which amounted 1061 bp in his study on common carp.

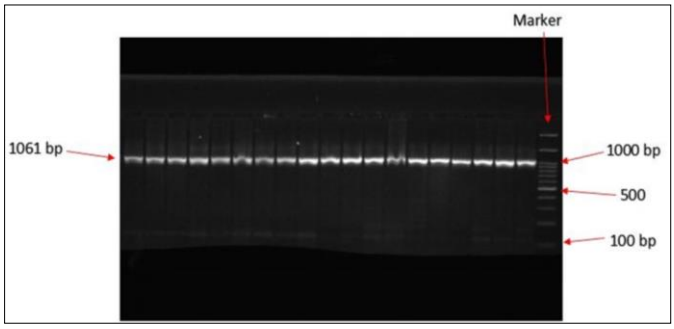

Fig 1: Extracted fragment from insulin like growth factor of hormone gene

3.2 Genotypes in insulin like hormone gene using RFLP Technique

Genotypes in insulin like hormone gene in carp were identified by using Restriction Fragment Length Polymorphism(RFLP) with the existence of the Restriction enzyme BstEll (Eco9ll) and the product was migrated by agarose gel 1.5 % for one hour and half and adjust the voltage 70 volt, 40 m. Am with marker (100 – 3000 bp) as in Figure 2.

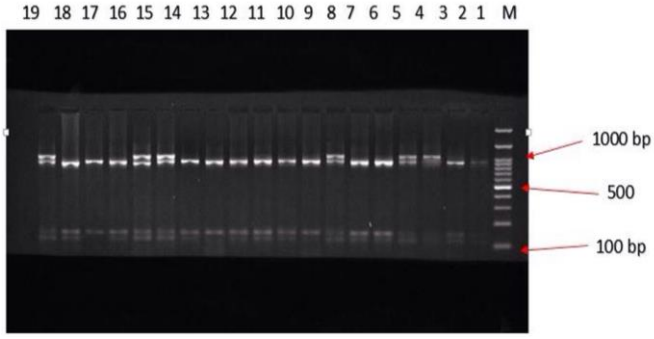

Fig 2: Bundles formed after the BstEII enzyme restriction process to determine the genotypes of IGF-I gene

Restriction was performed with BstEll, enzyme, where identifying the sensitive location within a specific sequence of cutting location, cutting is done in 3759 T>G location of the second Intron (accession no. AF465830,1) where indicates of two bundles the first was 913 bp and the second 148 bp in size followed the wild genotype (TT), where cutting was performed in both Alleles (no mutation in both Alleles), as for existence of three bundles (19061,913,148 bp) implies the Heterozygous genotype (TG), existence of mutation in one

Allele (changing the nitrogenaes base T to G in one Allele) and there existed one bundles of 1061 bp in size, that implies symmetric homozygous genotype (GG) containing the mutation in both Alleles.

3.3 Distribution of IGF-I gene genotypes in common carp

Table 2 demonstrated numbers and percentages of fish genotypes distribution, where they were amounted 69.47 % for fish carrying TT genotype, 29.47 % for fish carrying TG genotype and 1.06% for fish carrying GG genotype with significant difference between genotypes.

Table 2: Numbers and percentages of genotypes and the allele frequency of IGF-I gene in common carp.

Percentage (%)	the number	Genotype
69.47	66	TT
29.47	28	TG
1.06	1	GG
100%	95	Total
10.735**	---	Chai square value)χ^2(

Results of statistical analysis (Table 3) showed existence of significance differences ($p<0.05$) in moisture percentage with the genotypes difference, whereas, the TG genotype recorded 82.50% and 79.55 % in TT genotype. As for body protein percentage, the TT genotype achieved higher value amounted 15.95% while lower value in TG genotype and amounted 9.65% with significance differences ($P<0.05$) between genotypes. TG genotype recorded fat percentage 3.90 % higher than TT genotype amounted 3.75% with no significance differences, As for ash, results were 1.78 and 1.57 % in TT and TG genotype, respectively no significance differences were recorded.

Table 3: Chemical composition of the body components in common carp (standard error ± average).

Genotype			Adjective (%)
Moral level	TG	TT	
*	0.17± 82.50 A	0.17± 79.55 B	Moisture
*	0.60 ± 9.65 B	0.80 ± 15.95 A	Protein
N.S	0.36±3.90 A	0.25±3.75 A	Fat
N.S	0.09 ± 1.57 A	0.35 ± 1.78 A	Ash

The averages with different characters within the same row differ significantly between them.
*Significant ($P<0.05$): N.S. not significant

The chemical structure is affected by external factors including environment, nutrition system and interior conditions including genetics and could be associated with six, age and size [19]. The body liquids are considered mean for food transfer and metabolism, water is the main element in these liquids and it is required for natural performance for many biological and protein molecules could maintain their original shape and the usual functions with water existence. Water percentage in body is different largely where moisture percentage in fish bodies between 70 – 80% available in two shapes of tissues either connected to proteins or free and there existed inverse relation between water and fat contents in fish that total percentages for the two almost 80% [20] and fat included large collection of composites and fat identifies that they are part of any biological material able to be extracted by low polarized solvents and in case of fish tissue. Fat contents along chain of amino acid. similar to that smaller percentage of other components, and in many of fish types there existed fat accumulation during nutrition season and decrease during lay egg, As for fat fish such as the sardines and herring, the main location for storing fat is muscles [21]. Storing energy in animals basically in fat when the existed available energy from food stored as fat and used during periods of decreasing the available energy in the body and store either in liver or in some cases in fatly tissues [22] the significance increase which the current study results in body protein related to the great role played by the hormone to form muscles thus the occurred mutation has an effect to decrease the protein in fish carrying it and could achieve economic and nutritional benefits via increasing the protein percentage in the body and its influence on the consumer. As for decrease of moisture in fish carrying the TT genotype, is a natural product of increasing the protein and there is revers relation between the protein and moisture in the body [23].

