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Association of follistatin gene polymorphism with growth common carp *Cyprinus carpio* L. traits of fry

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Abstract

The present study was conducted at the Nutrition Laboratory, Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah. The study aimed to investigate the association of follistatin gene polymorphism with growth traits of fry common carp *Cyprinus carpio L*. The study lasted eight weeks (56 days). Results of sequencing and Single Nucleotide Polymorphism (SNP) showed two genotypes AA and AG at the nitrogenous base 344 of the follistatin gene. Genotypes frequencies were 34.61% and 65.38% respectively. The growth parameters of common carp fish were studied, included body weight (BW), total length (TL), standard length (SL), head length (HL), body height (BH), caudal peduncle height (CPH), and eye diameter (ED). All growth parameters associated insignificantly with the two genotypes of follistatin gene during the experimental period. We can conclude from this study that the growth characteristics is not affected by the genetical differences between the genotypes. Study on larger samples of fish catches from different locations would give more truthful results in the application of the marker-assisted selection (MAS) strategy.

Keywords: common carp, follistatin gene, growth parameters, polymorphism

I. Introduction

There is a clear trend in various parts of the world to increase the intensity of fish production to meet the growing demand, especially since the level of global fisheries production is stable, and this is encouraged by the great development in fish care and breeding techniques. As well as the improvement of fish farm conditions, including increasing the ability to control water properties, improving the efficiency of dealing with diseases, and developing fish feed manufacturing processes for different stages and types of fish (Al-Jamal, 2016). Growth is one of the most important factors for commercial success in animals, including fish. The body growth trait in fish is of great importance for individual survival and reproduction and has implications for population, ecology and evolution. Somatic growth is controlled by the GH/IGF endocrine axis and is influenced by nutrition, reproductive hormones as well as abiotic factors such as temperature and salinity (Canosa and Bertucci, 2023). Fish growth analysis has become an essential part of research in fisheries biology, aquaculture and physiology (Carcamo *et al*., 2019).

Common carp is a very important species for aquaculture in many Asian and some European countries. It has been introduced practically everywhere outside its original geographic and climatic range. The application of better management practices can contribute significantly to the material and financial development of its producers and to the health of the environment (FAO, 2011). There are a number of environmental factors, such as temperature, oxygen concentration, salinity, and photoperiod,

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that affect the growth rate. Recent data indicate that genotypes hormones, and physiological conditions of the individual are also internal growth regulators (Dutta, 1994).

Follistatin (FST) is a cysteine-rich monomeric glycoprotein, originally isolated from bovine and porcine follicular fluid in 1987 (Ueno *et al*., 1987; Robertson *et al*., 1987). Follistatin plays important roles in modulating the action of activin through its binding property (Murakami *et al*., 2012). Regulates skeletal muscle growth by inhibiting proliferation and terminal differentiation inhibin-activin-follistatin is part of the muscle cell axis, as a competitive binding protein MSTN, FST can inhibit its function and accelerate muscle growth in vivo (Amother *et al*., 2004). The majority of these functions are facilitated by the affinity of follistatin for activin, as the effects of activin are neutralized by its binding to follistatin. As such, the interaction between follistatin and activin represents a powerful regulatory mechanism that affects a variety of cellular processes within the body (Phillips and de Kretser, 1998).

The present study aimed to determine the genetic makeup of the follistatin gene in common carp and to correlate the genetic polymorphisms of the gene with productive traits.

II. Materials and Methods

One hundred common carp *Cyprinus carpio* fish, weighing between (8-36)g, were obtained from the Agricultural Research and Experimental Station in Al-Hartha (Aquaculture Unit of the College of Agriculture). The open culture system was designed in the fish nutrition laboratory at the Department of Fisheries and Marine Resources, using 10 aquaria with dimensions of 60 x 40 x 30cm; the capacity of each was 50 liters. The ponds are equipped with electric air pumps for aeration and supplying the water with dissolved oxygen, as well as heating devices (heater) to maintain the water temperature at +25°C, which is the appropriate temperature for fish (Guderley and Blier, 1988). The fishes were distributed randomly, with 8 fish in each tank and the experiment lasted for 56 days. Fishes were fed a standard diet and were marked by colored threads. The environmental conditions were similar in all ponds (table, 1).

