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GENE POLYMORPHISM 1RELATIONSHIP OF APOLIPOPROTEIN A-WITH SOME GROWTH TRAITS OF COMMON CARP (*CYPRINUS CARPIO* L .)

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ABSTRACT

The study was conducted to investigate the relationship between the polymorphisms of A, B, C, D and E of the apolipoprotein A-1 (ApoA-1) gene with some traits (daily and total weight gain rate and feed conversion rate and efficiency) in 70 samples of common carp (*Cyprinus carpio*). There were significant differences in the growth traits of the genotypes A, B, C and D of the apolipoprotein A-1 gene compared to the genotype E. The results showed that the genotype C was significantly superior (p≤0.05) in the daily and total weight gain rates and reached the highest value of 0.56 and 39.42 g/fish, respectively, while the genotype E decreased to the lowest value of 0.14 and 9.50 g/fish, respectively. On the other hand, the feed conversion rate recorded highest significant effect (p≤0.05) at the genotype C and reached 5.13 Compared with the E genotype which recorded 9.6, the highest significant effect (p≤0.05) on the feed conversion efficiency trait was at the C genotype at a rate of 19.49% compared to the E genotype which reached 10.41%. We conclude from the above that the ApoA-1 gene may have an effect in improving the productive growth traits in common carp fish through its effect on the quantitative traits associated with the genetic phenotypes.

Keywords: Common carp. Quantitative traits. Phenotypes. Growth traits. ApoA-1.

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INTRODUCTION

Apolipoprotein is considered an important component of plasma lipoproteins, which has a function in promoting fat transfer, regulating enzyme activity, and directing the binding of lipoproteins in plasma to receptors (Simon, et al. 2011), as well as energy storage (Shen, et al. 2008). ApoA-1 regulates lipid deposition in the liver (Jobling 1993,Wang, et al. 2016.). reported that the levels of expression were significantly different from ApoA-1b in common carp before and after Re-feeding and heavy and light groups suggest that ApoA-1b plays an important role in regulating growth. Kondo et al. (2005) noted little similarity between fish lipoproteins and their mammalian counterparts. Analyses of tissue expression of apoA-1 in some fish species showed that the liver was consistently the highest expressed, but more variable expression levels were observed in the intestine, gills, and skin (Villarroel, et al. 2007, Dietrich et al. 2014). Apolipoproteins influence food intake in fish, control of neuronal diseases, lipid metabolism and transport, as well as the multiple roles of these proteins. The ApoA-1 gene is the major component of high-density lipoproteins (HDL), which are major plasma proteins that perform diverse functions. However, their presence and roles are not well understood in many fish species. Previously, these ApoA-1 proteins were only known for lipid transport and metabolism, but later they were recognized to perform important immune

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functions (Park et al. 2021). Considered diet is one of the main factors affecting fat content and its composition in muscle tissue of common carp (Mráz, & Pickova, 2011). Since fish are variable-temperature animals and fats are used as a primary source of energy, unlike mammals that primarily use carbohydrates, lipoproteins may have a unique role in fish compared to other vertebrates (Watanabe, 1982). Fish uses fats instead of carbohydrates as the main source of energy physiologically fat metabolism and lipoprotein functions are very important for homeostasis (Kondo et al. 2005). Where fish derive energy mainly from fats, which play an important role in the metabolism and growth of fish, therefore the process of fat metabolism appears to be more important to maintain the homeostasis balance in external organism's heat Ectotherms such as fish, more than those found in Homeotherms heat congeners (Kondo et al. 2005). Some studies have indicated that ApoA-1 is very important for fish growth and should be taken into account when developing a suitable aquaculture diet (Qu, et al. 2014). Fat in the liver appears to be regulated by the rate of fat synthesis and the rate of secretion of lipoprotein ,fat accumulates in the liver as droplets when the manufacturing rate is higher than the rate of excretion ,and the low rate of lipoprotein secretion may be responsible for high levels of fat in the liver in some fish such as puffer and flounder .More studies on lipoproteins are needed to clarify the mechanism of fat accumulation in fish (Amthauer, et al. 1989). Park et al. (2021) studied the transcriptional responses of the ApoAI gene in the fish Spotnape Ponyfish (Nuchequula nuchalis), which lives in different salinity environments, from estuaries to deep waters. He concluded that expression changes in the ApoAI gene in these fish may be linked to changes in lipid and glucose metabolism, and thus may stimulate different growth and development, leading to different body sizes in N. nuchalis. However ,studies on the functions of ApoA 1-in fat metabolism in fish have not been conducted on a large scale despite the fact that fish uses fat as the main source of energy ,and because of the importance of ApoA 1-in both metabolism and the normal immune system (Sahoo, et al. 2017). In addition to the known roles in fat transport and absorption, many fish apolipoproteins are needed for normal organism and embryonic cell growth and tissue regeneration (Lange, et al. 2005), and immunomodulation innate (Concha, et al. 2004). It has a certain protective effect on the healthy growth of fish (Villarroel, et al. 2007). As well as its antibacterial-positive and Gram-negative activity as well as its anti-pathogenic activity in fish (Johnston, et al. 2008). Therefore, the study aims to study the effect of the relationship of polymorphisms the phenotype in some the growth traits of common carp fish.