4. Conclusion

The present study results revealed polymorphism features in the insulin-like growth factor gene in common carp fish using the BstE11, enzyme, in the SNP identified at site T <G 3759. The values moisture and protein of were significantly affected ($P<0.05$). The moisture showed TG higher values than the wild structure, while the wild structure protein showed higher value than the hybrid structure. Their concentrations of ash and ash were not affected by different genotypes.

5. Acknowledgement

The authors express their deep gratitude to Dr. Hassan Saad Jawad\ Department of Animal Production- Faculty of Agriculture for his invaluable assistance in publishing the research and the Ministry of Agriculture for its support throughout the studies.

6. References

1. Khan S, Duan P, Yao L, Hou H. Shift-work mediated disruptions of Circadian rhythms and sleep homeostasis cause serious health problems.
2. Feng X, Yu X, Pang M, Liu H, Tong J. Molecular characterization and expression of three preprosomatostatin genes and their association with growth in common carp (*Cyprinus carpio*). Comparative Biochemistry and Physiology, Part B, 2016, 182:37-46.
3. Renaville R, Hammadi M, Portetelle D. Role of the somatotropic axis in the mammalian metabolism. Domestic Animal Endocrinology. 2002; 23:351-360.
4. Kavsan VM, Grebenjuk VA, Koval AP, Skorokhod AS, Roberts CJ, Leroith D. Isolation of a second nonallelic insulinlike growth factor I gene from the salmon genome. DNA Cell Biology. 1994; 13:555-559.
5. Ohlsson C, Mohan S, Sjogren K, Tivesten A, Isgaard J, Isaksson O, Jansson JO, Svensson J. The role of liver-derived insulin-like growth factor-I. Endocrine Reviews. 2009; 30:494-535.
6. Honda J, Manabe Y, Matsumura R, Takeuchi S, Takahashi S. IGF-I regulates pro-opiomelanocortin and GH gene expression in the mouse pituitary gland. Journal of Endocinology. 2003; 178:71-82.
7. Yada T, Kaiya H, Mutoh K, Azuma T, Hyodo S, Kangawa K. Ghrelin stimulates phagocytosis and superoxide production in fish leukocytes. Journal of Endocrinology. 2006; 189:57-65.
8. Schmid AC, Lutz I, Kloas W, Reinecke M. Thyroid hormone stimulates hepatic IGF-I mRNA expression in a bony fish, the tilapia *Oreochromis mossambicus*,in vitro and in vivo. General and Comparative Endocrinology.

2003; 130:129-134.
9. Riley LG, Hirano T, Grau EG. Estradiol-17beta and dihydrotestosterone differentially regulate vitellogenin and insulin-like growth factor-I production in primary hepatocytes of the tilapia *Oreochromis mossambicus*. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology. 2004; 138:177-186.
10. Castillo J, Codina M, Martínez ML, Navarro I, Gutiérrez J. Metabolic and mitogenic effects of IGF-I and insulin on muscle cells of rainbow trout. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2004; 283:R647-R652.
11. Pozios KC, Ding J, Degger B, Upton Z, Duan C. IGFs stimulate zebrafish cell proliferation by activating MAP kinase and PI3-kinasesignaling pathways. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2001; 280: R1230-R1239.
12. Weber G, Sullivan CV. Effects of insulin-like growth factorI on in vitro Wnal oocyte maturation and ovarian steroidogenesis in striped bass, Morone saxatilis. Biology of Reproduction. 2000; 63:1049-1057.
13. Mccormick SD. Endocrine control of osmoregulation in teleost fish. American Zoologist. 2001; 41:781-794.
14. Sambrook J, Fritschi EF, Maniatis T. Molecular cloning: a laboratorymanual, Cold Spring Harbor Laboratory Press, New York, 1989.
15. Feng X, Yu X, Tong J. Novel single nucleotide polymorphisms of the insulin-like growth factor-I gene and their associations with growth traits in common carp (Cyprinus carpio). International Journal of Molecular Sciences. 2014; 15:22471-22482.
16. AOAC. Official Methods of Analysis, 13th ed. Association of Official Analytical Chemists. Washington. 1980, 376-384
17. SAS. SAS User's Guide: Statistics Version 6th ed., SAS Institute Inc, 2012.
18. Duncan DB. Multiple Range and Multiple F Tests. Biometrics. 1955; 11:1.
19. Huss H. Quality and Quality changes in fresh fish. Rome: FAO Fisheries Technical Paper. 1995, 348.
20. Laviola G, Adriani W, Gaudino C, Marino R, Keller F. Paradoxical effects of prenatal acetylcholinesterase blockade on neuro-behavioral development and drug-induced stereotypies in reeler mutant mice. Psychopharmacology. 2006; 187:331-344.
21. Nestel PJN. Fish oil and cardiovascular disease: lipids and arterial function. The American Journal of Clinical Nutrition. 2000; 71:228-231.
22. Arts M, Ackman R, Holub B. Essential fatty acids in aquatic ecosystems: A crucial link between diet and human health and evolution. Ca. Journal of Fisheries and Aquatic Science. 2001; 58:122-137.
23. Cook JT, McNiven MA, Richardson GF, Sutterlin AM. Growth rate, body composition and feed digestibility conversion of growth-enhanced transgenic Atlantic salmon *Salmo salar*. Aquaculture. 2000; 188:15-32.