Table 1. The physical and chemical properties of the water used in experimental tanks of common carp fish during 56 days of experiment

Temperature (º C)	Salinity (ppt)	Dissolved oxygen (mg/L)	pH
22-24.36	0.4-0.9	6.36-9.38	6.80-8.74

Genomic DNA Extraction

The fish were anesthetized using clove powder at a concentration of 400 mg/L (Hassan *et al*., 2021). A sample of 0.5 ml of blood were collected from the heart muscle of all trial fish. These samples were collected in EDTA tubes and kept in freezer (- 18 °C) for DNA extraction by using DNA extraction kit (Geneaid, Korea), Then DNA samples were examined extracted using electrophoresis, where1% agarose gel was used and voltages of 70 and 65 mA.

PCR amplification

A primer was designed for this study as shown in Table 2 and was prepared by the Korean company Macrogen. The mixture was prepared from all the materials for the polymerase chain reaction (PCR) and the final concentration of the components was 25 microliters, Thermal cycle with the following profile: Initial denaturation at 95 C° for 5 minutes, then 35 cycles of 95 C° for 30 seconds, 55 C° for 30 seconds,72 C° for 45 seconds,72 C° for7 minutes.

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Table 2. Sequence of primer and the region covered of the Follistatin gene

Fragment code	Primer pair	Fragment size	Adhesion temperature (Ta, C)	Note
Folfs FST	F: 5'- AGGGACCAACTTTATGATCACC - 3' R: 5'- TGGACATGATTCCTTGCCCT - 3'	798 bp	55	Design in this study

Statistical analysis

The statistical program SPSS version 26 (2019) was used to the analyze the differences due to genotypes of follistatin gene. An independent Samples t Test was conducted to compare the genotype groups.

III. Results and Discussion

Polymorphisms

The results (Sequence) for the studied segment of follistatin with a size of 798 were established using the sequencing technology and the results were aligned on the global website of the Gene Bank (https://www.ncbi.nlm.nih.gov). It showed a match with the follistatin gene Figure (1) in common carp fish. The results of the current study showed the presence of two mutations in the studied region and after analyzing it, a mutation appeared at the nitrogenous base with the sequence 344 and each mutation resulted in two genetic structures, which are AA and AG (Figure 2).

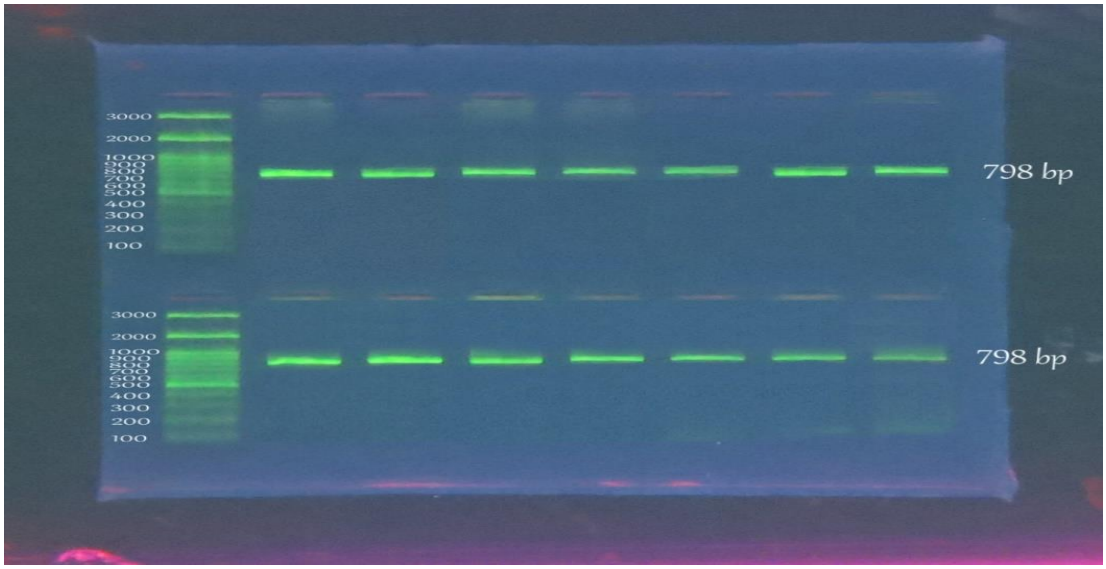

Figure (1) FST Gone migration of 75 pairs and more using 15% Caron Marker and 70 and 65 milliamps (use DNA Latter of 100 - 3000-pairs) from Geneaid