MATERIALS AND METHODS

Experimental design

Common carp were obtained with weights ranging from 70-90 g from one of a fish farm. Fish were transported to the laboratory by a car equipped with an air and water pump to circulate the water and placed in large metal tank. Sterilization of fish was carried out using a 5 g / liter salt to remove bacteria, fungi and parasites. The fish were transferred to twelve glass tank filled with water of 60 liters, water was changed daily by 20 liters with liquefied water stock for 48 hours to dispose of chlorine, all the the glass tank were equipped with air pumps to provide continuous oxygen and 100 watts of temperature to ensure the proper temperature for the growth of common carp fish (Naser, et al. 2024).

Fish acclimation: Each fish was independently numbered by a Chinese numbering device, a pistol with a needle fitted with numbers and a DYMO printer for

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printing numbers on small pieces of tape. Fish were distributed randomly and evenly on tanks with five fish/tank to acclimate them to the new conditions, a diet with protein content 27.06% was used for three meals a day, feeding 3% of body weight at the start of the experiment, depending on the appetite and acceptance of the fish, feeding was stopped 24 hours before the start of the experiment (6), the acclimation period lasted 15-day rounding off.

Studied Traits

Total Weight Gain (T.W.G.): Known as the amount of difference between the final weight and the initial weight of the fish was calculated as follows (Uten, 1979):

T.W.G. = F.W. – I.W.

As: (F.W) = Final weight (g

Daily Growth Rate (D.G.R.)

Which is the amount of calculated weight gain per fish per day (g / day) It was calculated from the equation cited (Jobling, 1993):

D.G.R. = $(W_2 - W_1) / (T_2 - T_1)$

As:

W1 = initial weight (g)

W2 = Final weight (g)

T2 - T1 = Duration for the experiment or between the two weights Days.

Feed Conversion Ratio (F.C.R)

It is calculated according to the method mentioned (Uten, 1979):

$$Font\ type\ and\ size = \frac{Weight\ of\ dry\ feed\ intake\ (g\ /\ fish)}{Wet\ weight\ gains\ for\ fish\ (g\ /\ fish}$$

Feed Conversion Efficiency (F.C.E)

It is calculated according to the method mentioned (Uten, 1979)

$$Feed\ Conversion\ Efficiency = \frac{\text{Wet weight gains for fish (g/fish)}}{\text{Weight of dry feed intake (g/fish)}} \times 100$$

RESULTS AND DISCUSSION

Extraction of the ApoA-1 gene

The ApoA-1 gene (Figure 4-1) was completed as a single band of 750 bp was reached after it was amplified by polymerase chain reaction (PCR), and a 5 µl sample of the PCR product was carried out in a 1% acrose gel, after the voltage was set at 100 V for 80 minutes with the use of DNA fragments of known sizes (Marker) 100-1500 bp to determine the sizes of the extracted fragments, and the concentrations of the DNA samples ranged between 116-68 ng/ml.