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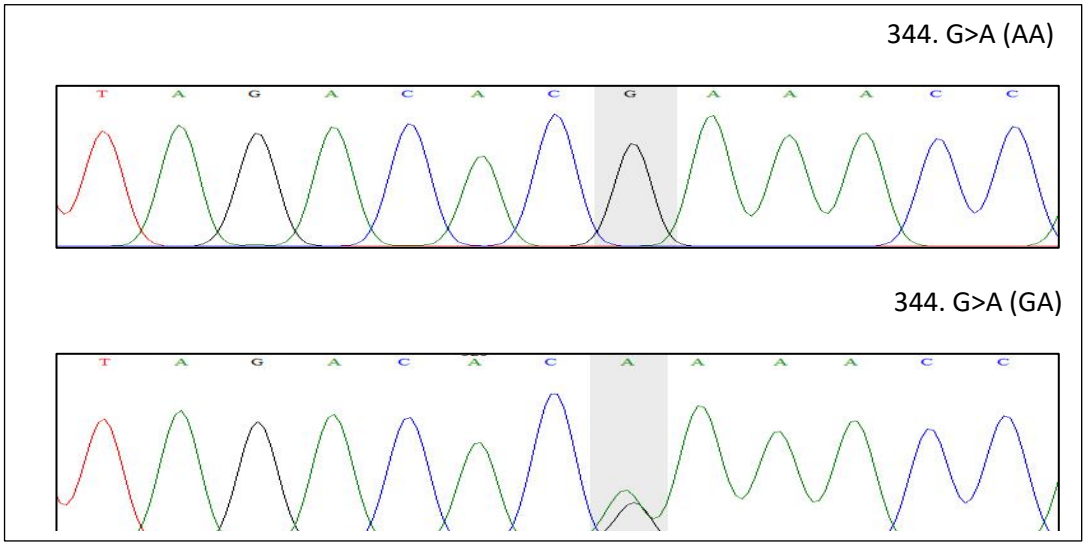

Figure (2) Mutation at nitrogenous base 344

Experimental conditions measurements

Growth parameters

In an effort to stimulate increased focus on identifying fish growth genes, before identifying potential candidate growth genes in finned fishes, it is essential to understand how allelic variation in a gene can influence its regulation. Candidate genes associated with growth Somatic morphology is a polygenic trait resulting from several physiological pathways that regulate energy metabolism and muscle growth (De-Santis and Jerry, 2007).

Figure 3, 4, 5, 6 & 7 show the initial body weight, total length, standard length, head length, body height, caudal peduncle depth, and eye diameter of the follistatin gene genotypes. The results of the statistical analysis showed no significant differences (0.05) in the body weight, total length, standard length, head length, body height, caudal peduncle depth, and eye diameter during the experimental period for the two follistatin gene genotypes. However, the follistatin gene has important but different roles in embryonic development in addition to the significant genetic effects on regulating early growth in fish. Applying this study to a larger sample of carp fish may give more truthful results. Subhi, (2020) indicated similar results when studying the relationship of the genetic features of the growth hormone receptor gene with some physiological and productive traits of common carp fish *Cyprinus carpio*. As well as Alwan (2018) when studying the genetic polymorphism of the mabostanin gene (T2230 and 2232) and its relationship with some physiological and growth traits of common carp fish as they concluded that applying a larger fish sample would give more accurate results in applying Gene-Assisted Selection (GAS) methods.

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Figure 3. Initial body weight, total length, standard length, head length, body height, caudal peduncle depth and eye diameter for follistatin gene genotypes