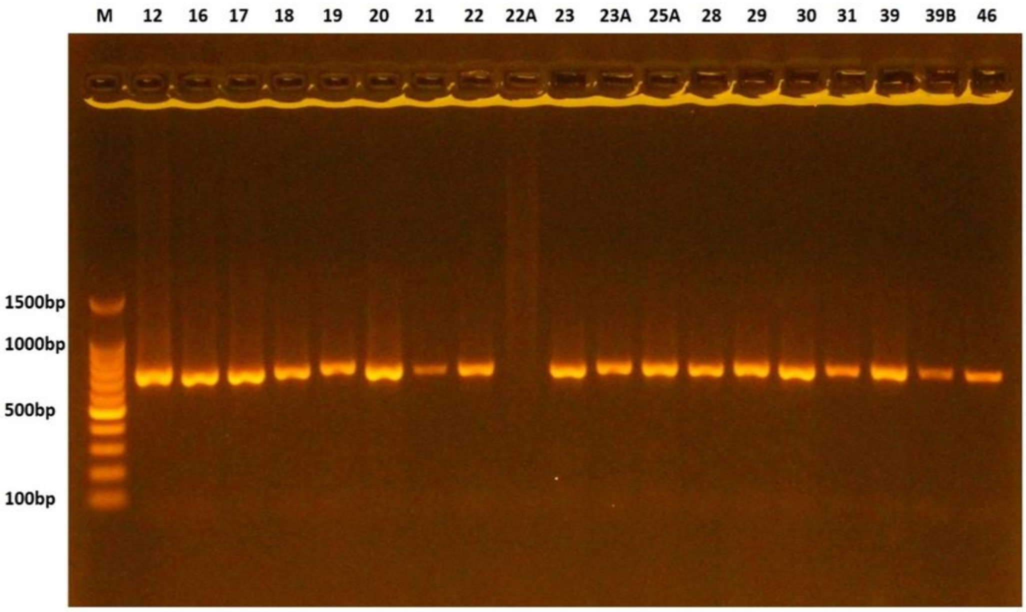

Figure 1. The extracted fragment of the ApoA-1 gene using PCR technology for common carp fish

Polymorphism of the ApoA-1 gene

Table (1-4) includes the numbers and percentages of the distribution of genetic phenotypes of common carp fish A, B, C, D, E and F, as the percentages of Polymorphism reached 44.78, 19.40, 28.36, 2.99, 2.99 and 1.49%, with numbers of 30, 13, 19, 2, 2 and 1, respectively. The results showed a highly significant superiority (P<0.01) for fish carrying the Polymorphism and the least for those carrying the Polymorphism F. The reason may be due to the adaptation of fish with the genetic phenotype A to environmental conditions and their unsuitability to their counterparts with the Polymorphism F, in addition to the small sample size.

Table 1. Number and percentage of the frequency of Polymorphism of the ApoA-1 gene in common carp

Pecentage	Numbers	Polymorphism
A	30	44.78
B	13	19.40
C	19	28.36
D	2	2.99
E	2	2.99
F	1	1.49
Total	67	100%
Chi sequare	---	61.32**
.(P<0.01) **		

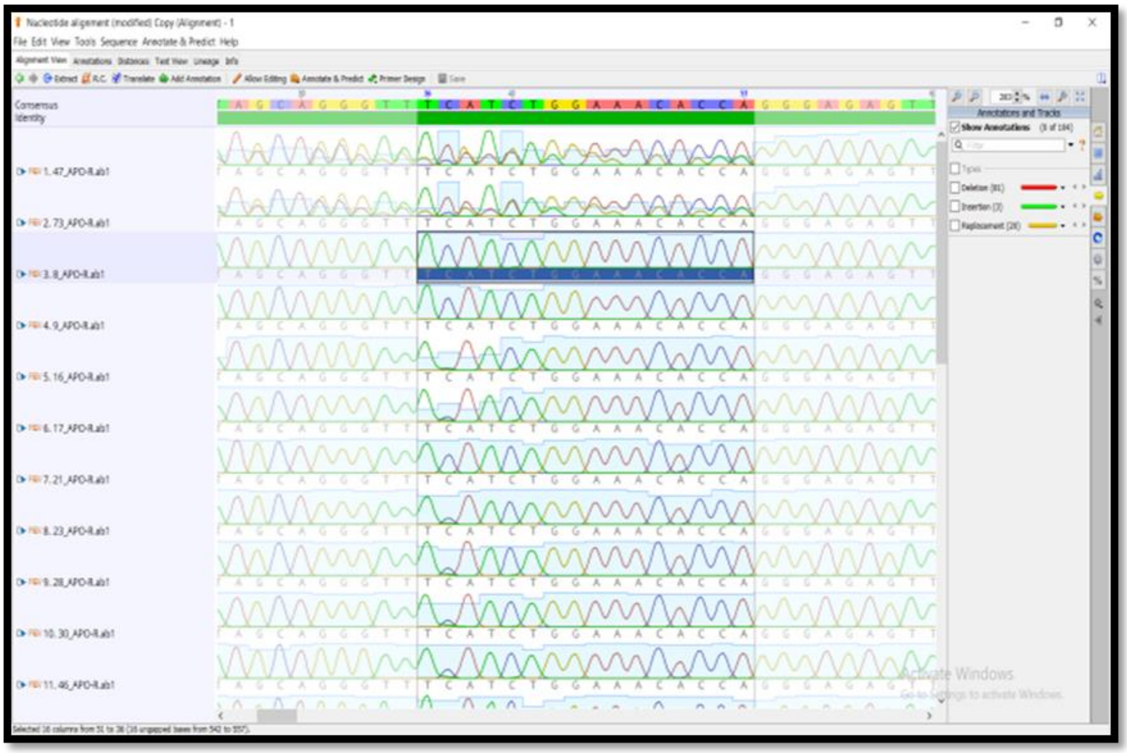

Figure 2. shows the genotypes A, B, C, D, E, and F of the ApoA-1 gene, which were identified using Geneious Software (version 10.1.3).

Table (2) includes effect of polymorphism of lipoprotein gene ApoA-1 in the traits rate of daily and total weight gain, feed conversion coefficient and feed conversion efficiency of common carp fish and for 70 days from the start of the experiment, significant superiority was observed (P<0.05) for phenotype A, B, C and D in all the growth traits mentioned on the

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phenotype E. An improveent was observed in the growth traits of the fish carrying the phenotype A, B, C and D probably due the presence of phenotype that are related to lipoprotein gene ApoA-1, that affects the genotype associated with quantitative traits that are affected for environmental and genetic factors. Quantitative traits can be highly multi-gene, ie, influenced by more than one gene, as that increasing the genetic expression of ApoA-1 by increasing the mRNA copies will increase its association with cholesterol and phosphates found within the cells, thus increasing HDL, and increasing the metabolic rate and thus improving the growth traits, so these fish are more innate immunity and resistance to pathogens, due decreased all growth traits of the phenotype E Perhaps as a result of the presence of the gene is a recessive pattern in the ApoA-1 gene, leading to low metabolism and low weight, phenotypes are influenced by genes as well as environmental factors, which may have been inappropriate for some fish because of the nature of its genotype bearing it, for example if fish can't obtain their basic nutrient needs, they can't produce specific proteins and therefore can't produce a specific phenotype. And this is one of the effects of environmental factors, differences in phenotype may sometimes be reversed because of the effect of a particular genotype on fish, affecting growth traits such as feed conversion efficiency and feed conversion coefficient. There may be more than one gene effect on phenotypic traits causing differences in rates of weight gain and growth, However, the effect of ApoA-1 gene remains important for the growth and health of common carp fish. The current study is consistent with (Shen, et al. 2008) He pointed out that the phosphate food fats increase the efficiency of the transfer of food such as fatty acids and fat from the intestine to the rest of the body possibly through the synthesis of enhanced lipoprotein. The study agrees with (Li, et al. 2018) when he conducted a study on polymorphism of SNPs for Apo-lipoprotein gene and its association with the growth properties of large bass mouth fish, it was found that Apo is involved in the regulation of lipid absorption, so the polymorphic sites in this gene may affect the digestion and absorption of fat in feed when feeding the bass of the largemouth bass, affecting fish growth and metabolism (Li, et al. 2018). Agree with the study of (Abdelghany, & Ahmad,. 2002) which have shown that an increase in the nutrition rate from the ideal level or when providing a greater amount of feed exceeds the level of saturation, the fish will not benefit from excess feed. As (Farhan, &. Naser, 2024) points out, the determinant factor for nutrient efficiency is saturation. As well as what (Chowdhury, 2011) the increase in the feeding rate of 3, 4 and 5% of body weight in *Oreochromis niloticus* cultured in ponds did not significantly affect weight gain, final weight and growth rate specific. On the other hand, the results are consistent with what was indicated by (Wang, et al. 2016) a study of significantly different expression levels of apoA-1 before and after refeeding and between light and heavy groups of common carp, which indicates that apoA-1 plays an important role in growth regulation. The increased expression of apoA-1 in *C. carpio* upon starvation for 20 days may indicate that fish need to consume their own fat to maintain normal metabolism during fasting. Therefore, fish synthesize more apoA-1 proteins to transport fat during fasting and convert it to energy absorption from external food after refeeding. A similar phenomenon of increased expression levels of apoA-1 during fasting has been observed in different human populations (Afolabi, et al. 2007). Therefore, the nutritional balance between energy and protein in the fish diet will regulate gene expression, which is crucial for supporting the expression of phenotypic productive traits, thus providing protective mechanisms against sudden, short-term and chronic environmental stress after good growth.