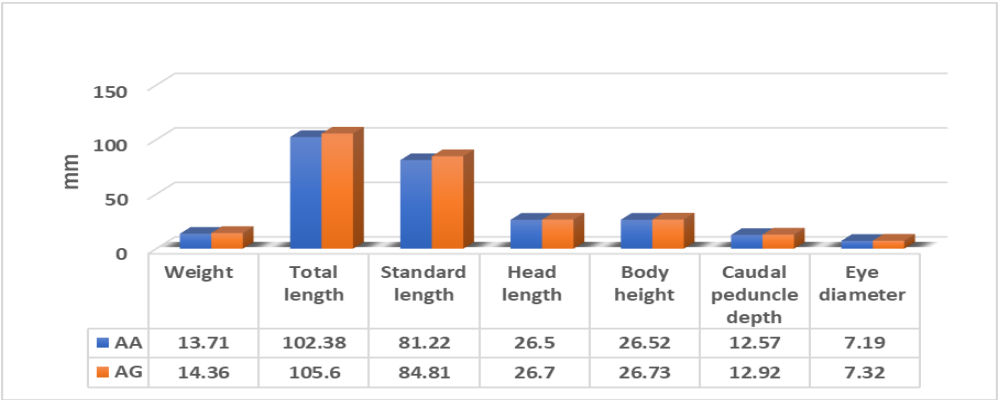

Figure 4. Average weight, total length, standard length, head length, body height, caudal peduncle depth and eye diameter for follistatin gene genotypes after 14 days of experiment

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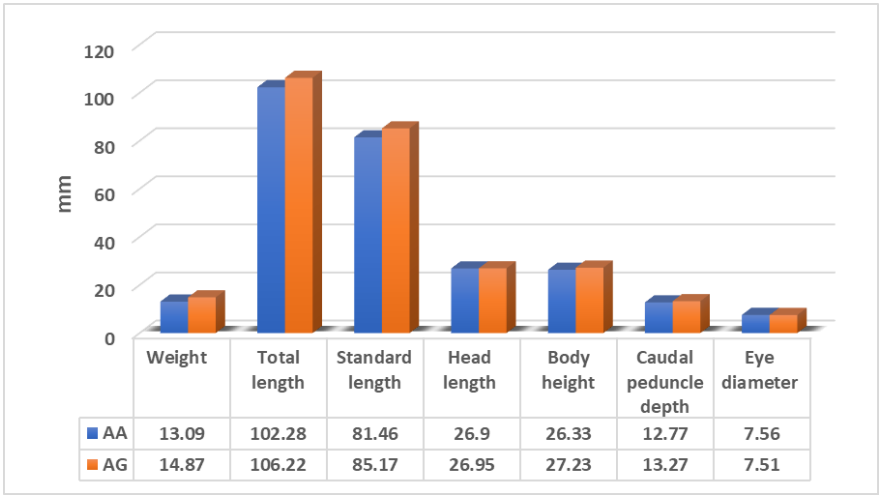

Figure 5. Average weight, total length, standard length, head length, body height, caudal peduncle depth and eye diameter for follistatin gene genotypes after 28 days of experiment

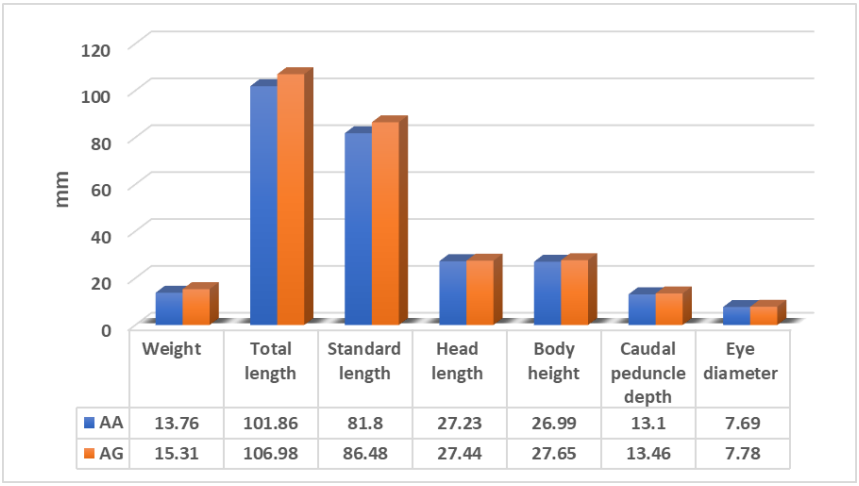

Figure 6. Average weight, total length, standard length, head length, body height, caudal peduncle depth and eye diameter for follistatin gene genotypes after 42 days of experiment