Table 2. Relationship of polymorphism lipoprotein gene ApoA-1 with daily and total weight gain rates and feed conversion coefficient and the feed conversion efficiency for common carp fish

Polymorphism	Averages ± standard error			
	Daily gain rates g/fish / day	Total gain rates g/fish	Feed conversion coefficient	Feed conversion efficiency %
A	0.49±0.03 a	34.4±2.60 a	7.69±1.01 A	13.00±1.43 a
B	0.54±0.05 a	38.07±4.06 a	5.68±0.63 A	17.60±2.07 a
C	0.56±0.06 a	39.42±4.40 a	5.13±1.54 A	19.49±2.25 a
D	0.45±0.06 a	31.5±4.50 a	6.8±0.97 A	15±2.14 a
E	0.14±0.05 b	9.50±3.5 b	9.6±23.65- B	10.41±2.05 b
significant level	*	*	*	*

The averages with different letters within the same column vary significantly between them (P<0.05) * N.S. Not significant.

CONCLUSIONS

The results of this study suggest that relativity must be considered when examining the association between polymorphisms A, B, C, D, and E of the Apolipoprotein A-1 (ApoA-1) gene and various growth parameters, including daily and total weight gain rates and feed conversion rate and efficiency. The ApoA-1 gene may influence the enhancement of productive growth features in common carp by impacting the quantitative traits linked to phenotypes.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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علاقة تعدد الوراثية لجين البروتين الدهني Apolipoprotein A-1 مع بعض صفات النمو لإسماك الكارب الشائع (*Cyprinus carpio* L.)

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المستخلص

أُجريت الدراسة لبحث علاقة تعدد المظاهر الوراثيةA وB وC وD وE لجين البروتين الدهني (ApoA-1 Apolipoprotein A-1) مع بعض الصفات (معدل الزيادة الوزنية اليومية والكلية ومعدَّل وكفاءة التحويل الغذائي) في 70 عينة من أسماك الكارب الشائع *Cyprinus carpio*. كانت هنالك فروق معنوية في صفات النمو للمظاهر الوراثية A وB وC و D لجين البروتين الدهني ApoA-1 بالمقارنة مع المظهر الوراثي E. إذ أظهَرَت النتائج تفوق المظهر الوراثي C معنوياً ($p \leq 0.05$) في معدَّلات الزيادة الوزنية اليومية والكلية وبَلَغَت أعلى قيمة 0.56 و39.42 غم/سمكة على التوالي، بينما انخفض المظهر الوراثي E إلى أدنى قيمة 0.14 و9.50 غم/سمكة على التوالي، من جهة أُخرى سَجل معدَّل التحويل الغذائي أعلى تأثير معنوي ($p \leq 0.05$) عند المظهر الوراثي C وبَلَغَ 5.13 مقارنةً مع المظهر الوراثي E الذي سجِّل 9.6، كان أعلى تأثير معنوي ($p<0.05$) في صفة كفاءة التحويل الغذائي عند المظهر الوراثي C بمعدل 19.49% بالمقارنة مع المظهر الوراثي E الذي بَلَغَ 10.41%. نستنتج مما تقدم أَنَّ جين ApoA-1 ربما لهُ تأثير في تحسين صفات النمو الانتاجية في أسماك الكارب الشائع عن طريق تأثيرهُ في الصفات الكمية المرتبطة بالمظاهر الوراثية.

كلمات مفتاحية: الكارب الشائع، الصفات الكمية، المظاهر الوراثية، صفات النمو، ApoA-1