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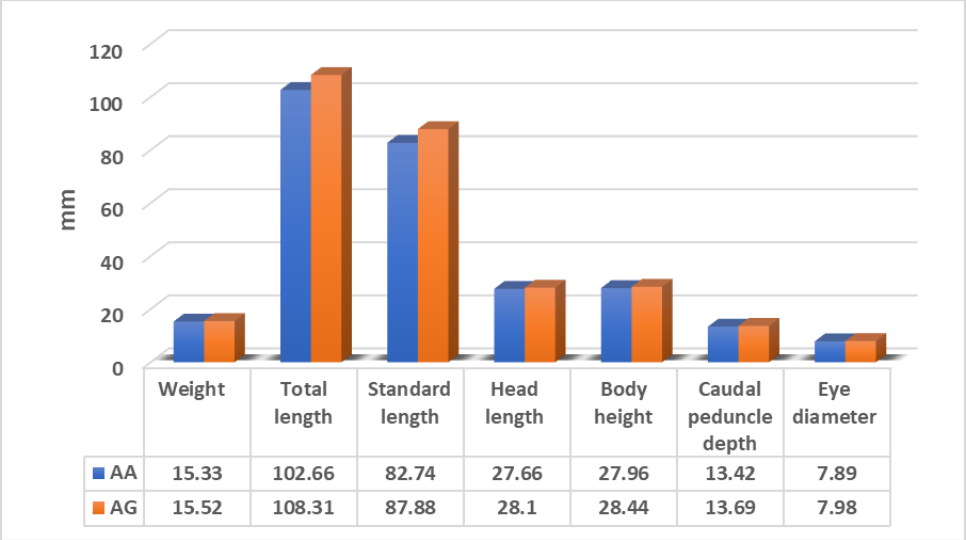

	Weight	Total length	Standard length	Head length	Body height	Caudal peduncle depth	Eye diameter
AA	15.33	102.66	82.74	27.66	27.96	13.42	7.89
AG	15.52	108.31	87.88	28.1	28.44	13.69	7.98

Figure 7. Average weight, total length, standard length, head length, body height, caudal peduncle depth and eye diameter for follistatin gene genotypes after 56 days of experiment

Morphological, physiological, behavioral and biochemical characteristics have been widely used to identify and classify fish species, however, the use of metric measurements (such as body length, body depth, head length, eye diameter and jaw length) is more common (Howe, 2002). Mean body weight was 12.98 g for the AA genotype and 13.70 g for the AG genotype at the beginning of the experiment. These weights increased to 13.71 g for the AA genotype and 14.36 g for the AG genotype at 14 days of the experiment. While the maximum weight recorded for the AG genotype 15.33 g and AA genotype 15.52 g at the end of the experiment. It is noted that the weight gain reached 1.63 g in the genotype AA and 2.54 g in the genotype AG during 56 days of the experiment. Saleh (2021) recorded a weight gain rate of 1.5 g in juvenile common carp fish during 56 days of the experiment. Kadhim (2022) recorded a weight gain rate of 1.86 g in juvenile common carp fish during 56 days. Whereas, Dawoud (2023) recorded a weight gain rate of 4.79 g in juvenile common carp fish during 56 days.

There are a number of factors that affect the ratio of SL, FL and TL of fish including the growth stage, food availability and quality, size range, health and general condition of the fish and preservation techniques in addition to other sampling procedures, sample size and length range that the analysis of covariance (Önsoy *et al.,* 2011). Gaygusuz *et al.(*2006) revealed no statistically significant differences ($p<0.05$) in the slope of length-length relationships for measurements between researchers for all fish species and no significant difference between personal measurements of different species, Jin *et al.* (2012) found that the relationship between eye cross and eye diameter with other commercial traits and QTL linkage group analysis suggests that eye diameter and eye cross can be used to aid indirect selection for body weight and has implications for future genetic studies and breeding of common carp.

IV. Conclusion

In conclusion, the current study introduced available information regarding the association of follistatin gene polymorphism and growth parameters of juvenile common carp during 56 days. Two genotypes were identified for the studied gene at the nitrogen base 344. The analysis of growth parameters including body weight and total length did not reveal a significant correlation with gene polymorphism during the study period. In case of getting realistic result, a large sample is needed in future studies. Which may enhance the potential for implementing marker-assisted selection (MAS) strategies in common carp

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breeding programs. Eventually, such research could contribute to optimizing growth performance and improving aquaculture practices.

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